

Influence of seasonal timing on thermal ecology and thermal reaction norm evolution in *Wyeomyia smithii*

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Abstract

Evolutionary changes in the seasonal timing of life-history events can alter a population's exposure to seasonally variable environmental factors. We illustrate this principle in *Wyeomyia smithii* by showing that: (1) geographic divergence in diapause timing reduces differences among populations in the thermal habitat experienced by nondiapause stages; and (2) the thermal habitat of the growing season is more divergent at high compared with low temperatures with respect to daily mean temperatures. Geographic variation in thermal reaction norms for development time was greater in a warm compared with a cool rearing treatment, mirroring the geographic trend in daily mean temperature. Geographic variation in body size was unrelated to geographic temperature variation, but was also unrelated to development time or fecundity. Our results suggest that proper interpretation of geographic trends may often require detailed knowledge of life-history timing.

Introduction

In seasonal environments, selection on a particular life-history stage or event depends strongly upon timing, or phenology. At a given geographic location, season-dependent selection applies to any life-history stage or event that occurs in a predictable and defined annual time window when selective factors are seasonally fluctuating. For example, selection on floral traits mediated by pollinators depends on the timing of flowering in desert cacti (Fleming *et al.*, 2001). Similarly, periods of dormancy in *Daphnia* often coincide with periods of high predation (Slusarczyk, 2001) so that selection on anti-predator responses depends on the timing of initiation and termination of dormancy. Migratory events or stages also fit into this framework, as the timing of migration is often intimately tied to seasonal environmental fluctuation (Dingle & Drake, 2007).

Just as selection is time dependent within a particular site with seasonal fluctuations, variation in selection among geographic sites depends on the seasonal context.

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Traits such as adult body size, propagule size, growth rate and development rate exhibit latitudinal and altitudinal trends in diverse animal and plant taxa (e.g. Galen *et al.*, 1991; Blanckenhorn & Fairbairn, 1995; Tracy & Walsberg, 2001; Gilchrist *et al.*, 2004). Latitude and altitude serve as geographic proxies for environmental variation, suggesting that these clines are the result of local adaptation (Endler, 1986). In seasonal environments, however, both environmental factors and the activity pattern of organisms vary throughout the year, and the timing of a particular life-history stage can greatly affect the selective environment experienced by that life-history stage. Thus, simple geographic proxies provide little insight into the actual selective factors driving such clines without knowledge of life-history timing and patterns of annual environmental variation. In addition, evolved differences in life-history timing among geographic populations will further modify how selection on a particular life-history stage varies with geography.

In response to geographic variation in seasonality, geographic populations often vary in the seasonal timing of life-history events or stages. For example, geographic clines in the timing of hibernation diapause (dormancy) are widespread in temperate insects. Studies in a number of species show that the timing of the onset and termination of diapause diverges between geographic populations

such that the length of active, or growing season decreases with increasing altitude or latitude (Tauber *et al.*, 1985; Taylor & Spalding, 1986).

Geographic variation in diapause timing affects geographic clines in selection in two ways. First, shifts in diapause timing change the length of the growing season, causing geographic variation in developmental time constraints. The consequences of this effect have been well studied both theoretically (Roff, 1980) and empirically (Masaki, 1972; Fischer & Fiedler, 2002; Laugen *et al.*, 2003; Burke *et al.*, 2005), particularly as they relate to geographic clines in development time. Second, changes in diapause timing can alter the environment experienced by a given life-history stage. The most obvious adaptive value of diapause timing is to mitigate the exposure of actively growing individuals to harsh winter conditions (Tauber *et al.*, 1985), but there is an additional consequence. The timing of diapause will also affect the environment experienced by actively growing (i.e. nondiapause) individuals by changing the window of exposure to seasonally fluctuating environmental factors.

Temperature is an important selective factor that varies seasonally and geographically, and diapause timing will impact patterns of selection on and adaptation of thermal reaction norms. For traits that are temperature sensitive such as body size and development time in insects, selection mediated by temperature acts on the relationship between trait value and temperature, termed a thermal norm of reaction or reaction norm. Theoretical models predict that the strength of selection on trait value at a particular temperature is proportional to the frequency at which that temperature is experienced in the natural environment (Gilchrist, 1995, 2000; Kingsolver & Gomulkiewicz, 2003). Consequently, the frequency distribution of temperatures at a given site describes the relative strength of selection applied across temperatures of a thermal reaction norm (Kingsolver & Gomulkiewicz, 2003). Changes in diapause timing effectively change the frequency distribution of temperatures experienced during active growth and reproduction, altering thermal selection on nondormant life-history stages. Similarly, geographic variation in diapause timing will influence geographic variation in the temperature frequency distribution of the growing season. In this way, geographic clines in thermal selection may depend on geographic clines in diapause timing. Despite these clear ecological and evolutionary implications, the influence of diapause timing on exposure to seasonally fluctuating environments is rarely considered (but see Bradshaw *et al.*, 2004). To our knowledge, this phenomenon has not been the direct focus of any theoretical or empirical studies.

