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The effect of fluctuating temperatures on ectotherm life-history traits: comparisons among geographic populations of *Wyeomyia smithii*

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ABSTRACT

Hypothesis: The effects of temperature variation on life-history traits depend on both mean developmental temperature and geographic population of origin.

Organism: Pitcher plant mosquitoes, *Wyeomyia smithii*.

Field sites: We established laboratory colonies from three geographic populations spanning a latitudinal and altitudinal gradient in eastern North America. Sites at lower latitudes or altitudes experience both higher mean temperatures and greater thermal variability during the growing season.

Methods: For each of the three sampled populations we analysed the effects of rearing temperature, population, and sex on survival, development time, and mass at pupation for mosquito larvae reared at 16, 20, and 27°C constant temperatures. We also measured the same variables in two fluctuating temperature treatments with means of 20 and 27°C. Using mixed linear models, we tested for the effects of mean temperature, temperature variation, population, and sex including all data except those from 16°C constant.

Results: Temperature variation did not have a significant effect on survival to pupation at 20 or 27°C constant. However, low survival at 16°C constant compared with high survival after transient exposure to the same temperature implies that duration of exposure may affect survival to pupation. The effects of temperature variation on both development time and pupal mass depended on mean temperature. Differences between constant and fluctuating temperatures for both these traits were predicted by the non-linear relationship between development rate and temperature (the Kaufmann effect). Moreover, this effect appears to explain geographic variation in the relationship between temperature variation and the measured life-history traits.

Keywords: environmental variability, Kaufmann effect, life-history evolution, reaction norm, temperature.

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INTRODUCTION

Most organisms inhabit environments that vary within and across generations. Many theoretical and empirical studies have investigated the evolution of plasticity and acclimation in response to such environmental variability (e.g. Levins, 1963; Huey and Kingsolver, 1989; Gilchrist, 1995; Bell, 1997; Bennett and Lenski, 1997; Kingsolver and Huey, 1998; Woods and Harrison, 2002). The vast majority of these studies, however, focus on the relationship between phenotype and the mean value of an environmental variable – that is, the familiar form of a norm of reaction. Less well appreciated and less frequently addressed is the relationship between environmental variability and phenotype, such as a norm of reaction for trait value across two environments with the same mean but different variance for a given environmental factor. If present, the phenotypic effects of environmental variance may be an important component of local adaptation. Additionally, interactions between the effects of environmental mean and variance may complicate comparative analyses across geography.

Temperature is one of the most widely studied and physiologically influential environmental factors, and illustrates the potential evolutionary importance of environmental variance. Temperature-dependence of physiology affects nearly every aspect of the ecology and evolution of life histories in ectotherms (e.g. Blanckenhorn, 2000; Huey and Berrigan, 2001; Fischer *et al.*, 2003; Stillwell and Fox, 2005; Colinet *et al.*, 2007). Many studies have explored differences in the relationship between life-history traits and mean temperature among geographic populations that occur along latitudinal and altitudinal clines (e.g. Blanckenhorn and Fairbairn, 1995; Fischer and Fiedler, 2002; Hallas *et al.*, 2002; Berner *et al.*, 2004; Bradshaw *et al.*, 2004; Castaneda *et al.*, 2004; Burke *et al.*, 2005). These studies provide important evidence for patterns of geographical adaptation to local thermal conditions (Endler, 1986).

Most terrestrial and many aquatic ectotherms experience a broad range of developmental temperatures that vary diurnally and seasonally. Because temperature typically has nonlinear effects on growth rate, development rate, and survival probabilities in ectotherms, temperature fluctuations within generations can have important impacts on life-history traits, such as adult body size and development time (Huey and Berrigan, 2001; Kingsolver *et al.*, 2004). A substantial literature documents how fluctuating temperatures alter development time in insects and other ectotherms (Hagstrum and Milliken, 1991; Worner, 1992).

In understanding geographic patterns of thermal adaptation, it is important to recognize that both mean temperature and temperature variation often vary along climatic gradients. For example, the amplitude of diurnal and annual temperature fluctuations varies with altitude and latitude (Taylor, 1981). Thus, geographic populations arrayed along latitudinal and altitudinal clines will experience different annual mean temperatures and different degrees of temperature variation. Consequently, comparisons among geographic populations or among microhabitats that focus solely on mean temperature may ignore important geographic differences in response to temperature variation.

How does environmental variability contribute to geographic clines in temperature-sensitive traits? Several studies have examined the effects of temperature variation within populations (e.g. Lamb, 1961; Bradshaw, 1980; Behrens *et al.*, 1983; Dallwitz, 1984; Elliott and Kieckhefer, 1989; Kieckhefer and Elliott, 1989; Hagstrum and Milliken, 1991; Petavy *et al.*, 2001, 2004; Davis *et al.*, 2006). At least one study has examined temperature variation $\times$ genotype interactions within a single population (Brakefield and Kesbeke, 1997). To our knowledge, however, no single study has directly tested for differences in response to temperature variation among geographic populations along a climatic gradient.

