

ADAPTATION TO TEMPERATE CLIMATES

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Abstract.—Only model organisms live in a world of endless summer. Fitness at temperate latitudes reflects the ability of organisms in nature to exploit the favorable season, to mitigate the effects of the unfavorable season, and to make the timely switch from one life style to the other. Herein, we define fitness as R_y , the year-long cohort replacement rate across all four seasons, of the mosquito, *Wyeomyia smithii*, reared in its natural microhabitat in processor-controlled environment rooms. First, we exposed cohorts of *W. smithii*, from southern, midlatitude, and northern populations (30–50°N) to southern and northern thermal years during which we factored out evolved differences in photoperiodic response. We found clear evidence of evolved differences in heat and cold tolerance among populations. Relative cold tolerance of northern populations became apparent when populations were stressed to the brink of extinction; relative heat tolerance of southern populations became apparent when the adverse effects of heat could accumulate over several generations. Second, we exposed southern, midlatitude, and northern populations to natural, midlatitude day lengths in a thermally benign midlatitude thermal year. We found that evolved differences in photoperiodic response (1) prevented the timely entry of southern populations into diapause resulting in a 74% decline in fitness, and (2) forced northern populations to endure a warm-season diapause resulting in an 88% decline in fitness. We argue that reciprocal transplants across latitudes in nature always confound the effects of the thermal and photic environment on fitness. Yet, to our knowledge, no one has previously held the thermal year constant while varying the photic year. This distinction is crucial in evaluating the potential impact of climate change. Because global warming in the Northern Hemisphere is proceeding faster at northern than at southern latitudes and because this change represents an amelioration of the thermal environment and a concomitant increase in the duration of the growing season, we conclude that there should be more rapid evolution of photoperiodic response than of thermal tolerance as a consequence of global warming among northern, temperate ectotherms.

Key words.—Global climate change, life-history evolution, reciprocal transplants, seasonal selection, stress tolerance, thermal and photoperiodic adaptation.

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We are concerned with factors that affect the adaptive radiation of species in the temperate zone. “At the global scale perhaps the most striking pattern of the distribution of terrestrial organisms is the latitudinal cline in species diversity. . . . Given the parallel cline in climate, it is perhaps inevitable that some of the most frequent correlations examined [between some measure of fitness and environment] are with some measure of temperature” (Clarke 1996). In central and eastern North America, temperature varies with latitude and season (Fig. 1). Three patterns emerge from the isotherms in Figure 1: With increasing latitude, there is a decrease in the duration and intensity of summer heat, there is an increase in the duration and intensity of winter cold, and there is a decrease in the length of the favorable or growing season (the 15°C isotherm declines from eight to zero months between the Gulf Coast and the Canadian border). Any organism dispersing over this latitudinal gradient encounters and must accommodate or adapt to all three of these patterns.

When confronted with temperatures that approach the limits of tolerance, organisms may avoid thermal exigencies through behavioral or ecological means (Haufe and Burgess 1956; Muul 1965; Porter et al. 1973; Kingsolver 1983a,b; Masaki 1986; Garland et al. 1991; Lynch 1992; Costanzo et al. 1995; Johnston and Bennett 1996; Dahlggaard et al. 2001) as well as by confronting them directly through immediate or evolved modifications in physiological response (Bratts-

from 1968; Parsons 1981; Hochachka and Somero 1984; Garland et al. 1991; van Berkum 1986, 1988; Jenkins and Hoffmann 1999; Dahlggaard et al. 2001; Gibert et al. 2001; Hoffmann et al. 2001, 2002). Herein, we define adaptive thermal responses as genetic changes in thermal optima or limits of tolerance that covary with the thermal environment and can be shown to improve fitness in those environments.