The pitcher plant mosquito, *Wyeomyia smithii*, exhibits a well-characterized cline in diapause timing across both latitude and altitude (Bradshaw & Lounibos, 1977). Here, we examine how geographic variation for diapause

timing affects geographic variation in the thermal environment of the growing season and geographic divergence in thermal reaction norms for life-history traits. The length of the growing season decreases with increasing altitude and latitude in *W. smithii* (Bradshaw & Lounibos, 1977), limiting exposure of actively growing individuals to cold winter temperatures. Thus, we hypothesized that geographic variation in the timing of hibernal diapause reduces geographic variation in the thermal environment experienced during the growing season.

For several geographic populations, we combined available diapause timing data with long-term weather records to determine the seasonal time window and temperature conditions associated with active growth and reproduction. In addition, we tested for geographic variation in thermal reaction norms for development time, body size and fecundity, life-history traits that often vary substantially across latitude and altitude in insects. Because of logistical issues we were only able to measure fecundity at a single temperature, but these data prove useful for assessing whether body size impacts fitness via fecundity. We compare geographic variation in the thermal environment with geographic variation for thermal reaction norms to explore the consequences of local adaptation of diapause timing.

Methods

Study organism and sample sites

Wyeomyia smithii obligately oviposits into the water-filled leaves of the purple pitcher plant, *Sarracenia purpurea*, and the geographic distribution of *W. smithii* tracks that of *S. purpurea* from the Gulf Coast of Florida to Newfoundland (Armbruster *et al.*, 1998). The initiation and termination of diapause in *W. smithii* is cued by photoperiod (Bradshaw & Lounibos, 1972), and geographic populations demonstrate a cline of increasing critical photoperiod (CP, the photoperiod at which 50% of a sample initiates or terminates diapause) with increasing latitude and altitude (Bradshaw & Lounibos, 1977). Diapause occurs in the larval stage and critical photoperiod for initiation and termination is symmetrical (Bradshaw & Lounibos, 1977).

We collected approximately 1000 larvae from each of four geographic populations (Table 1) during the spring and autumn of 2004, sampling a minimum of 50 pitcher plants per population. With the exception of the Massachusetts population (MAS), CP has previously been estimated for each sampled population (FL, NC Coast and NC Mtn are WI, GS and DB, respectively, in Bradshaw & Lounibos (1977). Here, we computed an estimate of CP for MAS using eqn 1 of Bradshaw & Lounibos (1977). Field observations of diapause timing agree well with this estimate (A. Ellison, personal communication). Phylogeographic data suggest that

Table 1 Geographic locations and critical photoperiods for the four *Wyeomyia smithii* study populations.

Geographic Location	Latitude/longitude (deg)	Altitude (m)	Critical photoperiod (h)
Florida gulf coast (FL)	30°N 85°W	10	12.25*
North Carolina coast (NC Coast)	34°N 78°W	20	12.75*
North Carolina mountains (NC Mtn.)	35°N 83°W	900	14.35*
Central Massachusetts (MAS)	42°N 72°W	265	14.50†

*From Bradshaw & Lounibos (1977).

†Calculated from eqn 1 in Bradshaw & Lounibos (1977); see *Methods*.

these populations cluster into two distinct clades based on morphology (Bradshaw & Lounibos, 1977), allozyme (Armbruster *et al.*, 1998) and mtDNA variation (W.E. Bradshaw, unpublished data). All geographic populations of *W. smithii* are considered a single species (Bradshaw & Lounibos, 1977). FL and NC Coast fall into a southern clade, whereas NC Mtn and MAS fall into a northern clade. Hereafter, we refer to FL and NC Coast as southern clade populations and NC Mtn and MAS as northern clade populations to reflect this phylogeographic clustering. We did not choose these populations to specifically test the effects of altitude or latitude. Rather, we chose populations that provided replication within clade (northern and southern) and exhibited substantial differences in environmental temperature (see *Results*).

Temperature data

To characterize the thermal environment of each population we obtained weather data from 1950 to 2001 including daily maximum and minimum temperatures from nearby (< 5 km) weather stations. We calculated daily mean temperatures as the mean of the daily maximum and minimum. In comparison with actual mean temperatures, this calculation is generally biased during warmer months particularly at lower latitudes, but generally by no more than 1 °C (for August 2000, temperature means calculated from daily maxima and minima were on average biased by 0.69 and -0.10 °C in Wilmington, NC, and Portland, ME respectively). Data from temperature loggers placed in pitcher plant leaves at each site indicate that when there is no snow cover, thermal conditions at the actual field sites are highly correlated with nearby weather station data (G. Ragland, unpublished data; Bradshaw *et al.*, 2000). Snow cover moderates exposure of diapausing larvae to temperatures below freezing (Bradshaw *et al.*, 2004), but this does not affect temperatures experienced during the growing season.

