

DOES ENVIRONMENTAL ROBUSTNESS PLAY A ROLE IN FLUCTUATING ENVIRONMENTS?

Tarmo Ketola,^{1,2} Vanessa M. Kellermann,^{3,4} Volker Loeschcke,³ Andrés López-Sepulcre,⁵ and Torsten N. Kristensen^{3,6,7}

¹Centre of Excellence in Biological Interactions, Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FI-40014, Finland

²E-mail: tketola@cc.jyu.fi

³Department of Bioscience, Integrative Ecology and Evolution, Aarhus University, Ny Munkegade 114, Building 1540, DK-8000 Aarhus C, Denmark

⁴Department of Biological Sciences, Monash University, Clayton 3800, Victoria, Australia

⁵Laboratoire d'Ecologie et Evolution, CNRS UMR7625. Ecole Normale Supérieure de Paris, 46 Rue d'Ulm, 75230 Paris Cedex 05, France

⁶Department of Molecular Biology and Genetics, Aarhus University, Blichers Allé 20 DK-8830, Tjele, Denmark

⁷Department of Biotechnology, Chemistry and Environmental Engineering – Section of Biology and Environmental Science, Aalborg University, Sohngårdsholmsvej 57, DK-9000, Denmark

Received April 11, 2013

Accepted September 26, 2013

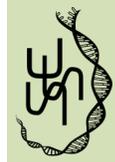
Data Archived: Dryad doi:10.5061/dryad.1pg07

Fluctuating environments are expected to select for individuals that have highest geometric fitness over the experienced environments. This leads to the prediction that genetically determined environmental robustness in fitness, and average fitness across environments should be positively genetically correlated to fitness in fluctuating environments. Because quantitative genetic experiments resolving these predictions are missing, we used a full-sib, half-sib breeding design to estimate genetic variance for egg-to-adult viability in *Drosophila melanogaster* exposed to two constant or fluctuating temperatures that were above the species' optimum temperature, during development. Viability in two constant environments (25°C or 30°C) was used to estimate breeding values for environmental robustness of viability (i.e., reaction norm slope) and overall viability (reaction norm elevation). These breeding values were regressed against breeding values of viability at two different fluctuating temperatures (with a mean of 25°C or 30°C). Our results based on genetic correlations show that average egg-to-adult viability across different constant thermal environments, and not the environmental robustness, was the most important factor for explaining the fitness in fluctuating thermal environments. Our results suggest that the role of environmental robustness in adapting to fluctuating environments might be smaller than anticipated.

KEY WORDS: Constant versus fluctuating temperature, genetic correlation, genotype-by-environment interaction, phenotypic plasticity, reaction norm.

Fluctuating environments are thought to select individuals that are viable across environments. In theory, high viability in fluctuating environments could be achieved by two mutually nonexclusive means; having high average viability across the environments

and having high environmental robustness in viability (i.e., low amounts of variation in viability between environments), their relative importance being dependent on the proportion that they confer to viability in fluctuating environments (Scheiner 1993;



Liefting et al. 2009; Masel and Siegal 2009). Typically, the association between genotype and the environment has been described by reaction norms (i.e., the genotype-specific viability across environments). In the case of two environments, the slope describes the level of environmental robustness in viability, whereas the elevation describes the average viability of the genotype across two environments. Determining the extent to which fluctuating environments select for high reaction norm elevation or a flatter reaction norm slope provides insight into the theoretical basis of the evolution of environmental robustness and phenotypic plasticity (Scheiner 1993; Liefting et al. 2009; Masel and Siegal 2009). It also has important implications for predicting the outcome of climate change induced thermal variation on biota (IPCC 2007), and explaining the maintenance of genetic variation in fitness (Hunt et al. 2004). Moreover, if the genetically determined rank of individuals is sensitive to the environment, it has direct consequences for the determination of fitness and on sexual selection (Qvarnström 2001; Hunt et al. 2004; Mills et al. 2007).

In order for reaction norm parameters to respond to selection, they must have a genetic basis. Evolutionary potential is traditionally quantified by the heritability of the trait, and there have been repeated attempts to quantify the heritability of reaction norm slope and elevation, in different traits (Scheiner and Lyman 1989; Nussey et al. 2005, 2007; Brommer et al. 2008; Charmantier et al. 2008; reviewed in Scheiner 1993; Windig et al. 2004). These studies show, that in general the reaction norm elevation contains more heritable variation than the slope, but that both reaction norm parameters should contain enough genetic variation to respond to selection. Evolutionary changes in traits have traditionally been predicted by the Breeder's equation (e.g., Falconer and Mackay 1996), where the heritabilities and genetic correlations of traits play a key role, alongside the strength of selection on each of the traits. Recently, Robertson Price identity (Price 1970) has been suggested to be a more correct way for predicting evolutionary changes in traits, especially when the potential selection pressures are uncertain and when not all fitness components have been measured (Morrissey et al. 2010, 2012). Robertson Price identity is the trait's genetic covariation with fitness, which predicts the effect of selection for fitness on correlated traits. Therefore, by the genetic covariation between reaction norm parameters for fitness (here: slope and elevation of egg-to-adult viability measured in constant environments) and fitness in fluctuating environments (here egg-to-adult viability in fluctuating environments) can be used to predict whether fluctuating environments select for high reaction norm elevation or a reduced slope. However, it is noteworthy that this interpretation assumes that fitness in fluctuating environments can be predicted by the reaction norm parameters that are estimated in constant environments. Tolerance of temperature transitions, bet-hedging or rapid expression of stress proteins when environments become