Effects of thermal variability on life-history differences among populations also have important practical implications. From the perspective of experimental design, if phenotypic responses to temperature variation differ among populations, the choice of fluctuating temperature rearing environments may alter among-population comparisons of mean temperature effects. Moreover, constant temperature treatments in the laboratory may not accurately reflect comparative differences under ecologically realistic conditions.

Here we test for geographic variation in the effects of fluctuating temperatures among populations of the pitcher plant mosquito, *Wyeomyia smithii*. The three study populations span a gradient in both mean temperature and temperature variation in the eastern United States. We measured survival to, time to, and mass at pupation in several temperature rearing environments. By using two diurnally fluctuating temperatures, one of our experiments simulated typical cool and warm diurnal temperature cycles measured in the field. In an additional experiment, we applied three constant temperature rearing environments, two equal to the means of the fluctuating environments. Comparison between the constant treatments and fluctuating treatments with the same mean provide a direct, commonly used test for the effects of temperature variation (Beck, 1983; Hagstrum and Milliken, 1991). We also include data from a relatively low constant rearing treatment with no comparable fluctuating treatment to illustrate the importance of duration of exposure and thermal threshold effects. Our results illustrate the effects of fluctuating temperatures on body size and development time, and document population differences in responses to fluctuating temperatures.

MATERIALS AND METHODS

Study organism

The pitcher plant mosquito, *Wyeomyia smithii*, obligately oviposits into the leaves of the purple pitcher plant, *Sarracenia purpurea*. Both plant and mosquito range from the panhandle of Florida north to Newfoundland along the eastern seaboard and into the Great Lakes region of North America, covering a broad range of thermal and seasonal habitats (Bradshaw *et al.*, 2000). Larval hibernal diapause, or dormancy, is cued by photoperiod, and geographic populations demonstrate a cline in photoperiodic response. Bracketed between diapause termination and initiation, the length of the growing season declines with increasing latitude or altitude (Bradshaw and Lounibos, 1977).

In the spring of 2004, we established separate laboratory colonies from collections of approximately 1000 larvae from each of three geographic populations (Table 1).

Table 1. Geographic and temperature data for three populations of *Wyeomyia smithii*

Population	Latitude/longitude	Altitude (m)	$\overline{T}_{ANN}$	$\hat{\sigma}_{ANN}$	$\overline{T}_{GRW}$	$\hat{\sigma}_{GRW}$
FL	30°N, 85°W	10	19.3	12.7	23.0	9.1
NC coast	34°N, 78°W	20	17.5	14.4	23.5	9.8
NC Mtn.	35°N, 83°W	900	11.2	14.0	18.0	5.9

Note: Temperature data include annual average daily mean ($\overline{T}_{ANN}$), standard deviation of the annual average ($\hat{\sigma}_{ANN}$), average daily mean of the growing season ($\overline{T}_{GRW}$), and standard deviation of the growing season average ($\hat{\sigma}_{GRW}$). Temperature units are degrees centigrade, and means and standard deviations were calculated from 35 years of weather data obtained from weather stations <2 km from each site.

Phylogeographic data suggest that FL and NC coast populations cluster together in a southern clade, while the NC Mtn. population falls into a more distantly related northern clade (Armbruster *et al.*, 1998; W. Bradshaw, unpublished data). Compared with the NC coast and FL populations, the NC Mtn. population experiences lower daily mean temperatures averaged across an entire year. All populations experience similar temperature variation on this annual scale (similar standard deviations of the means; Table 1). However, only temperatures experienced during active growth will affect life-history traits during non-diapause development (i.e. development that does not initiate from or terminate in a diapause stage). Thus, the thermal environment of the growing season is arguably the most critical component of direct, temperature-mediated selection on the life-history traits of actively growing individuals (Ragland and Kingsolver, 2007). Using the critical photoperiod of each population [the photoperiod at which 50% of a sample enters or terminates diapause; values estimated in Bradshaw and Lounibos (1977)] to define the growing season as in Ragland and Kingsolver (2007), we estimated average daily mean temperature and the standard deviation of this mean for the growing season alone. During the growing season, the NC Mtn. population experiences both the lowest mean temperature and least variable temperature conditions: the standard deviation of the mean is nearly 40% lower than the values for the NC coast and FL populations (Table 1). These data suggest that the NC coast and FL populations experience a similar thermal environment, whereas the NC Mtn. population experiences a cooler, less variable thermal environment during the growing season.