Excluding years for which there were any missing data from any month of the year at any site (leaving 35 years

of data), we estimated frequency distributions (binned into 1 °C intervals) of daily mean temperatures for: (1) the entire year; and (2) only the growing season predicted by CP for each population. CP-corrected frequency distributions were computed by including temperature data only for days of the year longer than the CP at each geographic location. A previous study suggests that *W. smithii* includes incoming light during twilight periods in its perception of day length (Bradshaw & Phillips, 1980), so we included civil twilight in our estimates. Excluding civil twilight did not qualitatively change the results. Choosing the last day longer than the CP as a fall cut-off for the growing season is somewhat arbitrary as *W. smithii* is photosensitive at least one instar before the diapausing instar and must develop through to diapause (Bradshaw & Lounibos, 1972); however, extending this cut-off by 2.5 weeks did not qualitatively change the results.

Colony establishment and maintenance

Larvae sampled from each population were reared to adulthood under standard laboratory conditions (L : D 16 h : 8 h, temperature oscillating as a sine curve from 13 to 29 °C) as in Hard *et al.* (1992). Adults were allowed to oviposit into freshly cut pitcher plant leaves in five gallon mating cages and the resulting eggs were reared to the diapause stage at L : D 8 h : 16 h (shorter than the shortest photoperiod necessary to induce diapause in all populations) and 20 °C. Individuals selected for the next generation represented a haphazard sample across the entire oviposition period of each mating cage. Once all populations were synchronized in diapause, we switched diapausing larvae back to standard, long-day conditions and reared them to adulthood to produce eggs for the next generation. We initiated experimental cohorts with hatching larvae from the F3 laboratory generation. Laboratory colonies were maintained with at least 500 mating individuals per population per generation.

Experimental conditions

Our experiment was designed to test for the influence of temperature, clade and population on development time, pupal mass and fecundity. Temperature treatments were applied as levels of a crossed factor using temperature-controlled growth chambers, and larvae from each population were reared in cohorts, introducing a random effect. Development time and pupal mass were measured on individuals, whereas fecundity was measured on cohorts.

If diapause timing evolves towards progressively shorter growing seasons with increasing altitude and latitude, the temperature frequency distribution of the growing season should become increasingly truncated (at low temperatures) at higher latitude/altitude. The coldest

temperatures of the growing season occur near the transitions between diapause and active growth. Therefore, such a geographic trend in diapause timing should reduce geographic variation primarily in the thermal conditions associated with these transitional periods. As the growing season generally includes the warmest temperatures of the year (i.e. the summer) in many insect species including *W. smithii*, transitions between diapause and nondiapause stages usually occur in the spring or autumn.

Accordingly, we chose two fluctuating temperature rearing conditions that reflected both warm summer-like conditions and cool, spring-like conditions. The warm treatment simulates a hot summer day in the south ($T_{ave} = 27\text{ }^{\circ}\text{C}$, $T_{min} = 23\text{ }^{\circ}\text{C}$, $T_{max} = 39\text{ }^{\circ}\text{C}$) and the cool treatment simulates a typical cool summer day in the north ($T_{ave} = 20\text{ }^{\circ}\text{C}$, $T_{min} = 16\text{ }^{\circ}\text{C}$, $T_{max} = 32\text{ }^{\circ}\text{C}$). Each treatment was designed to mimic diurnal temperature fluctuations measured by temperature loggers in the field and each had identical diurnal profiles offset by $7\text{ }^{\circ}\text{C}$. Figure 1 shows the relationship between the experimental treatments and actual diurnal temperature variation measured in pitcher plant leaves in a southern (NC coast) and northern (NC Mtn) clade population.

Eggs were collected every 3 days from colony cages and checked for hatching daily. Newly hatched larvae were haphazardly selected from a well-mixed Petri dish and placed in 170 mL of distilled water in $150 \times 25\text{ mm}^2$ culture dishes, 25 larvae per dish (one dish = one cohort). Each dish was assigned to one of two Percival 36-VL environmental chambers simulating the warm and cool temperature treatments. Each chamber was set at 16 h : 8 h L : D to simulate long-day conditions. Use of a single long-day light cycle is a standard practice

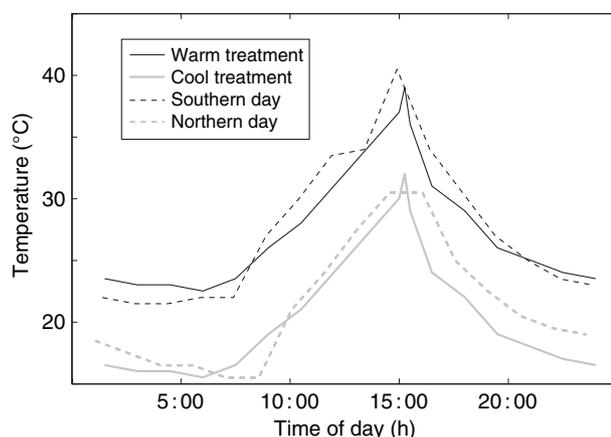


Fig. 1 Diurnal temperature cycle for experimental 'warm' (black solid line) and 'cool' (grey solid line) rearing treatments and diurnal fluctuations of a typical early summer day at the NC coast (southern clade; black dashed line) and NC Mtn (northern clade; grey dashed line) populations.