stressful are important for survival and fitness in fluctuating environments (Cohen 1966; Berrigan and Scheiner 2004; Sørensen et al. 2003; Ketola et al. 2004; Gabriel 2005; King and Masel 2007; Arnoldini et al. 2012). However, none of these factors might be reflected in reaction norm parameters estimated in constant environments. If this is the case, the genetic correlations between reaction norm parameters and viability at fluctuating temperatures might be much smaller than expected.

Despite the strong theoretical interest in reaction norms, their pervasive role in various fields of biology (e.g. Qvarnström 2001; Hunt et al. 2004; DeWitt and Scheiner 2004), and a number of studies exploring the genetic potential (i.e. heritability) of reaction norm parameters (Scheiner and Lyman 1989; Nussey et al. 2005, 2007; Brommer et al. 2008; Charmantier et al. 2008; reviewed in Scheiner 1993; Windig et al. 2004), there is a lack of studies investigating whether genetically determined reaction norm parameters are genetically correlated with fitness in fluctuating environments. Although there have been a few attempts to do this in natural populations (e.g. Brommer et al. 2005; Nussey et al. 2005), the statistical methods used in these studies may not have been optimal (Postma 2006; Hadfield et al. 2010). By using the methodology outlined by Hadfield et al. (2010), we were able to circumvent statistical problems associated with breeding value regressions.

We used a standard full-sib, half-sib breeding design (Lynch and Walsh 1998) to explore the quantitative genetic basis of egg-to-adult viability of *Drosophila melanogaster* in constant and fluctuating thermal environments. Unlike adults capable of behavioral thermoregulation, eggs, larvae, and pupae are directly subjected to heat stress when exposed to high temperatures in nature. Thus, egg-to-adult survival is a target of strong temperature-dependent selection and encompasses much of the natural life time fitness of this short lived insect species (Cossins and Bowler 1987). In our experiment, eggs from a number of paternal half-sib families were split into four temperatures: two stable (25°C and 30°C) and two fluctuating environments (means of 25°C and 30°C). Note that all of the experienced temperatures fall above the temperature that is considered optimal for *D. melanogaster* (David et al. 2004; J. Overgaard, pers. comm.). With this design we can test (1) if egg-to-adult viability in fluctuating environments is genetically positively correlated to reaction norm elevation for egg-to-adult viability and negatively to reaction norm slope; and (2) how well reaction norm slope and elevation of egg-to-adult viability estimated at constant temperatures can predict egg-to-adult viability in fluctuating environments.

Materials and Methods

We used a mass bred *D. melanogaster* population collected in Southern Tasmania, Australia (43.15°S, 147.00°E). Before the

experiment, flies were maintained at approximately 1000 individuals under a 12:12 h light/dark cycle at 25°C, 50% relative humidity, and an oat–sugar–yeast–agar medium for 20 generations. Before the experiment, larval density was controlled by picking 30 eggs into each of 100 vials containing 10 mL of yeasted medium. Emerging virgin adults were collected three times daily until all flies emerged.

Our experiment follows a standard paternal full-sib, half-sib breeding design (Lynch and Walsh 1998). First, we set up 140 vials with one sire and five females 3–5 days of age. We allowed flies to mate for 30 hours before splitting them into separate egg-laying vials with medium filled spoons. For each female, we split the eggs collected from the spoons into 12 vials containing 10 eggs each. We placed three replicate vials per female in each of four treatments: (1) constant 25°C; (2) constant 30°C; (3) fluctuating temperature with a mean of 25°C (18 h at 23°C + 6 h at 31°C); and (4) fluctuating temperature with a mean of 30°C (18 h at 28°C + 6 h at 36°C). We collected eggs over 3 days and randomized vials across treatments. We also randomized the location of the trays and racks in the cabinets daily. Note that temperatures were always above the temperature that is considered optimal for this species. For example, temperature that maximizes ovariole number is 22.2°C, whereas for morphological traits the temperature is even lower (David et al. 2004). For egg-to-adult viability, 19°C seems to be the optimal temperature (J. Overgaard, pers. comm.). Three days after the mass hatching event (the time where the number of hatching individuals peaked), adult individuals hatching from the vials were counted to quantify egg-to-adult viability. For more details, see Ketola et al. (2012).