Field-collected larvae were reared in 170 ml distilled water in 150×25 mm culture dishes under standard long-day conditions (16 : 8 h light/dark) and a temperature regime fluctuating (sinusoidally) between 13 and 29°C. Food consisted of a standard suspension of 4 : 1 guinea pig chow to freeze-dried brine shrimp (Hard *et al.*, 1992). Pupae were transferred to 19-litre mating cages and once eclosion commenced each cage was supplied weekly with a freshly cut pitcher plant leaf for oviposition and a sponge moistened with honey-water for adult nutrition. Eggs were collected every 3 days, placed in culture dishes, and transferred to diapause-inducing conditions (8 : 16 h light/dark, 20°C constant temperature) until all adults in a cage had died. Once all individuals had developed to the diapausing larval instar under standard feeding conditions, we moved the larvae to standard long-day conditions to initiate the next generation. We maintained breeding populations at a minimum of 500 individuals, representing a constant proportion from each egg collection.

Constant temperature experiment

On the day of hatch, 25 haphazardly selected first instar larvae (= one cohort) from the F_2 laboratory generation of each population were transferred to 150×25 mm culture dishes with 170 ml distilled water. To maintain *ad libitum* food conditions, we transferred larvae to a new culture dish each week, supplying the new dish with a fresh aliquot of 0.05 $g \cdot ml^{-1}$ standard food suspension. Cohorts were started with 1.00-ml food suspension; 1.75, 2.5, and 3.0 ml were added each week respectively for the next 3 weeks, and 2.5 ml every week thereafter to simulate food capture in a pitcher plant (Bradshaw and Holzapfel, 1986). Constant temperature rearing treatments were at 16, 20, and 27°C. Cohorts were haphazardly assigned to temperature treatments for a total of six cohorts in each temperature treatment for each population. For larvae surviving to pupation we recorded time to pupation, mass at pupation, and sex.

Fluctuating temperature experiment

Methods and results for fluctuating temperatures appear elsewhere (Ragland and Kingsolver, 2007) and are similar to those for constant temperatures. Briefly, cohorts of 25 larvae originating from the F_3 laboratory generation of each population included in the constant temperature experiment were randomly assigned (12 cohorts per population × treatment combination) to one of two temperature rearing treatments, each with equal variance and the same diurnal profile: (1) fluctuation from 16 to 32°C with a mean of 20°C, and (2) fluctuation from 23 to 39°C with a mean of 27°C. Actual temperature–time profiles were designed to mimic a cool summer day most typical of the NC Mtn. population and a hot summer day most typical of the NC coast or FL populations (see Figure 1 in Ragland and Kingsolver, 2007). Cohorts were maintained with the same feeding conditions described for the constant temperature experiment.

Statistical analyses

In the constant temperature experiment, survival was scored as either successful pupation (1) or failure to pupate (0), and these data were analysed using mixed-model logistic regression (implemented in SAS version 9.1, Proc Glimmix, SAS Institute, 2004) including cohort as a random effect and temperature and population as fixed effects. Time to pupation and mass at pupation scored for those individuals surviving to pupation were analysed via separate mixed-model analyses of variance (ANOVA; SAS Proc Mixed) with cohort as a random effect and population, temperature, and sex as fixed effects. Time to pupation was natural log-transformed to improve normality, while pupal mass was transformed as ln(mass + 1) to prevent negative values. Sex was excluded from the survival analysis because we could not sex individuals before pupation, and was included in the other analyses because sex has a large influence on pupal mass and development time. We implemented each analysis using maximum likelihood and calculated AIC (Akaike information criterion) scores for each model (Johnson and Omland, 2004). Using a forward-selection process, we sequentially added main effects, two-way, three-way, and four-way interactions, retaining only effects that reduced the AIC. In the Results section, we present *F*-statistics only for the retained model terms. *Post-hoc* pairwise comparisons were performed using linear contrasts and associated *F* statistics. Where noted, significance of multiple comparisons were corrected using Fisher's LSD.

To examine constant versus fluctuating temperature effects, we compiled a data set that included all data from the fluctuating temperature experiment for the NC Mtn., NC coast, and FL populations and data from the 20 and 27°C temperature treatments from the constant temperature experiment. Survival, time to pupation, and mass at pupation were analysed as above with the addition of a fixed effect for temperature fluctuation (constant or fluctuating). Since constant and fluctuating temperature experiments were performed at different times and on different generations, there was a potentially confounding temporal effect. However, temporal block effects were likely minimal because the same (powdered) stock food formulation was used in the same dilutions prepared in an identical manner for both experiments, the experimental chambers held temperature at ± 0.1°C precision, and inbreeding in the laboratory stock colonies was minimized by maintaining large breeding populations. In addition, a temporal block effect would affect all populations equally, so it would not bias estimates of an interaction between the effects of temperature variation and population of origin.