In a seasonal context, most organisms exploit the warm summer through active growth, development, and reproduction, and avoid the cold winter through migration or dormancy. The adaptive problem is in the timing of these seasonal events: developing long enough to maximize the opportunities of summer but entering dormancy soon enough to mitigate the exigencies of winter (Levins 1969; Cohen 1970; Taylor 1980). To anticipate the changing seasons, many plants and animals use daylength (photoperiod) to cue the transition from active development to migration or dormancy (Withrow 1959; Anonymous 1960; Bünning 1964; Aschoff 1965; Menaker 1971; Dingle 1996; Dunlap et al. 2004). For these organisms, photoperiodic response becomes the mechanism by which they effect the timing of seasonal events.

In the northern hemisphere, as one proceeds northwards in latitude or upwards in elevation, the favorable season becomes shorter, winter arrives earlier, and spring arrives later. Hence, at more northern latitudes, organisms should switch from active summer development to winter dormancy earlier in the year when days are longer. Among a wide variety of arthropods, the switching day length or critical photoperiod cuing seasonal activities increases regularly with increasing latitude or altitude, thereby providing an apparent adaptive

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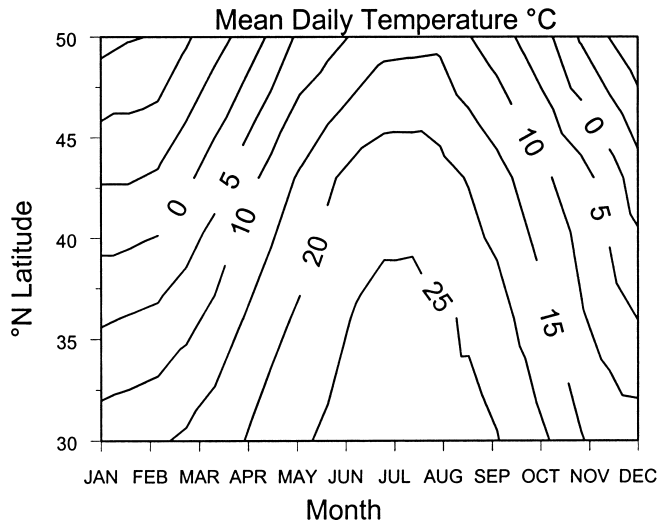


FIG. 1. Seasonal and latitudinal variation of mean daily air temperature at 25 weather stations in central and eastern North America. The data were obtained from ESSA (1968) at localities shown in Bradshaw et al. (2000, Fig. 2) and smoothed with the contour algorithm in Axum 4.0 (TriMetrix 1994).

photoperiodic response to the latitudinal gradient in season length (Andrewartha 1952; Danilevskii 1965; Bradshaw 1976; Taylor and Spalding 1986; Danks 1987; Bradshaw and Holzapfel 2001). Herein, we define adaptive photoperiodic responses as genetic changes in photoperiodic response that covary with the seasonal environment and can be shown to improve fitness in those environments.

By bringing organisms from diverse climates into the laboratory, it can be shown that they differ in thermal and photoperiodic responses; but, even if correlated with climatic gradients, (1) do these differences affect fitness under conditions experienced in nature, and (2) are the responses independent of each other? The answer to the first question might be provided by performing reciprocal transplants among localities of origin and, assuming all other ecological factors were constant, assessing some appropriate composite index of fitness in the native versus foreign habitat. The answer to the second question is more elusive. Reciprocal transplants over latitudinal gradients confound thermal and photoperiodic responses. Between the vernal and autumnal equinoxes, day lengths are longer in the north than in the south (Taylor and Spalding 1986). Hence, transplanting organisms between latitudes not only imposes longer or shorter growing seasons but also compounds the problem by providing inappropriate photic cues. Discordance between the genetically hard-wired photoperiodic response of locally adapted organisms and a novel seasonal and photic environment could therefore lead to reduced fitness, even in the absence of altered thermal extremes.