DATA ANALYSIS

We estimated quantitative genetic parameters using random effects modeling with the R package MCMCglmm (version 2.15). Link to data and the R syntax of all calculations is presented in the electronic supplement. The model was defined as a multiple-trait response (viability at each treatment) and included treatment as fixed effect, and as random effects maternal, vial, and environmental variances:

$$Y = XB + Z_s S + Z_m M + Z_v V + E \quad (\text{model 1}).$$

Here Y is the matrix of viability observations assigned as a binomial trait (viable or not), where each column represents a value for viability in each of the four treatments. Note that each individual will only have a value for its corresponding treatment and have missing values for the rest. Fixed effects are represented in matrix B , with X corresponding to the incidence matrix of those effects. Z_s , Z_m , and Z_v correspond to incidence matrices of sire, dam (nested within sire), and rearing vial, and S , M , V , and E are the 4×4 variance–covariance matrices for the sire, dam,

vial, and residual effects for the four treatments. Vial and residual effects were assumed to be independent (i.e., V and E are diagonal matrices), and residual variances were fixed to one as the analysis is a binary trait analysis. Sire and maternal effects were allowed to covary among treatments, to allow for the estimation of genetic correlations. This analysis was run for 50,000 MCMC iterations and a burn-in period of 25,000, with uninformative priors. We thinned the sample to every 10th values, providing a total of 2500 posterior samples. From the above model fit, we obtained posterior samples of sire breeding values for egg-to-adult viability for all four temperature regimes. All calculations relating the four breeding values of sires were estimated using the entire posterior distribution, repeating all calculations for each of the 2500 MCMC posterior samples, as recommended by Hadfield et al. (2010).

The breeding values for egg-to-adult viability in the two constant environments (treatments 1 and 3) were transformed into a breeding value for the elevation (the mean of the two breeding values) and a breeding value for the slope (the difference between breeding values). (Figure 1) This allows transforming breeding values from two constant temperatures into a more theoretically tractable form that describes the reaction norm shape, that is, the elevation and the slope. These new breeding values for elevation and slope were used as independent explanatory variables in a linear regression analysis explaining the breeding values for egg-to-adult viability in either (X) of the two fluctuating environments:

$$BV_{\text{fluctuating } X^\circ C, i} \sim \beta_{0, X^\circ C} + \beta_{1, X^\circ C} e_i + \beta_{2, X^\circ C} S_i,$$

where BV stands for breeding value of individual i , β represents the regression coefficients and

$$e_i = \frac{BV_{\text{constant } 25^\circ C, i} + BV_{\text{constant } 30^\circ C, i}}{2},$$

$$S_i = BV_{\text{constant } 25^\circ C, i} - BV_{\text{constant } 30^\circ C, i}.$$

To test environmental robustness per se, we also used absolute reaction norms. This was done because reaction norm slope with sign can indicate also if genotype is more specialized to a hot or a cold environment. Results from this analysis can be found in the electronic supplementary material.

The calculations resulted in 2500 posterior values for each coefficient describing the effect of elevation and slope on the two breeding values for the fluctuating temperature treatments. To be able to compare regression strength, the results were expressed as standardized partial correlation coefficients (Sokal and Rohlf 1995). We also obtained posterior distributions for the adjusted R^2 as a measure of model fit, and calculated heritabilities of the elevation and the slope.

To calculate heritabilities for elevation and slope, we calculated the variance components using standard formulas for the variance of linear combinations of random variables (e.g., Keeney