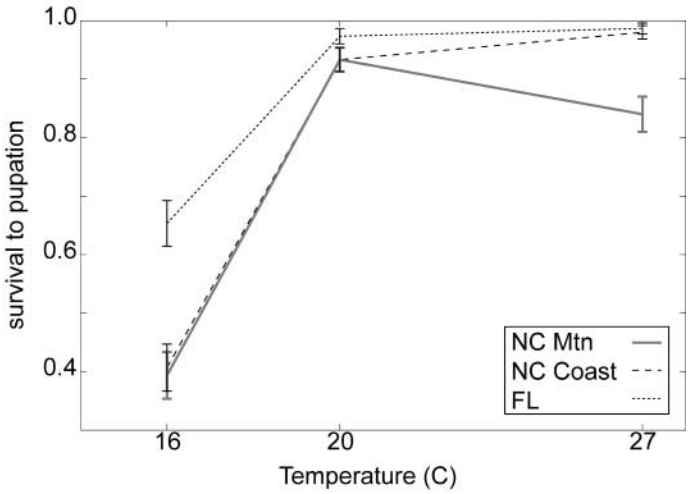

Fig. 1. Mean (± standard error) survival to pupation (proportion) across temperatures for each geographic population.

RESULTS

Constant temperatures

Survival to pupation was relatively high at the 20 and 27°C rearing temperatures and declined precipitously at 16°C for all populations (Fig. 1). Population, temperature, and their interaction had a significant influence on survival to pupation (Table 2). At 27°C, the FL and NC coast larvae had higher mean survival than the NC Mtn. larvae ($F_{1,45} = 22.78$, $P < 0.001$), whereas at 16°C the FL larvae had higher survival than those from the NC populations ($F_{1,45} = 24.93$, $P < 0.001$). There were no significant differences among populations at 20°C.

Development time and mass of pupae are conditioned on the survival responses. Particularly at 16°C, development time and pupal mass reflect the surviving subset of the total number of individuals included at the outset of the experiment. Increasing temperature led to decreasing development time, and males developed faster than females at all temperatures (Fig. 2a, b). Mixed-model ANOVA revealed significant main effects of temperature and sex (Table 2). The main effect of population was non-significant, but the population × temperature and sex × temperature interactions were significant (Table 2), indicating differences in the temperature–development time relationship between the sexes and populations. All populations developed at similar rates (no significant pairwise differences) at 16 and 20°C, but at 27°C females and males from NC Mtn. developed more slowly than the average value of the NC coast and FL populations (averaged across the sexes: $F_{1,45} = 19.24$, $P < 0.001$).

Females attained a larger mass at pupation than males, and all populations followed the temperature–size rule typical of most insects, decreasing in mass with increasing temperature in both sexes (Fig. 2c, d). There were significant effects of population, temperature, and sex (Table 2). Trends in the mass–temperature relationship were complex, with no consistent patterns among populations, sexes or temperatures; the interaction effects of

Table 2. Mixed-model ANOVA table for analysis of constant temperatures

Trait	Effect	d.f.	*F*-value	*P*-value
Survival	$Temp_{mn}$	2,45	112.29	<0.001
	Pop	2,45	12.74	<0.001
	Pop × $Temp_{mn}$	4,45	3.41	0.0161
Development time	$Temp_{mn}$	2,45	1115.80	<0.001
	Pop	2,45	0.73	0.490
	Sex	1,45	218.51	<0.001
	Pop × $Temp_{mn}$	4,45	5.23	0.002
	Sex × $Temp_{mn}$	2,45	5.87	0.005
Pupal mass	$Temp_{mn}$	2,45	351.02	<0.001
	Pop	2,45	3.47	0.0390
	Sex	1,45	1670.83	<0.001
	Pop × $Temp_{mn}$	4,45	7.70	<0.001
	Pop × Sex	2,45	19.05	<0.001
	Sex × $Temp_{mn}$	2,45	3.69	0.0320

Note: $Temp_{mn}$ is the effect of mean temperature.