Herein, we perform "reciprocal transplants" in controlled environment rooms where we are able to separately manipulate the thermal and photic environments while holding the natural microecology constant. We test two predictions. First, if dispersal through temperate North America has involved adaptive thermal responses, then northern populations should achieve higher fitness than southern populations in a northern

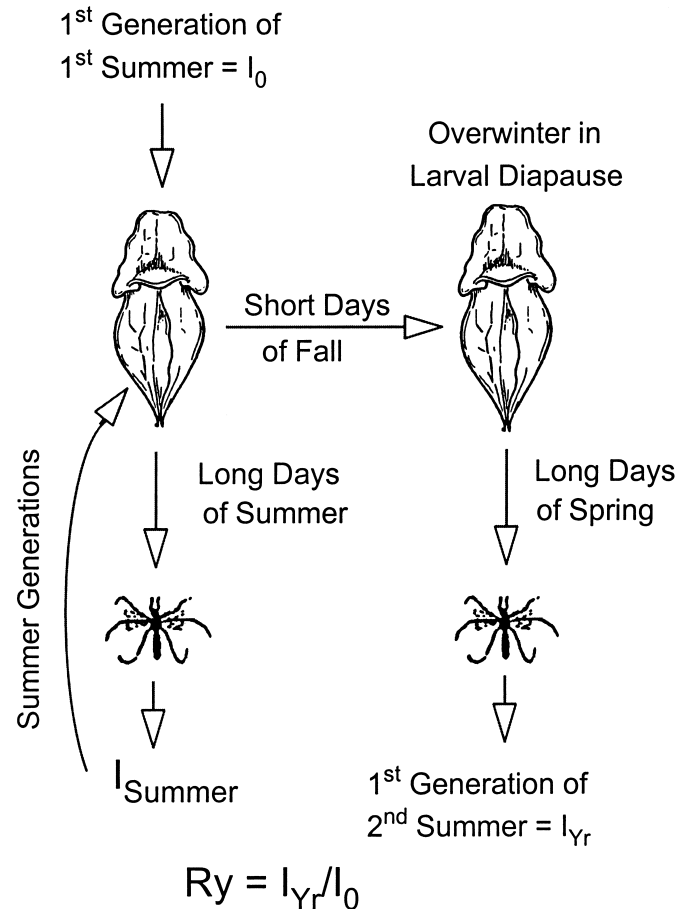


FIG. 2. Life cycle of *Wyeomyia smithii* and definition of the year-long replacement rate, R_y . Starting with the first summer generation (offspring of the overwintering generation), first-instar larvae (I_0) develop in pitcher-plant leaves and, under the influence of long summer days, emerge as adults. The adult females renew the cycle with their first-instar progeny (I_{Summer}). Under the influence of short fall days, larvae enter diapause and overwinter in the leaves. The long days of spring stimulate the resumption of development and the production of the second summer's first instars (I_{Yr}). The year-long replacement rate is then defined as the number of first instars hatching into the first generation of the second summer per first instar hatching into the first generation of the first summer, $R_y = I_{Yr}/I_0$.

thermal year when adaptive photoperiodic responses have been factored out, and vice versa in a southern thermal year. Second, if dispersal through temperate North America has involved adaptive photoperiodic responses, then southern and northern populations should realize lower fitness in a mid-latitude thermal year if they must use midlatitude day lengths to switch from development to diapause than if they are provided with photoperiodic cues that enforce the switch from development to diapause at the optimal time of the intermediate thermal year. We test these predictions with the pitcher-plant mosquito, *Wyeomyia smithii*.

The Pitcher-Plant Mosquito, Wyeomyia smithii

Wyeomyia smithii oviposits into and completes its entire preadult development within the water-filled leaves of the carnivorous pitcher plant, *Sarracenia purpurea* (Fig. 2). The very captive nature of this larval habitat exposes them to the