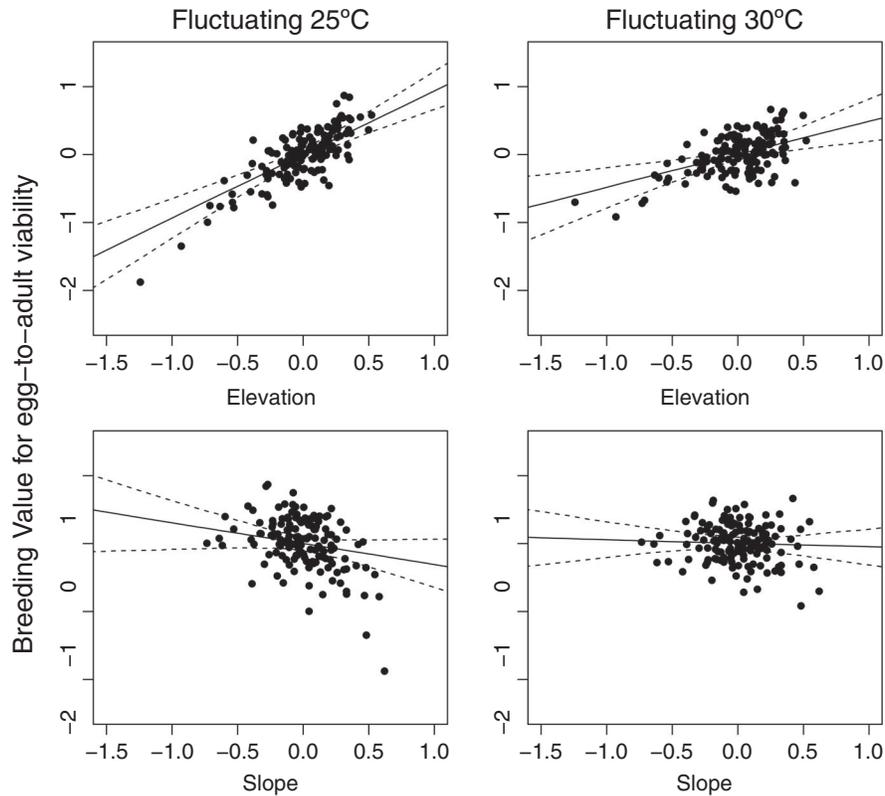


Figure 1. Scatters of breeding values of egg-to-adult viability in fluctuating 25°C (left panels) and fluctuating 30°C (right panels) against breeding values of reaction norm elevation (top panels) and reaction norm slope (lower panels). Breeding values for reaction norm elevation and slope were estimated at constant 25°C and 30°C. Breeding values for egg-to-adult viability were analyzed from separate but related individuals in each of the environments.

and Keeping 1951, see ESM). Before calculation of the heritabilities, residual variation was transformed to logit link-specific variance (~ 3.290). Heritabilities were calculated as four times the sire variances divided by the total phenotypic variances.

Results

To predict if fluctuating temperatures are associated with high reaction norm elevation or a less steep reaction norm slope requires estimates of genetic correlations between reaction norm parameters measured at constant temperatures and egg-to-adult viabilities at fluctuating temperatures. Genetic correlations between reaction norm elevation and egg-to-adult viability in both fluctuating thermal environments were clearly positive (Tables 1, 2). However, reaction norm slope was only weakly and negatively correlated with egg-to-adult viability at fluctuating 25°C, and not at all with egg-to-adult viability at fluctuating 30°C (Table 2). Because reaction norm slope can also reflect a genotype's optimum temperatures, we also calculated as absolute (unsigned) slopes for reaction norms. However, genetic correlations between egg-to-adult viability at fluctuating temperatures and absolute reaction norm slope were clearly nonsignificant (see ESM).

Genetic correlations are most often used to describe genetic relationships. However, the “genetic regressions” (analogous to phenotypic regressions) are useful as they can reveal independent effects of traits on a dependent variable. A regression analyses of breeding values showed that reaction norm elevation was clearly positively correlated with the egg-to-adult viability in both fluctuating temperatures (Table 2; ESM Table 2). However, reaction norm slope was negatively correlated to egg-to-adult viability at fluctuating 25°C, but not at fluctuating 30°C (Table 2). In contrast, absolute reaction norm slopes did not affect egg-to-adult viability in fluctuating environments (ESM Table 1). The explanatory power of regression analyses (R^2) revealed that elevation and slope explained 45–50% of the egg-to-adult viability at fluctuating 25°C, whereas at fluctuating 30°C the explanatory power of the model was ca. 20% (Table 2, ESM Table 2).

The heritability of the reaction norm elevation was moderate ($h^2 = 0.28$; 95% CI = 0.17–0.44), whereas a lower heritability was detected for the slope ($h^2 = 0.14$, 95% CI = 0.10–0.19). Heritabilities of egg-to-adult viability at fluctuating 25°C and 30°C were 0.267 (95% CI = 0.180–0.437) and 0.172 (95% CI = 0.126–0.282), respectively (published in Ketola et al. 2012).

Table 1. Genetic covariance matrix of egg-to-adult viability for the two fluctuating temperature treatments and the elevation and slope of the two constant temperatures. The diagonal shows the additive genetic variances, the lower triangle shows additive genetic covariances, and the upper triangle the genetic correlations. 95% credible intervals are shown in brackets.

	Fluctuating 25°C	Fluctuating 30°C	Elevation	Slope
Fluctuating 25°C	0.395 (0.259, 0.591)	0.431 (0.155, 0.662)	0.685 (0.492, 0.813)	−0.249 (−0.497, 0.042)
Fluctuating 30°C	0.135 (0.039, 0.259)	0.246 (0.153, 0.360)	0.451 (0.199, 0.661)	−0.054 (−0.289, 0.186)
Elevation	0.194 (0.103, 0.325)	0.102 (0.036, 0.192)	0.209 (0.125, 0.331)	−0.123 (−0.426, 0.182)
Slope	−0.080 (−0.175, 0.012)	−0.013 (−0.078, 0.049)	−0.028 (−0.102, 0.041)	0.258 (0.183, 0.361)

Table 2. Standardized partial correlation coefficients between the elevation or slope and the breeding value of egg-to-adult viability at both fluctuating temperature treatments. 95% credible intervals are shown in brackets. Significant departure from zero: * <0.05 or *** <0.001.