when comparing direct development of geographic populations of *W. smithii* (e.g. Bradshaw *et al.*, 2004) and other insect species (e.g. Tauber & Tauber, 1987). Day lengths more than an hour longer than the critical photoperiod have negligible effects on development rates in *W. smithii* (Bradshaw & Lounibos, 1972, 1977; W.E. Bradshaw, personal communication). In addition, a day length at least as long as the longest day of the year proportionally represents the same time point in the season for all populations because of the symmetry of critical photoperiod for diapause initiation and termination in *W. smithii*. A total of 12 dishes were assigned to each treatment per population and all dishes for a given population \times treatment combination were initiated within a 10-day window.

Once per week, larvae from each dish were transferred into a fresh dish of distilled water and fed with a 0.05-g mL^{-1} suspension of standard diet for *W. smithii* (4 : 1 guinea pig chow to freeze-dried brine shrimp). Larvae were fed weekly, and for the first 3 weeks, we progressively changed the amount of food to maintain *ad libitum* conditions without fouling the water. Food suspensions (1.0, 1.75, 2.5 and 3.0 mL) were added in weeks one to four and 2.5 mL weekly thereafter. Pilot studies suggested that adding additional food did not substantially change development time or final mass, and there were no statistically significant interactions among food treatments and rearing temperatures for either pupal mass or development time (based on mixed model ANOVA, $\alpha = 0.05$; G. Ragland, unpublished data). Additionally, data from Bradshaw & Holzapfel (1986) show that there is no interaction between larval density and population of origin for generation time and replacement rate, suggesting that effects of larval density do not vary consistently among geographic populations. Sex, time to pupation, and mass at pupation were recorded for all individuals that survived to pupation; survival in all treatment \times population combinations was high (95% on average).

Fecundity was measured in a subset of the experimental cohorts. We measured cohort fecundity by allowing pupae from four dishes (closest to each other in hatching date; $n = 100$ total) to eclose into 5.6-L mating cages, yielding three replicate mating cohorts per population. The bottom of each mating cage was covered with moistened paper towels, and periodic measurements confirmed that this maintained 80–85% relative humidity. Every 6 days, cages were provided with a fresh sponge moistened with honey water for adult nutrition and a freshly cut pitcher plant leaf for oviposition. Eggs were collected every 3 days until the last adult in a cage had died. We initiated mating cohorts in both the warm and cool treatments, but high mortality in the warm treatment mating cages because of logistical difficulties precluded measurements of fecundity. Here, we report fecundity data only for the cool rearing treatment.

Statistical analyses

Pupal mass and development time data were analysed in separate linear mixed model ANOVAs with dish effects as a random factor. In this design, dish effects serve as the error term in all *F*-tests of fixed effects (Kuehl, 1994). Both dependent variables were natural log transformed to improve normality and homoscedasticity. An AIC score was calculated from the maximum-likelihood value of models containing all possible combinations of the fixed factors clade (northern and southern), population nested within clade, sex, temperature and all two- and three-way interactions. As the experiment was designed to draw inferences about specific populations whose thermal environments were well characterized, we specified population effects as fixed rather than random. We present the best model selected by (minimizing) the AIC and *F*-statistics associated with each term in that model. To test whether the average linear relationship between temperature and each response variable differed between the northern clade (NC Mtn and MAS) and southern clade (FL and NC Coast) populations in: (1) average value across temperatures; and (2) slope, we estimated linear contrasts from the selected model. These tests assess parameters of the norm of reaction for trait value (development time or pupal mass) vs. temperature. They are particularly useful for probing complex interactions including three or more main effects. Methods for testing the significance of slope parameters of reaction norms using orthogonal polynomial contrasts are described by Huey *et al.* (1999). The tests we apply here are similar, but rather than testing whether a given slope parameter is different from zero we generated contrast coefficients to test whether a difference between slope parameters is different from zero (standard methods in Kuehl, 1994). We performed all analyses in SAS version 9.1 using Proc Mixed (SAS Institute Inc., 2004).

Cohort fecundities were calculated as the total number of eggs produced divided by the number of females for each mating cage. We performed ANOVA (SAS Proc Mixed) with clade and population nested within clade as fixed factors to test for population differences in fecundity (untransformed data were reasonably homoscedastic and normal). To estimate the effect of body size on cohort fecundity, we performed ANCOVA (SAS Proc Mixed) with clade and population nested within clade as fixed factors and average female body size as the covariate. No interaction effects including body size were significant, confirming parallelism.

Results

Thermal environment

Considering the entire year, frequencies of low temperatures were highly divergent among populations (Fig. 2a). Northern clade populations clearly experience higher frequencies of freezing temperatures than do southern clade populations. After accounting for geographic differences in diapause timing, however, frequencies of temperatures below 10 °C were much more similar among populations during the growing season. Moreover, none of the populations experience daily mean temperature below 0 °C during active growth and reproduction.