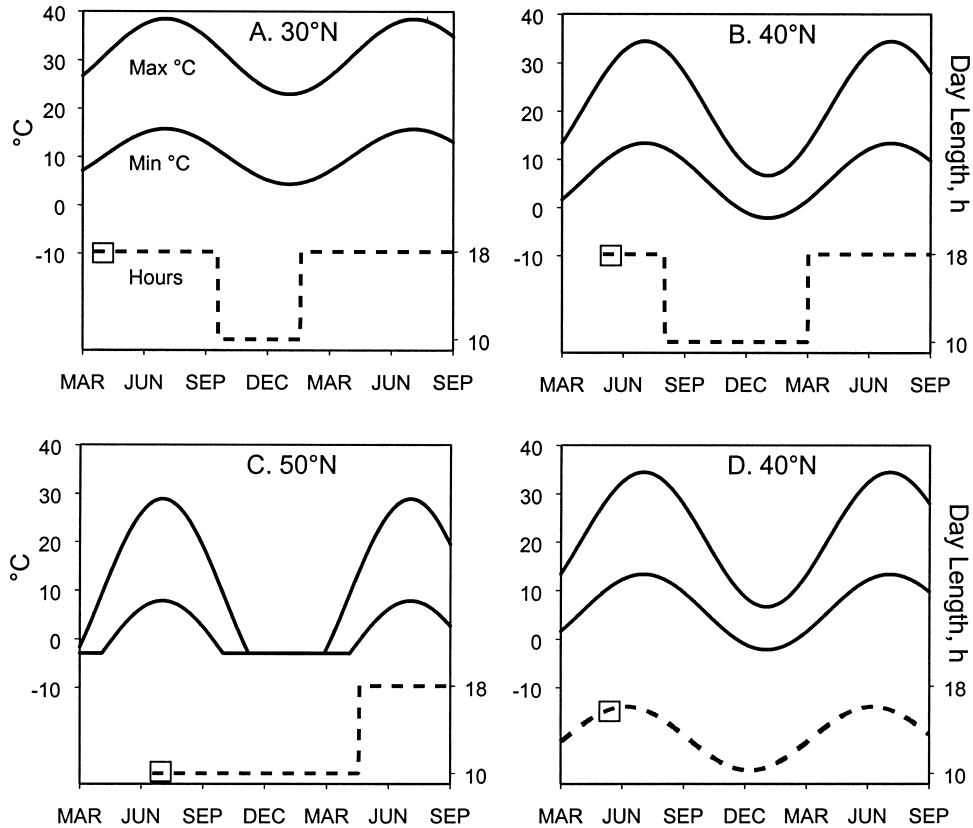


FIG. 3. Simulated southern (30°N), midlatitude (40°N), and northern (50°N) thermal years showing maximal (Max) and minimal (Min) daily temperatures over 18 months of experimental time. The line at the bottom of each plot shows day lengths as unambiguously long days (L:D = 18:6) that promote continuous development and reproduction in all populations, unambiguously short days (L:D = 10:14) that initiate and maintain diapause in all populations, or the naturally variable day lengths of 40°N. In the 50°N thermal year (C), the absolute lower limit of temperature was set at -3°C to mimic winter temperature under the snow at high latitudes. The box on each day-length plot shows the 30 days over which experimental cohorts were initiated. The ticks on the horizontal axes indicate midmonth. Details of experimental conditions are given in the Appendix.

direct vagaries of local weather. *Wyeomyia smithii* are aquatic as larvae and pitcher plants occur in humid bogs, swamps, and wet pine savannahs in which the weak-flying adults remain close to the emergent vegetation. Consequently, *W. smithii* live in a highly consistent habitat over a wide climatic gradient from the Gulf of Mexico (30°N) north to Labrador and Saskatchewan (54°N) (Darsie and Ward 1981; Bradshaw 1983). Southern populations complete five or more generations per year whereas the extreme northern populations are univoltine (Bradshaw 1983). Throughout their range, *W. smithii* overwinter within the leaves of their host as larvae in a diapause (Fig. 2) that is initiated, maintained, and terminated by photoperiod (Bradshaw and Lounibos 1977). *Wyeomyia smithii* is a typical long-day insect: ecologically relevant long days avert or terminate diapause; short days induce and maintain diapause. The critical photoperiod mediating the switch from active development to diapause is highly correlated ($R^2 > 90\%$) with latitude and altitude (Bradshaw 1976; Bradshaw and Holzapfel 2001). *Wyeomyia smithii* is therefore an ideal animal in which to study thermal and photoperiodic adaptation to climate.