	Elevation coefficient	Slope coefficient	Adjusted R^2
Fluctuating 25°C	0.677 (0.491, 0.804)***	−0.222 (−0.454, 0.029)*	0.496 (0.287–0.677)
Fluctuating 30°C	0.446 (0.189, 0.661)***	−0.002 (−0.246, 0.236)	0.203 (0.034–0.449)

Discussion

We found that egg-to-adult viability in two fluctuating environments was strongly and positively genetically correlated with reaction norm elevation. Although theories frequently highlight the importance of reaction norm slope, we found that it played only a minor role in egg-to-adult viability in fluctuating environments. This is an important observation considering the strong focus on environmental robustness, reaction norm shape, and plasticity in the evolutionary literature (Levins 1968; Lynch and Gabriel 1987; Huey and Kingsolver 1993; Kassen 2002; de Witt and Scheiner 2004).

Our results are similar to those of a recent experimental evolution study on the bacteria *Serratia marcescens*, in which the shape of the thermal tolerance curve changed very little, whereas the tolerance curve elevation clearly increased as a result of evolution in fluctuating thermal environments (Ketola et al. 2013). A similar result was also found in *Escherichia coli* where mean performance rather than robustness was selected upon in fluctuating temperatures (Leroi et al. 1994). Similarly, Scheiner and Yampolsky (1998) found that the elevation but not the slope of the reaction norm evolved in *Daphnia pulex* under fluctuating temperatures. To explain their results, Scheiner and Yampolsky (1998) suggested that the reaction norm elevation contained more genetic variation than the reaction norm slope, allowing for a stronger response to selection. Our study supports this hypothesis as both heritabilities and levels of genetic variation were higher for the reaction norm elevation than for the reaction norm slope. Somewhat lower heritability of slope, compared to elevation, might be explained by slope having been under stronger selection (Fisher 1930; Mousseau and Roff 1987). Alternatively, the low heritability in the slope of thermal reaction norm can be explained by smaller amounts of genes conferring to slope than elevation (Houle 1998; Walsh and Blows 2009).

The fact that the temperatures, fluctuating above the optimum, were associated more strongly with the elevation, compared to slope, challenges theoretical predictions of thermal reaction norm evolution. Many of the theoretical models have been built with the underlying assumption of a fixed area under the tolerance curve (e.g., Lynch and Gabriel 1987; Gilchrist 1995). This implies that the only way to change the breadth of the tolerance curve is via lowered performance at optimal temperatures. Existence of such trade-offs and restrictions are supposed to prevent the evolution of genotypes that would be superior in all environments. However, the costs of evolving a higher elevation of the thermal tolerance curve can also be realized in other non-thermal environments, or in other unmeasured traits (sensu Huey and Hertz 1984). Moreover, evolution of “super genotypes” that excel under all circumstances is also effectively prevented by the inability of a single genotype being selected in all possible environments, just because of time and spatial constraints (Whitlock 1996). These possibilities make the evolution of an apparently “cost free” generalists, that is, evolution of higher elevation of reaction norm (Kassen 2002; Buckling 2006) much less paradoxical. It should be noted, however, that if extended selection for greater elevation depletes heritable genetic variation in elevation, the level of environmental robustness could play an important role in fluctuating environments, as fitness increase is no longer possible via changes in the elevation.

Although egg-to-adult viabilities at both fluctuating temperatures were strongly and positively correlated with reaction norm elevation, we found a weak negative genetic correlation between egg-to-adult viability in fluctuating 25°C and the signed reaction norm slope (Tables 1, 2). However, when the slopes were fitted without a sign (absolute slope; ESM Table 1), this effect completely disappeared. Thus, the tentative negative correlation between egg-to-adult viability at fluctuating 25°C and reaction

norm slope possibly reflects negative selection on high-temperature tolerance at fluctuating 25°C. This could be manifested because of a hot-cold trade-off in viability. Individuals with a higher fitness under hot environments do poorly at cooler temperatures (23°C, in 25°C fluctuating temperature regime). Consequently, the observed negative genetic correlation between egg-to-adult viability at fluctuating 25°C cannot be interpreted as evidence for selection for increased environmental robustness per se, but more likely as a signature of selection on tolerance to low temperatures. This result also underlines the fact that the biological impacts of fluctuating temperatures are affected also by the mean temperatures. The mean of thermal fluctuations effectively determines the area of the tolerance curve that is selected by fluctuations. This can significantly alter how thermal selection affects biological processes (Brakefield and Kesbeke 1997; Laakso et al. 2003, 2004; Bozinovic et al. 2011).