Accounting for diapause timing also suggests a pattern of high vs. low temperature variation during the growing season that is opposite to the pattern observed for the entire year. Growing season frequency distributions show a clear peak at about 20 and 27 °C for northern and southern clade populations respectively (Fig. 2b). Averaged across populations within clades, northern clade populations experience daily means of 20 °C about three times more frequently than southern clade popu-

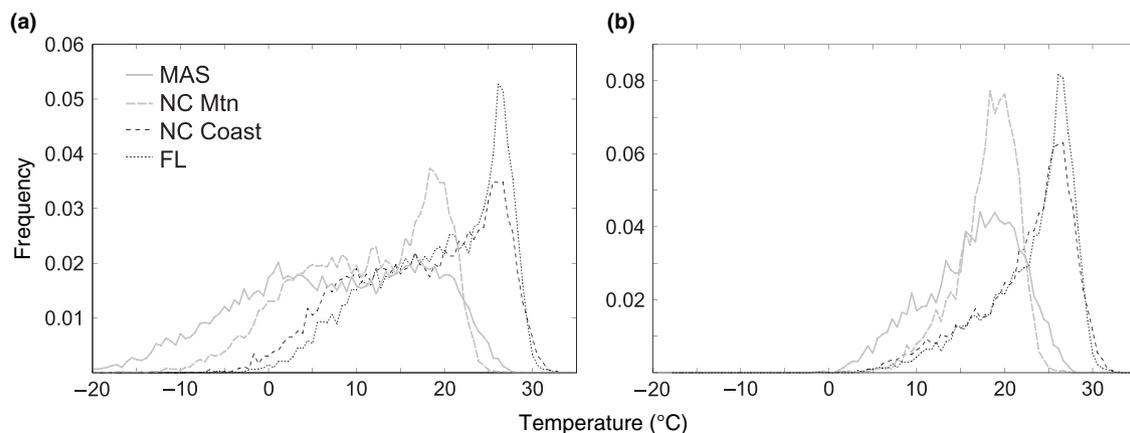


Fig. 2 Frequency distributions of daily mean temperatures for each geographic location as estimated from the 35-year weather data set for an entire year (a) and for the growing season alone (b), as defined by diapause timing.

lations. However, daily means of 27 °C occur more than an order of magnitude less frequently in northern compared with southern clade populations. These comparisons suggest that on a scale of daily mean temperature, the thermal environment of the growing season is more divergent among populations at high compared with low temperatures, whereas the reverse is true when considering the entire year (Fig. 2a). In addition, compared with distributions of mean temperatures for the growing season alone, clear separation between northern and southern clade populations in the modes (peaks) was markedly less pronounced for distributions for the entire year (Fig. 2a).

Development time and body mass

Temperature, sex, population of origin and clade all had significant effects on development time (Table 2). The clade \times temperature interaction was also significant, indicating that the slope of the thermal reaction norm for development time varied between clades. Although there was a significant pop \times sex \times temperature three-way interaction, clade \times sex \times temperature interaction effects were not significant, indicating that the temperature–development time relationship is not sex-specific across clades. Males developed faster than females at all temperatures (Fig. 3a,c), a pattern typical of many insects, including *W. smithii* (Holzapfel & Bradshaw, 2002). Northern clade populations developed more slowly than southern clade populations, averaged across temperatures and sexes ($F_{1,86} = 41.72$, $P < 0.001$; Fig. 3a,c). All populations exhibited the typical negative

relationship between development time and temperature, but southern clade populations had on average a more negative slope than northern clade populations ($F_{1,86} = 11.78$, $P < 0.001$). Despite differences in slope, however, the rank order of population mean development time did not significantly change across temperatures.

Geographic patterns for pupal mass were more complex. Pupal mass was also significantly affected by temperature, sex and population of origin, but the main effects of clade were nonsignificant (Table 2). The interaction effects of population \times temperature, population \times sex and population \times sex \times temperature were significant, indicating variable relationships between mass and temperature across sexes and across populations within clades. Although clade \times sex and clade \times sex \times temperature interactions were significant, there were no detectable differences in the slope of the temperature–mass relationship between clades within females ($F_{1,85} = 2.07$, $P = 0.15$; Fig. 3b) or males ($F_{1,85} = 2.93$, $P = 0.091$; Fig. 3d). Trends in average mass were sex specific. Averaged across temperatures, southern clade females were slightly larger than northern clade females ($F_{1,85} = 18.43$, $P < 0.001$; Fig. 3b). By contrast, southern clade males were on average slightly smaller than northern clade males ($F_{1,85} = 25.18$, $P < 0.001$; Fig. 3d), although this difference was largely determined by the relatively small size of one southern population (FL). Overall, there was no obvious relationship between latitude/altitude of origin and pupal mass in either males or females (Fig. 3b,d).