Experimental Approach

The essential elements of our experimental design are two-fold. First, we are able to conduct experiments and evaluate

fitness while rearing *W. smithii* in their natural microhabitat, the leaves of intact pitcher plants. Second, with our processor-controlled environment rooms, we are able to recreate the daily and seasonal thermal and photic environments independently. We are able to factor out evolved differences in photoperiodic response. Although the median or critical photoperiod is tightly correlated with latitude of origin in *W. smithii*, all populations will enter and maintain diapause if exposed to unambiguously short days (L:D = 10:14) and will avert or terminate diapause if exposed to unambiguously long days (L:D = 18:6) (Wegis et al. 1997). For example, we can expose southern, intermediate, and northern populations of *W. smithii* to a southern and a northern thermal year (Fig. 3A,C) and, by switching day lengths from long to short, we can impose diapause in all populations simultaneously at the latitudinally appropriate time. In so doing, we remove adaptive (evolved) differences in photoperiodic response among populations. Even if thermal tolerance were modified by day length, each population would still perceive long or short days at the time of year appropriate for the latitudinally specific thermal environment. Differences in fitness between southern and northern populations in a southern or northern thermal year would then reflect differences in adaptive thermal response to either the duration or intensity of summer heat or winter cold.

TABLE 1. Origin of experimental populations.

Population origin	Ref ¹	Region	°N Latitude	°W Longitude	m Altitude
N Central Florida	WI	South	30.1	85.0	10
NW Florida	CR	South	30.7	86.6	40
S Alabama	LI	South	30.5	87.5	15
S Alabama	CI	South	31.2	88.3	150
E Shore Maryland	NP	Midlat	38.3	75.5	20
S New Jersey	HV	Midlat	39.7	74.5	10
Central New Jersey	MM	Midlat	39.8	74.6	10
Central New Jersey	PB	Midlat	39.9	74.4	10
N Maine	KC	North	46.2	68.3	365
N Wisconsin	ML	North	46.0	89.6	500
W Newfoundland	GM	North	49.6	57.9	55
NW Ontario	DL	North	49.5	94.1	405

¹ Reference to specific populations used in prior studies from this lab.

We are also able to factor out evolved differences in thermal response (Fig. 3B,D). We can expose southern, intermediate, and northern populations to a less-stressful, geographically intermediate thermal year and either enforce the appropriate timing of diapause and development with long and short days as above (Fig. 3B), or program the gradual seasonal changes in ambient day length of the intermediate latitude (Fig. 3D). If differences among populations in photoperiodic response are adaptive, then both northern and southern populations, but not intermediate populations, should realize reduced fitness in the naturally variable as opposed to the fixed day lengths.

Fitness in a seasonal environment involves the abilities to exploit the favorable season, to mitigate the effects of the unfavorable season, and to make a timely transition between the two lifestyles. Fitness in a seasonal environment cannot be evaluated from performance over a single generation in a single season. Hence, as our composite index of fitness, we use R_y , the per capita, year-long replacement rate, which equals the number of descendants hatching into the summer generation one year hence per individual hatching into the summer generation in the current year (Fig. 2). Herein, we create southern (30°N, Florida), midlatitude (40°N, New Jersey), and northern (50°N, Ontario) thermal years (Fig. 3). We determine R_y of populations of *W. smithii* originating from southern (30–31°N), intermediate (38–40°N), or northern (46–50°N) regions (Table 1) in these thermal years. To maximize the independence of replicates within climatic zones, we chose to use four replicate populations within a narrow range of latitudes (Table 1), rather than using replicate lines within populations.

We test two specific predictions. First, if there has been thermal adaptation in *W. smithii*, then when we factor out genetic differences among populations in photoperiodic response (Fig. 3A,B,C), R_y of southern relative to northern populations should decrease with increasing latitude of the thermal year. Second, if genetic differences in photoperiodic response are adaptive, then R_y in a midlatitude thermal year should be lower for southern and northern but not midlatitude populations with naturally variable, midlatitude day lengths (Fig. 3D) than with fixed day lengths (Fig. 3B) promoting development and reproduction and enforcing diapause in all populations at the appropriate, midlatitude time of year. The

critical test in each case is the significance of the interaction term in a two-way ANOVA.