However, the fact that flies experienced a certain area of non-linear tolerance curve (e.g., two fluctuating environments with differing means) can also be a potential confounding factor affecting the interpretation of our results. Nonlinearity is especially problematic for the interpretation of the results if the optimum temperature falls between the temperatures that individuals experience when temperatures fluctuate. This is because such a scenario would maximize the effect of curvature of the reaction norm on the selection response leading to a low predictive power of linear reaction norm (see Ruel and Ayres 1999; Ragland and Kingsolver 2008). However, the optimum for egg-to-adult viability is close to 19°C in *D. melanogaster* (David et al. 2004; J. Overgaard, pers. comm.). Therefore, it is clear that the temperatures flies are exposed to in the fluctuating regimes were always at the declining part of the tolerance curve.

Although the reaction norm elevation was significantly linked to egg-to-adult viability in fluctuating thermal environments, only half of the genetic variation for egg-to-adult viability at fluctuating 25°C was explained by reaction norm parameters. The explanatory power was even lower for egg-to-adult viability at fluctuating 30°C (20%; Table 2). Regardless, if the slope was signed or absolute, the explanatory power or regression analyses remained rather similar. Thus, our results suggest that fitness estimates obtained in constant thermal environments may not be very good predictors of fitness at fluctuating temperatures. The discrepancy between estimates obtained in constant and fluctuating thermal environments could be due to coverage and limited number of fluctuating temperature regimes used for estimating the reaction norm parameters. However, biologically such low explanatory power could emerge because of distinct types of adaptations in constant and fluctuating environments (Hochachka and Somero 2002). For example long-term evolution in fluctuating temperature was not found to affect tolerance curves of the ciliate *Tetrahymena thermophila*, but fluctuating temperatures in-

creased inducible heat shock protein expression during heat shock (Ketola et al. 2004). It would be tempting to argue that higher thermal fluctuations could lead to increased selection on reaction norm slope. However, in addition to the fact that 6 hours at 36°C is already very near lethal temperatures, selection could be complicated by the existence of inducible responses, the most likely response to fluctuating environments (DeWitt and Lagerhans 2004). This can effectively diminish selection on reaction norm slope. This again highlights the need to measure fitness traits also in fluctuating thermal environments to make accurate predictions on the impact of fluctuating temperatures on the trait evolution (Sarup and Loeschcke 2010; Schulte et al. 2011).

To summarize, contrary to the common idea of a strong role of environmental robustness in fluctuating environments, our data suggest that robustness plays a minor role for genetically determined viability in fluctuating temperatures in an experiment where all of the experienced temperatures fell above optimum. Only the average viability over the constant thermal environments was clearly genetically correlated with viability in fluctuating environments. With respect to the predicted increases of thermal fluctuations related to climate change, our results suggest that overall high viability seems to be beneficial even if environments fluctuate. Therefore, it is expected that climate change induced thermal variations will select for overall more viable individuals with similarly narrow tolerance curves.

ACKNOWLEDGMENTS

The authors thank D. Andersen, L. van Koll, M. Thybring, P. Sarup, C. Vermeulen, J. Witt, L. Andersen, N. Le, K. S. Pedersen, and J. Overgaard for help in the laboratory, A. A. Hoffmann for providing the flies, the Academy of Finland for funding for TK and the Danish research council for frame grants to VL, and a Steno stipend to TNK and the Aarhus Stress Network for supporting the visit of TK to Aarhus University.

LITERATURE CITED

- Arnoldini, M., R. Mostowy, S. Bonhoeffer, and M. Ackermann. 2012. Evolution of stress response in the face of unreliable environmental signals. *PLoS Comput. Biol.* 8:e1002627.
- Berrigan, D., and S. M. Schneiner. 2004. Modelling the evolution of phenotypic plasticity. In: T. J. DeWitt and S. M. Schneiner, eds. 2004. Phenotypic plasticity. Functional and conceptual approaches. Oxford Univ. Press, Oxford, U.K.
- Bozinovic, F., D. A. Bastías, F. Boher, S. Clavijo-Baquet, S. A. Estay, and M. J. Angilletta. 2011. The mean and the variance of environmental temperature interact to determine physiological tolerance and fitness. *Phys. Biochem. Zool.* 84:543–552.
- Brakefield, P. M., and F. Kesbeke. 1997. Genotype-environment interactions for insect growth in constant and fluctuating temperature regimes. *Proc. R. Soc. Lond. B.* 264:717–723.
- Brommer, J. E., J. Merilä, B. C. Sheldon, and L. Gustafsson. 2005. Natural selection and genetic variation for reproductive reaction norms in a wild bird population. *Evolution* 59:1362–1371.