Development time and pupal mass were moderately correlated *within* populations and rearing temperatures (average $r^2 < 0.5$; data not shown), but development time was not strongly related to pupal mass in either sex *across* populations. Despite developing more rapidly (Fig. 3a), southern clade females were slightly larger than northern clade females (Fig. 3b). In addition, even though males from both southern clade populations developed at similar rates at each rearing temperature (Fig. 3c), NC Coast males were significantly larger than FL males averaged across rearing temperatures ($F_{1,85} = 9.83$, $P = 0.002$; Fig. 3d). These data suggest that mean pupal mass and mean development time have evolved independently across populations.

Fecundity

An ANOVA revealed a significant effect of clade on cohort fecundity ($F_{1,8} = 25.89$, $P < 0.001$), whereas population-within-clade effects were nonsignificant ($F_{2,8} = 0.07$, $P = 0.93$). The results of the ANCOVA suggested that pupal mass had a marginally nonsignificant effect ($F_{1,7} = 4.73$, $P = 0.066$), accounting for 28% of the variance (compared with 65% explained by clade effects) in fecundity. Further, females from northern clade populations were slightly smaller than females from

Table 2 ANOVA results from mixed model analyses of development time and pupal mass.

Trait	Effect	d.f.*	F-value	P-value
Development time	Clade	1	41.72	< 0.001
	Pop (clade)	2	6.01	0.004
	Temp	1	275.88	< 0.001
	Sex	1	817.18	< 0.001
	Clade \times temp	1	11.78	< 0.001
	Pop \times temp \times sex (clade)	9	2.83	0.006
Pupal mass	Clade	1	0.16	0.6887
	Pop (clade)	2	11.88	< 0.001
	Temp	1	603.35	< 0.001
	Sex	1	5268.35	< 0.001
	Clade \times sex	1	88.68	< 0.001
	Pop \times temp (clade)	2	5.60	0.005
	Pop \times sex (clade)	2	8.01	< 0.001
	Pop \times sex \times temp (clade)	4	5.63	< 0.001
Clade \times sex \times temp	2	5.60	0.005	

Interaction terms excluded from the best model via AIC are excluded from the table. Parentheses reflect nesting relationships (e.g. 'Pop (clade)' is population nested within clade).

*Denominator d.f. are 86 and 85 for analyses of development time and body mass respectively.

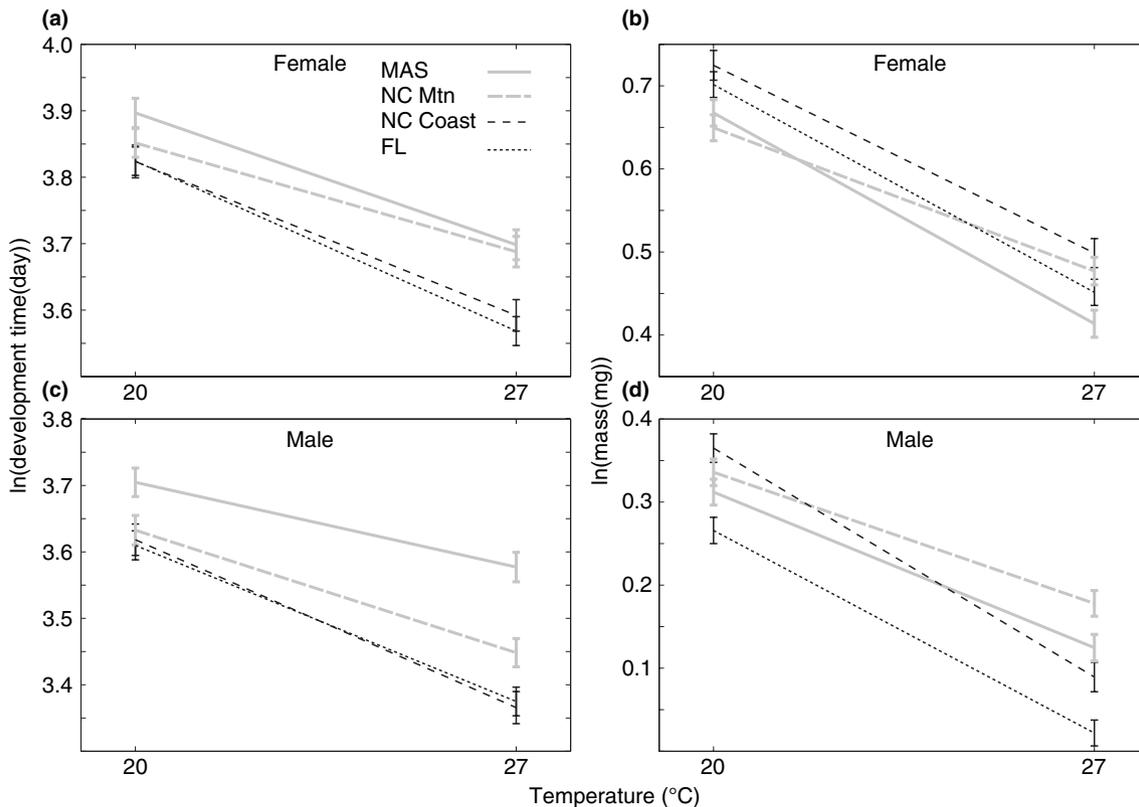


Fig. 3 Least-squared means \pm SE of experimental cohorts for log-transformed development time from egg hatch to pupation (a,c) and pupal mass (b,d) vs. average rearing temperature in females (a,b) and males (c,d). Black and grey lines represent southern (FL, short dashed; NC coast, long dashed) and northern (MAS, solid; NC Mtn, dashed) clade populations respectively.