MATERIALS AND METHODS

Temperatures Measured in Northern and Southern Leaves

Our experimental temperatures (Fig. 3) were based on long-term climatic atlas data and the correlation between temperatures in a single leaf and airport temperature in northwest Florida (see Appendix). To obtain a broader evaluation of leaf temperatures in northern and southern climates, we used Watchdog 100-Temp 2K (Spectrum Technologies, available at: www.specmeters.com) data loggers (16 mm diameter × 6 mm high). Eight recorders were placed in water-filled pitcher-plant leaves in a northern kettle-hole bog in northern Wisconsin (46°N, ML in Table 1): two plants each in the ecotone between the open bog mat and surrounding forest on the northern and southern edges of the bog, and two plants each on the open bog mat at the southern and northern edges of the bog. Six recorders were placed in pitcher-plant leaves in a southern pine savannah in northern Florida (30°N, near WI in Table 1): two plants each with a northern and a southern aspect, and two plants in the open portion of the savannah. The recorders were set out in the early summer or late fall to bracket the seasonal extremes of summer heat and winter cold.

Collection and Maintenance

Wyeomyia smithii were collected in the late spring when all of the genotypes in the population were available for sampling as overwintering larvae. Each population was run through two generations of ≥1000 animals to minimize any effects of the maternal field environment before the start of experiments. Stock populations were maintained in diapause on short days (L:D = 8:16) at 21°C until five weeks before the start of an experiment when they were transferred to long days (L:D = 18:6) with a fluctuating 13–29°C sine-wave thermoperiod and 80% RH to stimulate development and reared to adults. These adults provided the eggs that were transferred to short days (L:D = 8:16) at 21°C. On the day of hatch, first instars were transferred to the experimental controlled-environment rooms where they were used to set up experimental cohorts. The first instars not used to set up

cohorts remained on short days at 21°C to induce diapause in 2500 larvae/population and, eventually, to serve as parents of the next experimental larvae. When populations are cycled through diapause each generation, the photoperiodic response is stable for over 15 generations (Bradshaw et al. 2003). We completed all experiments within 10 laboratory generations.

Establishing Experimental Cohorts

A cohort consisted of 30 first-instar larvae set up in 20 ml of distilled water within 36 h of hatch in a single leaf on an intact plant of *Sarracenia purpurea*, with two plants placed in a 10 L terrarium. Terraria were placed 12 cm below two, 48 W cool-white fluorescent lamps run by remote zero-degree ballasts.

In nature, female mosquitoes selectively oviposit into freshly opened leaves and resources for their developing larvae consist of prey captured by the host leaf. Prey capture is a log-normal function of leaf age (Bradshaw 1983; Bradshaw and Holzapfel 1983, 1986). To mimic this pattern of prey capture and oviposition, cohorts of *W. smithii* were fed 38 freeze-dried adult *D. melanogaster* on the day the cohort was set up and then 150, 75, and 37 flies at weekly intervals thereafter for a total of 10 flies/larva. During periods of active development, cohorts were checked every four to five days (MWF) for pupae. Once pupation commenced, cohorts were checked until all larvae had pupated or died or until no pupae were found during three consecutive weeks. Pupae were removed to population cages (7.6L) that were provided with pesticide-free raisins for adult nutrition and a fresh-cut leaf of *Sarracenia purpurea* for oviposition. Cages were checked three times per week (MWF) for pupal exuviae to score survivorship to adult eclosion of each sex and for eggs to score fecundity. In the spring generation, eggs were transferred to distilled water and, after five days for embryonation, were checked three times per week (MWF) for hatch to score fertility until no first instars were found for one week.