- Brommer, J. E., K. Rattiste and A. J. Wilson. 2008. Exploring plasticity in the wild: laying date-temperature reaction norms in the common gull *Larus canus*. *Proc. R. Soc. Lond. B.* 275:687–693.
- Buckling A., M. A. Brochurst, M. Travisano, P. B. Rainey. 2006. Experimental adaptation to high and low quality environments under different scales of temporal variation. *J. Evol. Biol.* 20:296–300.
- Charmantier, A., R. H. McCleery, L. R. Cole, C. Perrins, L. E. B. Kruuk and B. C. Sheldon. 2008. Adaptive phenotypic plasticity in a response to climate change in a wild bird population. *Science* 320:800–803.
- Cohen, D. 1966. Optimizing reproduction in randomly varying environment. *J. Theor. Biol.* 12:119–129.
- Cossins, A. R., and K. Bowler. 1987. *Temperature biology of animals*. Chapman and Hall, New York.
- David J. R., P. Gibert, and B. Moreteau. 2004. Evolution of reaction norms. In: T. J. DeWitt and S. M. Schneiner eds. *Phenotypic plasticity. Functional and conceptual approaches*. Oxford Univ. Press, Oxford, U.K.
- DeWitt, T. J., and R. B. Lagerhans. 2004. Integrated solutions to environmental heterogeneity. In: T. J. DeWitt and S. M. Schneiner, eds. *Phenotypic plasticity. Functional and conceptual approaches*. Oxford Univ. Press, Oxford, U.K.
- DeWitt, T. J., and S. M. Schneiner, eds. 2004. *Phenotypic plasticity. Functional and conceptual approaches*. Oxford Univ. Press, Oxford, U.K.
- Falconer, D. S., and T. F. C. Mackay. 1996. *Introduction to quantitative genetics*, 4th edition. Longman, Harlow, UK.
- Fisher, R. A. 1930. *The genetical theory of natural selection*. Oxford Univ. Press, Oxford, U.K.
- Gabriel, W. 2005. How stress selects for reversible phenotypic plasticity. *J. Evol. Biol.* 18:873–883.
- Gilchrist, G. W. 1995. Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. *Am. Nat.* 146:252–270.
- Hadfield, J. D., A. J. Wilson, D. Garant, B. C. Sheldon, L. E. B. Kruuk. 2010. The misuse of BLUP in ecology and evolution. *Am. Nat.* 175:116–125.
- Hochachka, P. W., and G. N. Somero. 2002. *Biochemical adaptation: mechanism and processes in physiological evolution*. Oxford Univ. Press, New York, U.K.
- Houle, D. 1998. How should we explain variation in the genetic variance of traits? *Genetica* 102/103:241–53.
- Huey, R. B., and J. G. Kingsolver. 1993. Evolution of resistance to high temperature in ectotherms. *Am. Nat.* 142:S21–S46.
- Huey, R. B., and P. Hertz. 1984. Is a jack-of-all-temperatures a master of none? *Evolution* 38:441–444.
- Hunt J., L. F. Bussière, M. D. Jennions, and R. Brooks. 2004. What is genetic quality? *Trends Ecol. Evol.* 19:329–333.
- IPCC. 2007 Summary for policymakers. In: *Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, eds. Cambridge Univ. Press, Cambridge, U.K.
- Kassen, R. 2002. The experimental evolution of specialists generalists and the maintenance of diversity. *J. Evol. Biol.* 15:173–190.
- Kenney, J. F., and E. S. Keeping. 1951. *Mathematics of statistics*, Pt. 2, 2nd ed. Van Nostrand, Princeton, NJ.
- Ketola, T., J. Laakso, V. Kaitala, and S. Airaksinen. 2004. Evolution of Hsp90 expression in *Tetrahymena thermophila* (Protozoa, Ciliata) populations exposed to thermally variable environment. *Evolution* 58:741–748.
- Ketola, T., V. M. Kellermann, T. N. Kristensen, and V. Loeschcke. 2012. Constant, cycling hot and cold thermal environments: strong role on mean viability but not on genetic estimates. *J. Evol. Biol.* 25:1209–1215.
- Ketola, T., L. Mikonranta, J. Zhang, K. Saarinen, V.-P. Friman, A.-M. Örmälä, J. Mappes, and J. Laakso. 2013. Fluctuating temperature leads to evolution of thermal generalism and pre-adaptation to novel environments. *Evolution* 67:2936–2944.
- King, O. D., and J. Masel. 2007. The evolution of bet-hedging adaptations to rare scenarios. *Theor. Popul. Biol.* 72:560–575.
- Laakso, J., V. Kaitala, and E. Ranta. 2003. Nonlinear biological responses to disturbance: consequences on population dynamics. *Ecol. Model.* 162:247–258.
- . 2004. Nonlinear biological responses to disturbance affect population extinction risk. *Oikos*. 104:142–148.
- Leroi, A. M., R. E. Lenski, and A. F. Bennett. 1994. Evolutionary adaptation to temperature. III. Adaptation of *E. coli* to a temporally varying environment. *Evolution* 48:1222–1229.
- Levins, R. 1968. *Evolution in changing environments*. Princeton Univ. Press, Princeton, NJ.
- Liefing, M., A. A. Hoffmann, and J. Ellers. 2009. Plasticity versus environmental canalization: population differences in thermal responses along a latitudinal gradient in *Drosophila serrata*. *Evolution* 63:1954–1963.
- Lynch, M., and W. Gabriel. 1987. Environmental tolerance. *Am. Nat.* 129:283–303.
- Lynch, M., and B. Walsh. 1998. *Genetics and analysis of quantitative traits*. Sinauer Associates, Sunderland, MA.
- Masel, J., and M. L. Siegal. 2009. Robustness: mechanisms and consequences. *Trends Genet.* 25:395–403.
- Mills, S. C., R. V. Alatalo, E. Koskela, J. Mappes, T. Mappes, and T. A. Oksanen. 2007. Signal reliability compromised by genotype-by-environment interaction and potential mechanisms for its preservation. *Evolution* 61:1748–1757.
- Morrissey, M. B., L. E. B. Kruuk, and A. J. Wilson. 2010. The danger of applying the breeder's equation in observational studies of natural populations. *J. Evol. Biol.* 23:2277–2288.
- Morrissey, M. B., D. J. Parker, P. Korsten, J. M. Pemberton, L. E. B. Kruuk and A. J. Wilson. 2012. The prediction of adaptive evolution: empirical application of the secondary theorem of selection and comparison to the breeder's equation. *Evolution* 66:2399–2410.
- Mousseau, T. A., and Roff, D. A. 1987. Natural selection and the heritability of fitness components. *Heredity*. 59:181–197.
- Nussey, D. H., E. Postma, P. Gienapp, and M. E. Visser. 2005. Selection on heritable phenotypic plasticity in a wild bird population. *Science* 310:304–306.
- Nussey, D. H., A. J. Wilson, and J. E. Brommer. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. *J. Evol. Biol.* 20:831–844.
- Postma, E. 2006. Implications of the difference between true and predicted breeding values for the study of natural selection and micro-evolution. *J. Evol. Biol.* 19:309–320.
- Price, G. 1970. Selection and covariance. *Nature* 227:520–521.
- Qvarnström, A. 2001. Context dependent genetic benefits from mate choice. *Trends Ecol. Evol.* 16:5–7.
- Ragland, G. J., and J. G. Kingsolver. 2008. The effect of fluctuating temperatures on ectotherm life-history traits: comparison among geographic populations of *Wyeomyia smithii*. *Evol. Ecol. Res.* 10:29–44.
- Ruel, J. J., and M. P. Ayres. 1999. Jensen's inequality predicts effects of environmental variation. *Trends Ecol. Evol.* 14:361–366.
- Sarup, P., and V. Loeschcke. 2010. Developmental acclimation affects clinal variation in stress resistance traits in *Drosophila buzzatii*. *J. Evol. Biol.* 23:957–965.
- Scheiner, S. M. 1993. Genetics and evolution of phenotypic plasticity. *Ann. Rev. Ecol. Syst.* 24:35–68.