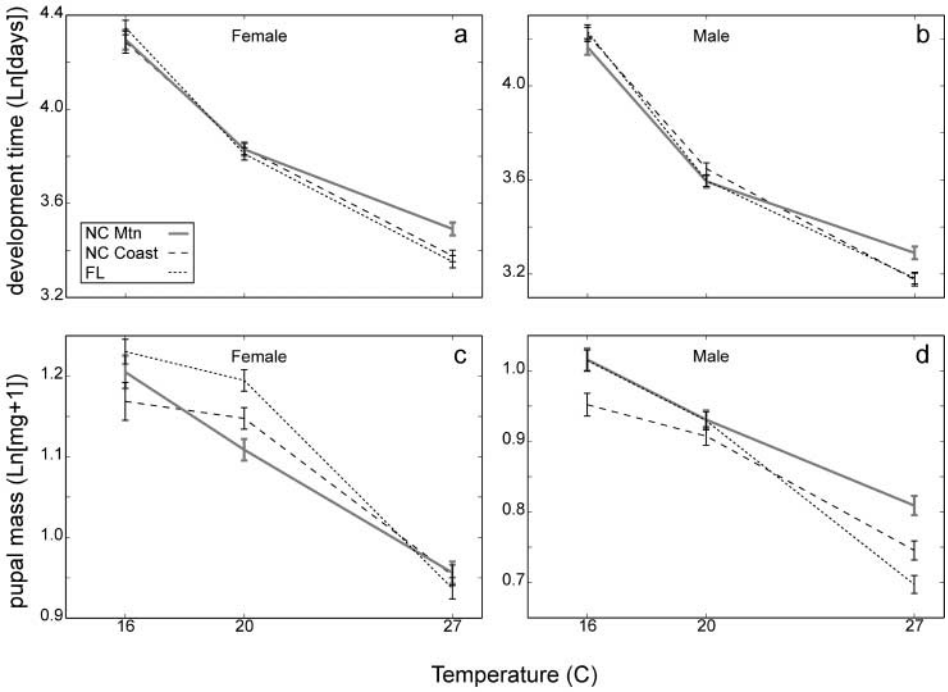

Fig. 2. Mean (± standard error) development time (a, b) and pupal mass (c, d) for females (a, c) and males (b, d) at constant temperatures of 20 and 27°C.

Table 3. Mixed-model ANOVA table for analysis of constant and fluctuating temperatures

Trait	Effect	d.f.	*F*-value	*P*-value
Survival	$Temp_{mn}$	1,99	0.58	0.447
	$Temp_{fl}$	1,99	0.74	0.393
	Pop	2,99	5.20	0.007
Development time	$Temp_{mn}$	1,96	908	<0.001
	$Temp_{fl}$	1,96	39.2	<0.001
	Pop	2,96	15.6	<0.001
	Sex	1,96	1011	<0.001
	$Temp_{mn} \times Temp_{fl}$	1,96	98.5	<0.001
	$Pop \times Temp_{mn}$	2,96	14.5	<0.001
Pupal mass	$Temp_{mn}$	1,92	814	<0.001
	$Temp_{fl}$	1,92	2.20	0.141
	Pop	2,92	3.28	0.0422
	Sex	1,92	4577	<0.001
	$Temp_{mn} \times Temp_{fl}$	1,92	28.3	<0.001
	$Temp_{mn} \times Pop$	2,92	15.5	<0.001
	$Temp_{fl} \times Sex$	1,92	4.78	0.0311
	$Pop \times Sex$	2,92	57.9	<0.001
	$Temp_{mn} \times Temp_{fl} \times Pop$	4,92	2.47	0.0502

Note: $Temp_{mn}$ and $Temp_{fl}$ are the effect of mean temperature and temperature variation, respectively.

population × sex, population × temperature, and sex × temperature were all significant (Table 2).

Constant versus fluctuating temperatures

Temperature fluctuation had no detectable effect on survival (Table 3), and no interaction terms including temperature fluctuation in the ANOVA model were significant (excluded from model via AIC). Thus, when reared at the same mean temperature, larval survival was similar in constant and fluctuating temperature conditions. Population effects were significant (Table 3), and in this model the differences between populations were the same as reported at 20 and 27°C in the analysis of constant temperatures alone (see previous section).

As in the analysis of constant temperatures, population, mean temperature, sex, and population × temperature interactions significantly affected development time (Table 3). Temperature fluctuation, the factor of primary interest, was significant as a main effect and also interacted with mean temperature (Table 3). No interaction terms including sex were significant, indicating that development time in males and females was similarly affected by temperature mean and fluctuation. Figure 3 shows the difference between development time at constant and fluctuating rearing temperatures (constant – fluctuating) as a function of average rearing temperature. Differences between fluctuating and constant temper-