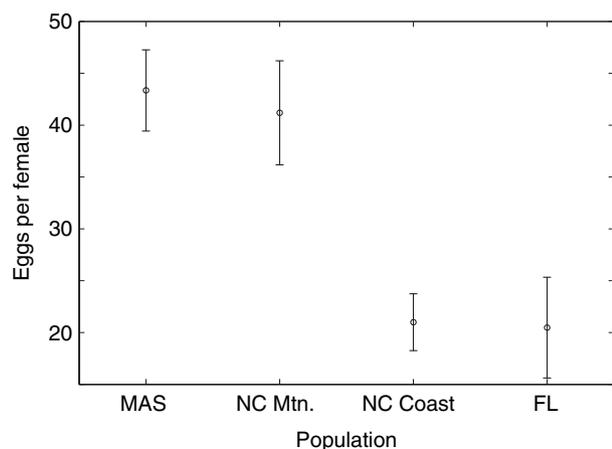


Fig. 4 Average fecundity \pm SE of experimental cohorts ($n \sim 100$ per cohort, three cohorts per population) from each population under the $T = 20^\circ\text{C}$ rearing treatment.

southern clade populations (Fig. 3b), yet northern clade populations achieved higher average cohort fecundity than southern clade populations (Fig. 4) in the cooler

rearing treatment. These results suggest that female pupal mass may moderately affect fecundity within populations but fails to explain geographic trends in fecundity across populations.

Discussion

In seasonal organisms with hibernial diapause, phenology evolves as a response to a seasonally fluctuating environment. Evolution may be driven by fluctuations in biotic resources such as food or oviposition sites, or fluctuations in abiotic factors such as temperature (Tauber *et al.*, 1985). Adult *W. smithii* exhibit a strong oviposition preference for freshly opened pitcher plant leaves (Bradshaw & Holzapfel, 1986), and *S. purpurea* only produce new leaves during the warmer months of the year. Thus, diapause phenology in *W. smithii* could be evolving in response to host availability rather than in response to temperature-mediated selection. Regardless of the specific selective factors, however, the result is that nondormant life-history stages experience more similar thermal habitats across geographic populations.

Exposure to low daily mean temperatures during active growth and reproduction in *W. smithii* is relatively

similar among populations because of evolved differences in diapause timing. As illustrated by the low ends of the temperature distributions in Fig. 2a,b, northern clade populations experience much colder temperatures than southern clade populations during the winter, but not during the growing season. Considering the entire year, winter minimum temperatures generally decline faster than summer maxima with increasing latitude, suggesting that adaptations to cold temperature should be more geographically variable than adaptations to high temperature (Addo-Bediako *et al.*, 2000). The evolution of diapause timing in *W. smithii* effectively counters this trend for nondiapause life-history stages, homogenizing the low temperature environment of the growing season. Positive linear relationships between critical photoperiod and latitude have been documented in a number of insect species (Tauber *et al.*, 1986; Taylor & Spalding, 1986), suggesting that the effects of diapause timing on the thermal environment of *W. smithii* may be common among insects with photoperiodically cued diapause.

With respect to daily mean temperature, variation among populations of *W. smithii* is actually more pronounced at the high compared with the low end of the temperature distribution for the growing season. Theory predicts that the strength of thermal selection at a particular temperature is proportional to the relative frequency at which that temperature is experienced (Gilchrist, 1995, 2000; Kingsolver & Gomulkiewicz, 2003). Thus, greater geographic variation in the relative frequency of a temperature or temperatures predicts greater geographic variation in thermal selection at that temperature or temperatures. Thermal reaction norms for life-history traits that are primarily associated with active growth and exhibit consistent geographic trends should therefore demonstrate greater geographic variation at higher temperatures in *W. smithii*.

Development time varies consistently with respect to both geography and phylogeography. Averaged across temperatures, southern clade populations developed faster than northern clade populations. Observations of pulses in *W. smithii* pupa across the growing season at each field site indicate that the Massachusetts and North Carolina mountain populations have one to two generations per year (A. Ellison, personal communication; G. Ragland, unpublished data), whereas the Florida and North Carolina coast populations have three or more generations per year (Bradshaw & Holzapfel, 1983, 1986). Thus, populations with the greatest voltinism exhibit the most rapid development, results consistent with selection to fit in additional generations in the south.