- Scheiner, S. M., and R. F. Lyman. 1989. The genetics of phenotypic plasticity I. Heritability. *J. Evol. Biol.* 2:95–107.
- Scheiner, S. M., and L. Y. Yampolsky. 1998. The evolution of *Daphnia pulex* in a temporally varying environment. *Genet. Res.* 72:25–37.
- Schulte, P. M., T. Healy, and N. A. Fanguie. 2011. Thermal performance curves, phenotypic plasticity and the timescales of temperature exposure. *Int. Comp. Biol.* 51:691–702.
- Sokal, R. R., and F. J. Rohlf. 1995 *Biometry*. 3rd ed. Freeman and Co., New York.
- Sørensen, J. G., T. N. Kristensen, and V. Loeschcke. 2003. The evolutionary and ecological role of heat shock proteins. *Ecol. Lett.* 6:1025–1037.
- Walsh, B., and M. W. Blows. 2009. Abundant genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. *Ann. Rev. Ecol. Evol. Syst.* 40:41–59.
- Whitlock, M. C. 1996. The red queen beats the jack-of-all trades: limitations on the evolution of phenotypic plasticity and niche breadth. *Am. Nat.* 148:S65–S77.
- Windig, J. J., C. G. F. De Kovel, and G. De Jong 2004. Genetics and mechanics of plasticity. In T. J. DeWitt and S. M. Schneiner, eds. *Phenotypic plasticity. Functional and conceptual approaches*. Oxford Univ. Press, Oxford, U.K.

Associate Editor: J. Hunt

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Genetic covariance matrix of egg-to-adult viability for the two fluctuating temperature nine treatments and the elevation and absolute slope of the two constant temperature treatments.

Table S2. Standardized partial correlation coefficients between the elevation or absolute slope and 15 the breeding value of egg-to-adult viability at both fluctuating temperature treatments.