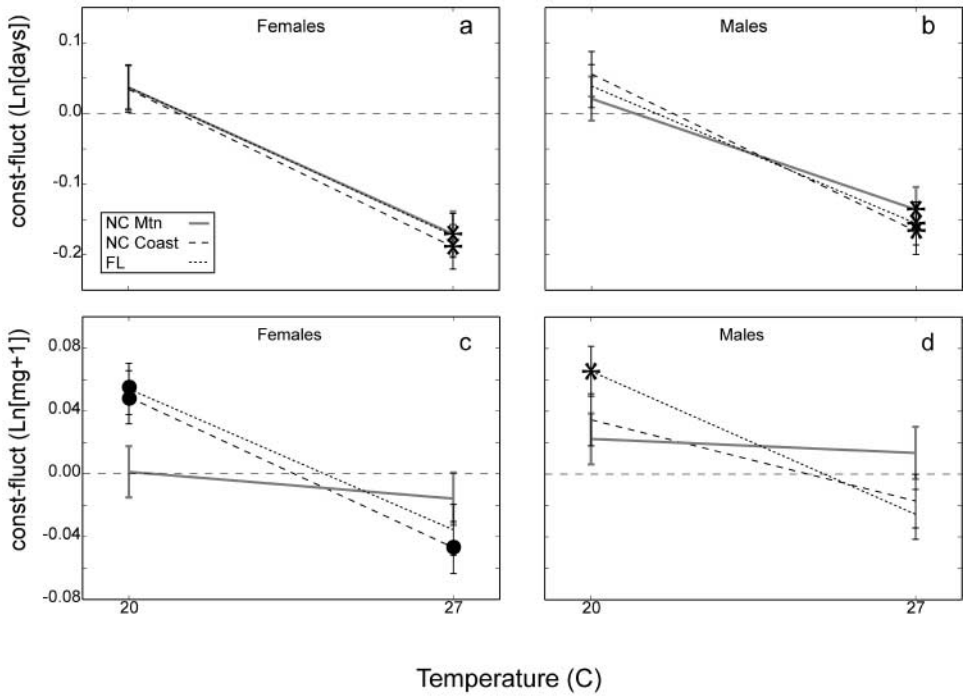

Fig. 3. Difference between constant temperatures and fluctuating temperatures with a comparable mean (constant – fluctuating) for development time (a, b) and pupal mass (c, d) of females (a, c) and males (b, d). The presence of a symbol indicates a value significantly different from zero at $P < 0.001$ (*) or $P < 0.01$ (●).

atures were non-significant (not different from zero) at $T_{ave} = 20°C$ in both sexes and for all populations. At $T_{ave} = 27°C$, however, development time was significantly shorter in the constant rearing treatment in both sexes and for all populations (Fig. 3a, b; corrections for multiple comparisons did not alter statistical significance). Interactions between temperature fluctuation and population were non-significant in the ANOVA, suggesting that fluctuating temperatures had equivalent effects on all populations.

Mean temperature and sex significantly affected pupal mass (Table 3). The main effect of population was marginally significant, while the interaction effects of population × sex and mean temperature × population were highly significant (Table 3). Temperature fluctuation was non-significant as a main effect in the ANOVA but significantly interacted with mean temperature and sex. In contrast with the results for development time, forward-selection via AIC indicated a significant three-way interaction between population, mean temperature, and temperature fluctuation (for the model including only main and two-way interaction effects in Table 3, −2log[likelihood] = −5536.4, number of parameters = 15, AIC = −5506.4; for the same model with the inclusion of the three-way interaction $Temp_{mn} \times Temp_{fl} \times Pop$, −2log[likelihood] = −5545.8, number of parameters = 19, AIC = −5507.8), though the associated *F*-statistic yields a *P*-value that falls directly on the $\alpha = 0.05$ significance threshold. Both female and male pupae from the FL and NC coast populations were generally larger at constant temperatures compared with fluctuating at a mean of 20°C, whereas this relationship was reversed at a mean temperature of 27°C

(Fig. 3c,d). After correcting for multiple comparisons, only the difference between constant and fluctuating temperatures for FL males at 20°C remained significantly different from zero, whereas all other differences that were formerly significant became marginally non-significant. The general trends for the FL and NC coast populations are qualitatively similar (FL and NC coast are also the most phylogeographically similar populations), and these trends are consistent across the sexes. In contrast, pupae from the NC Mtn. population were roughly the same size at constant and fluctuating temperatures in both sexes and at both mean temperatures. These differences among populations reflect the interaction between population, mean temperature, and temperature fluctuation.

DISCUSSION

Temperature fluctuations and survival

Compared with the results from an analysis of fluctuating temperatures, universally low survival to pupation at a constant temperature of 16°C implies a strong effect of duration of exposure. Larvae reared at temperatures fluctuating from 15.5 to 32°C and hovering at or below 16.5°C for more than 7 h at night show a 90% survival rate (fluctuating temperature regime about 20°C mean; survival data not shown), whereas larvae reared at 16°C constant show 40–60% survival (Fig. 1). Clearly, 16°C is not an acutely stressful temperature for *W. smithii*, or even chronically stressful on the scale of a diurnal temperature cycle. However, it appears that long-term chronic exposure to this temperature is stressful enough to cause high mortality. The lack of diurnal temperature fluctuation is not the sole factor responsible for this result, as survivorship at means of 20 and 27°C was comparable between constant and fluctuating temperatures (Table 2). Thus, an interaction between mean temperature and temperature variation must have contributed to the observed levels of mortality.