Fitness during Varying Thermal Years

The known timing of seasonal development in the field at 30°N (Bradshaw and Holzapfel 1986) and 50°N (Evans 1971) formed the basis of our experimental starting dates and starting photoperiods for the southern and northern thermal years; the starting dates and photoperiods for the midlatitude thermal year represent the medians between those for the northern and southern thermal years (Fig. 3). Oviposition by adults of the overwintering generation takes place mainly during April at 30°N and July at 50°N, so we used April 1, May 15, and July 1 as starting days for the southern, midlatitude, and northern thermal years, respectively. Above 49°N, *W. smithii* is univoltine (Evans 1971), therefore we started the year at 50°N on short days to enforce univoltinism in that simulated climate. At northern latitudes, snow provides a protective thermal blanket (Evans 1971; Paterson 1971; Heard 1994), and even northern *W. smithii* do not survive prolonged temperatures below -3°C in nature (Paterson 1971). Consequently, -3°C was set as an absolute lower limit to winter temperature in the northern thermal year (Fig. 3). Populations at lower latitudes are bi- to multivoltine (Bradshaw 1983); therefore, we started the simulated year on long days for the

southern and midlatitude years. At 30°N, larvae hatching early in September develop to pupae but those that hatch early in October enter diapause (Bradshaw and Holzapfel 1986) so that we switched photoperiods from long to short to enforce diapause on October 1 in the southern thermal year. At 40°N, collections of *W. smithii* in late September do not yield any pupae and few second or fourth instars, meaning that the population has already entered diapause in the third instar by that time (W. E. Bradshaw and D. Wilson, unpubl. obs. 1993; W. E. Bradshaw and P. A. Zani, unpubl. obs. 2002). Because the optimal time to enter diapause is about one generation before the onset of winter (Taylor 1980), we set August 15 (equals October 1 minus approximately one generation) as the switching date from long to short days to enforce diapause in the midlatitude thermal year. At the end of the simulated winter, we switched photoperiods from short to long to induce the resumption of development at the specific time of year appropriate for each latitude.

For each simulated thermal year, commencing on the starting date, we set up two cohorts for each of the 12 populations (Table 1) twice per week for four weeks (Fig. 3). The one-month spread of starting dates reflects the spread in oviposition of the first summer generation at 30°N (Bradshaw and Holzapfel 1986) and at 50°N (Evans 1971). Cohorts were fed and checked as above. Pupae were pooled within populations regardless of cohort starting date or pupation date, resulting in a total of 12 population cages in which adults could eclose and oviposit. Then, using the larvae that hatched from these eggs, we set up one new cohort in a new leaf twice per week (MF). This regimen simulated the development and emergence of a population into a habitat with a finite number of patches (pitcher-plant leaves) with maximum survivorship and minimum density-dependent effects within patches (Bradshaw and Holzapfel 1989). We continued this process through the summer and fall, and into the winter until all adults in the population cage were dead, at which time the overwintering generation of each population consisted entirely of larval cohorts in a number of leaves on intact plants in their terraria. We allowed the populations to persist through winter on short days, changing to long days the following spring and summer (Figs. 2 and 3). We also started examining leaves for pupae and pooled pupae within populations into population cages regardless of cohort start date (total of 12 cages per thermal year). Fitness (R_y) achieved by each population was then the total number of live-hatching progeny produced by the overwintering generation divided by the initial 240 first instars.

Prior to analyses, R_y , total pupae produced by the overwintering generation, and eggs per enclosed female were $\log(X+1)$ transformed; embryonic viability (% of eggs hatching) was arcsine square-root transformed. We subjected R_y to two-way ANOVA (Type III sums of squares) with all three thermal years and all three latitudes of origin as fixed effects and population as a random effect. The effect of thermal year and thermal year by latitude of origin interaction were tested as $F = MS(\text{effect})/MS(\text{error})$; the effect of latitude of origin was tested as $F = MS(\text{latitude})/MS(\text{population} \times \text{latitude})$. Our principal test involved the same ANOVA, but with the three latitudes of origin crossed with the northern and southern thermal years only, to determine whether there was a

significant latitude of origin by thermal-year interaction. We then sought to determine the source of the significant interaction term by performing similar ANOVAs on factors that might be affecting Ry. Following these ANOVAs, comparisons among thermal years were unplanned and were adjusted to maintain the experimentwise protection level at $P = 0.05$ using Ryan's Q (Day and Quinn 1989).