These results contrast somewhat with previous results showing that generation time from egg hatch to mean date of oviposition actually declines with increasing latitude from 30 to 42°N (Bradshaw & Holzapfel, 1983). However, Hard *et al.* (1993) found no consistent differences among geographic populations in development

time at temperatures fluctuating around a mean of 21 °C (similar to our results at 20 °C), suggesting that differences in generation time may be driven by variation in oviposition schedules. Southern populations are more iteroparous than northern populations (Bradshaw, 1986), thus extending the time period of oviposition. Faster development at higher temperatures may partially compensate for the effects iteropary on generation time, i.e. prolonging the oviposition period increases mean generation time, whereas faster development decreases generation time. Selection is strongly density dependent and generations are asynchronous in southern populations (Bradshaw & Holzapfel, 1986), probably diluting the selective advantage of a particular temperature–development time relationship. However, the fitness payoff of successfully completing an additional generation is substantial, and irrespective of density dependence there are a limited number of accumulated heat units (the physiological scale on which ectotherm development is measured) in a given growing season. Selection will thus favour those individuals that best exploit the thermal environment to maximize year-long replacement rate.

Geographic variation in development time reaction norms is more pronounced at higher temperatures, consistent with geographic variation in the thermal environment of the growing season. The rank order of population means did not change significantly across rearing temperatures, but development time reaction norms for southern clade populations had a significantly steeper negative slope on average than did those for northern clade populations (Fig. 3a,c). Differences between northern and southern clade populations were thus greater in magnitude in the warm compared with the cool rearing treatment. Moreover, data from an additional study indicate that northern and southern clade populations have statistically indistinguishable development times at a lower rearing temperature (16 °C constant; G. Ragland, unpublished data). Several studies present similar results in other insect species (Fischer & Fiedler, 2001; Norry *et al.*, 2001; Burke *et al.*, 2005), suggesting that evolutionary divergence in development time among populations may often be greater at higher rearing temperatures in temperate insects. Development time is a trait typically associated exclusively with nondormant life-history stages, so the thermal environment of the growing season rather than that of the entire year should be a better predictor of direct selection on development time. Our data are consistent with this hypothesis, as geographic variation in the thermal habitat best explains geographic variation in the development time–temperature relationship when evolved differences in diapause timing are accounted for.

Pupal mass vs. temperature reaction norms also differed among geographic populations but did not co-vary with development time. If body size and development time are positively genetically correlated, life-history models generally predict that selection will act

most strongly on development time (because encountering a catastrophic event such as a hard frost while not in diapause results in massive mortality) and that body size will evolve as a correlated character, often resulting in converse Bergmann's clines (size decreases with increasing latitude) in body size (Mousseau, 1997; Blanckenhorn & Demont, 2004). In *W. smithii*, however, a previous study shows that artificial selection on development time produces no correlated response in pupal mass (Bradshaw & Holzapfel, 1996), suggesting that these traits are not genetically correlated. Our results are consistent with those data, as body size and development time appear to evolve independently across populations.

Unconstrained by the life-history trade-off with development time, body size fails to show a consistent geographic trend, and geographic variation was roughly equivalent at cool and warm rearing temperatures. Significant differences in body size across populations in both sexes and at both rearing temperatures show that body size can evolve, but the lack of a geographic trend suggests that selection for body size does not vary consistently from south to north. Selection on female body size is often mediated through selection on fecundity (Roff, 2002; Kingsolver & Pfennig, 2004), and cohort fecundity in the cool rearing treatment varied significantly among populations. Within the single density level (25 larvae per dish) applied in our study, however, variation in body size did not strongly influence fecundity, nor does it explain fecundity differences among populations. Pupal size is negatively related to larval density (or resource availability) in *W. smithii*, and measured across a range of pupal sizes generated by a broad range of density treatments there is a positive relationship between female size and fecundity (Bradshaw & Holzapfel, 1992). However, the relationship between density and fecundity does not consistently vary with geography (Bradshaw, 1986), so the existence of these correlations does not alter our inferences about population differences based on a single density treatment. Our combined fecundity and body size measures suggest that fecundity selection is neither acting to maximize female body size in a given population nor to maintain an optimum female body size across populations. Given the lack of a consistent geographic trend, it is somewhat unsurprising that geographic variation in the thermal environment does not predict geographic variation in the thermal reaction norm for body size.

To summarize, our results illustrate how evolutionary differences in seasonal timing can impact geographic variation in selection on thermal reaction norms for life-history traits. In *W. smithii*, this is reflected in geographic variation in the slopes of thermal reaction norms for some life-history traits (development time) but not others (body size). Our analyses emphasize that proper interpretation of geographic patterns in thermal reaction norms requires an understanding of the phenological

context, a point largely neglected in the substantial literature on reaction norm evolution (e.g. Huey & Kingsolver, 1989; Angilletta *et al.*, 2002; Kingsolver & Gomulkiewicz, 2003).

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