Since 16°C is not acutely stressful, high mortality at 16°C suggests a physiological mechanism that involves the thermal dependency of growth and development. Development rate is determined by many underlying physiological processes that often vary in thermal sensitivity and in thermal thresholds, below or above which these processes are strongly inhibited (Beck, 1983). Rearing at a constant temperature that surpasses a thermal threshold of any underlying physiological process can thus result in developmental stagnation, and eventually mortality (Lin *et al.*, 1954; Howe, 1967; Beck, 1983), or partial mortality if there is population variation for thermal thresholds that overlaps the constant rearing temperature. Development time is greatly increased at 16°C compared with 20°C (Fig. 2a, b), indicating that 16°C approaches the lower thermal threshold for development as measured at constant temperatures. This observation agrees well with a previous estimate of 15°C for the lower developmental threshold in *W. smithii* (Evans and Brust, 1972). Moreover, we detected no significant effects of temperature fluctuation on survival at mean temperatures of 20 and 27°C for any population, a trend also consistent with thermal threshold effects that manifest only at lower temperatures. Davis *et al.* (2006) observed a similar pattern in green peach aphids. Aphid survival was markedly lower in constant than in fluctuating conditions at a low mean temperature (15°C), but comparable at intermediate mean temperatures (20–30°C). Collectively, these results suggest that mean temperature × temperature fluctuation interactions for survival may be driven by thermal threshold effects on development.

Temperature fluctuations, development time, and adult size

Differences in development time between constant and fluctuating temperatures agree well with predictions based on the non-linear relationship between development rate and temperature. A wealth of empirical data from ectotherms, especially insects, suggests that development rate curvilinearly and asymmetrically declines on either side of a temperature maximum such that the decline is more rapid at high than at low temperatures (Sharpe and DeMichele, 1977) (Fig. 4). Development time is the inverse of average development rate, so rapid development rates translate into short development times. The development rate–temperature curve predicts that where the curve is concave-down, or decelerating (at higher temperatures), development time will be shorter (faster average rate) at constant temperatures than at fluctuating temperatures about the same mean (Fig. 4, square symbols). Where the curve is concave-up, or accelerating (at lower temperatures), the reverse will be true (Fig. 4, circlular symbols). This property of the non-linear development rate function, termed 'Jensen's inequality' (a general property of non-linear functions) or the 'Kaufmann effect' [specific to temperature-dependent development (Worner, 1992; Ruel and Ayres, 1999)], also predicts that the magnitude of the difference in development rate between constant and fluctuating temperatures will be greater at higher rearing temperatures because curvature at these temperatures is more extreme (Fig. 4, difference between open and closed squares compared with difference between open and closed circles). Our results closely match these

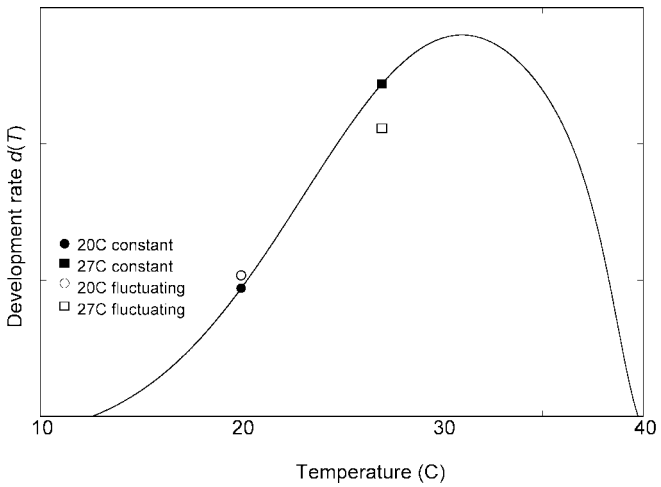

Fig. 4. Hypothetical function relating development rate (d) to environmental temperature (T). The equation:

$$d(T) = C \exp[-\alpha(T - T_{\max})^2 - \exp[\beta(T - T_{\max}) - 8]] + b$$

approximates the Sharpe-Schoolfield equation, modelling development rate as a function of temperature with an asymmetric decline from an intermediate maximum at $T = T_{\max}$ (modified from Frazier *et al.*, 2006). The y-axis scale is arbitrary, dependent upon the constant C; α and β determine the steepness and symmetry of the decline from the maximum; and b is the y-intercept. For the function depicted here, $T_{\max} = 31$, $\alpha = 0.008$, $\beta = 1$, and $b = -1.0 \times 10^{-4}$. Using this function to model development time at the mean rearing temperatures used in the current study, the symbols represent the predicted values of average daily development rate for the constant (closed symbols) and fluctuating (open symbols) thermal profiles applied at daily means of 20°C (circles) and 27°C (squares).

predictions: for both males and females in all populations, development time was shorter at constant than at fluctuating temperatures at a mean of 27°C, while the reverse was true at a mean of 20°C (Fig. 3a, b). The difference between constant and fluctuating was smaller in magnitude (and not significantly different from zero) at a mean of 20°C, again consistent with predictions based on the less extreme curvature of the development time response curve at lower temperatures (Fig. 4).

Our results for development time in *W. smithii* closely match observed patterns in other insects. In a survey of development time at constant versus fluctuating temperatures for 17 species, Hagstrum and Milliken (1991) found that development time at constant temperatures was typically shorter above means of 25–30°C and longer at lower mean temperatures. This consistency of results across species implies that the approximate temperature ranges in which the temperature–development rate function is accelerating and decelerating are evolutionarily conserved.

Because the relationship between mass at maturity and temperature is the result of the interaction between growth rate and differentiation rate (Van der Have and De Jong, 1996), the shape of the mass–temperature relationship is often more linear than that for development time (e.g. Gibert and De Jong, 2001) and may be more variable across species. Petavy *et al.* (2001) found that across a range of mean temperatures from 12 to 32°C, *Drosophila melanogaster* adult body size was always smaller in fluctuating than in constant temperature treatments. They suggested that these differences may have been driven by stress responses to extreme temperatures in the fluctuating treatments, but the observed trends are also predicted by the Kaufmann effect (concave-down reaction norm across the entire range of measured mean temperatures). The differences in pupal mass between constant and fluctuating temperatures in *W. smithii* are consistent with the Kaufmann effect as well. Pupal mass versus temperature plots for the FL and NC coast populations in Fig. 2c and 2d suggest a reaction norm that is concave-down at lower temperatures, particularly for females. As predicted by the Kaufmann effect, the general trend we observe is towards larger pupae at constant than at fluctuating temperatures (again, particularly for females) at a mean of 20°C (Fig. 3c, d). Differences between constant and fluctuating temperatures are statistically indistinguishable from zero at both mean temperatures for the NC Mtn. population. This pattern is also predicted by the Kaufmann effect, as the reaction norm relating mass to constant temperature is relatively linear (Fig. 2c, d).

Temperature fluctuations and geographic variation in life-history traits

If the consequences of temperature variation are primarily explained by the Kaufmann effect, we expect that these effects would vary most among populations when thermal reaction norms (trait value vs. mean temperature) are most divergent. Here we observe this pattern for the effects of temperature variation on development time and pupal mass in *W. smithii*. Based on the landmark temperatures measured in this study and in a separate analysis of fluctuating temperatures (Ragland and Kingsolver, 2007), the shape of the thermal reaction norm for development time appears to be much less variable among populations than for pupal mass (compare population variation in Fig. 3a, b to Fig. 3c, d). Paralleling this result, the effects of fluctuating temperatures were only statistically distinguishable among populations for pupal mass. This suggests that in addition to accounting for the effects of temperature variation within populations, the Kaufmann effect likely explains variation among populations at intermediate developmental temperatures.

Differences in thermal habitat among geographic populations of *W. smithii* appear to have driven local adaptation to mean temperature, producing differences in the effects of temperature variation primarily as a by-product. As shown by the mean and standard deviation values in Table 1, the FL and NC coast populations experience both a warmer and a more variable thermal habitat during the growing season than the NC Mtn. population integrated across the entire growing season. Southern clade populations (e.g. FL and NC coast) exhibit increased year-long replacement rate (Bradshaw *et al.*, 2004) and decreased development time at high temperatures (Ragland and Kingsolver, 2007) (Fig. 2a, b) compared with northern clade populations, whereas northern clade populations exhibit enhanced survival of cold winter temperatures (Bradshaw *et al.*, 2004) and increased fecundity (Ragland and Kingsolver, 2007) at low temperatures. However, the only detectable population differences in response to temperature variation appear to be caused by population variation in the shape of the relationship between pupal mass and mean temperature. If present, any physiological responses to temperature variation *per se* must be relatively stable across geography, at least across the range of relatively benign environments used in this study.

Geographic differences in the effects of temperature variation are also likely to arise in thermal environments in which either thermal thresholds for any underlying developmental processes or thresholds for stress response induction are regularly surpassed. Applying thermal environments with extreme mean temperature or increasing the amplitude of diurnal temperature fluctuations increases the magnitude of temperature variation effects (Hagstrum and Milliken, 1991; Petavy *et al.*, 2001). Here we infer this effect via the comparison between survival at 16°C and under fluctuating conditions that transiently reach 16°C. But, we are unable to test for population differences with our experimental design. If there is among-population variation in thermal thresholds, differences in the effects of temperature variation should become increasingly apparent the more frequently temperatures beyond these thresholds occur. As with population differences at more benign temperatures, population variation in thermal thresholds would primarily reflect adaptation to extreme temperature rather than adaptation to temperature variation *per se*.

Both Kaufmann and temperature threshold effects emphasize the importance of careful choice of thermal conditions in comparative life-history studies. Comparisons at constant temperatures may not necessarily reflect differences under more ecologically realistic conditions when the effects of population (or species, in a broader phylogenetic framework) of origin interact strongly with the effects of temperature variation. This is most likely to occur when thermal reaction norms are highly divergent among populations. Similarly, the application of relatively extreme constant temperatures may obscure population differences contingent upon developmental and stress response temperature thresholds. Applying ecologically realistic, fluctuating thermal conditions in comparative studies of evolutionary ecology avoids these potential pitfalls.

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