Fitness during Varying Photic Years

This experiment proceeded exactly as those above in the midlatitude thermal year except, instead of using only unambiguous long and short days to induce diapause and resume development, we also programmed in the photic year of 40°N (Fig. 3D). For these experiments, the midlatitude thermal year with unambiguous long and short days served as the control (Fig. 3B) and we evaluated fitness and performance as a proportion of this control.

Prior to ANOVA, Ry was $\log(Ry + 1)$ transformed and then subjected to two-way ANOVA (Type III sums of squares) with the two photic environments crossed with the three latitudes of origin as fixed effects and population as a random effect. The effect of latitude of origin was tested as $F = MS(\text{latitude})/MS(\text{latitude} \times \text{population})$. The effect of the photic environment and of photic environment by latitude of origin interaction was tested as $F = MS(\text{effect})/MS(\text{error})$. Following the significant photic environment by latitude interaction, we calculated the ratio of Ry(natural photoperiod): Ry(fixed photoperiods) and tested whether this ratio in southern and northern populations was <1.0 with a one-tailed t -test. We also tested whether this ratio in midlatitude populations differed from 1.0 with a two-tailed t -test. Because of the significant departure from 1.0 in the southern and northern populations, we tested the ratios against 1.0 for five other factors that might have contributed to the low values of Ry. These latter tests were unplanned; consequently we subjected the resulting P -values to a sequential Bonferroni (Rice 1989), separately for southern and northern populations, to maintain a five-comparison protection level of 0.05.

RESULTS

Temperatures Measured in Northern and Southern Leaves

Figure 4A–B, E–F shows examples of the most extreme and most moderate temperatures from among six leaves in the Florida savannah; temperatures are intermediate in the other four leaves. Leaves in the Florida population experience a highly fluctuating thermal environment both in the summer and in the winter, with summer maxima regularly exceeding 41°C and winter minima reaching –7°C but in parts of the savannah, never falling below 0°C.

Figure 4C–D, G–H shows the most extreme and most moderate temperatures from among eight leaves in the Wisconsin bog; temperatures are intermediate in the other six leaves. Leaves in the Wisconsin population experience a highly fluctuating thermal environment in the summer with summer maxima occasionally exceeding 41°C and a stable thermal environment during the winter when, in parts of the bog, winter minima rarely fall below –3°C.

Variation in the Thermal Year

These experiments were designed to determine the effects of a northern or a southern thermal year on the fitness of mosquito populations from southern, intermediate, and northern latitudes by factoring out genetic differences in photoperiodic response. When populations from all three latitudes were exposed to all three thermal years with fixed photoperiods, the year-long cohort replacement rate (Ry) was affected by latitude of origin, thermal year experienced, and their interaction (Fig. 5). Average Ry was significantly lower in the northern and southern thermal years than in the midlatitude thermal years (Table 3). These results show that the midlatitude thermal year was relatively benign for all populations and that the southern and northern thermal years were moderately to severely stressful for all populations.

Because our main hypotheses contrast performance in northern and southern thermal years, we performed two-way ANOVA of performance in northern and southern thermal years crossed with three latitudes of origin (Fig. 6). There was a significant interaction between thermal year and latitude of origin: Ry decreased with latitude of origin in the southern thermal year and increased with latitude of origin in the northern thermal year (Fig. 6A). To determine the cause of these opposing latitudinal trends in Ry, we examined adult emergence, female fecundity, and embryonic viability during the spring. The same pattern observed for Ry was repeated in adult emergence during the simulated spring (Fig. 6B). Neither thermal year, latitude of origin, nor their interaction had a significant effect on either average female fecundity or embryonic viability (Fig. 6C,D). When we combined female fecundity and embryonic viability into net female reproductive success, there was still no significant effect of thermal year, latitude of population origin, or their interaction (Table 2f).

These results show that there are opposing latitudinal trends among populations in fitness achieved in southern and northern thermal years. The absolute number of adults emerging in the spring is the main determinant of this effect.

In the northern year, we enforced univoltinism on all populations so that adult emergence in the spring represented developmental success of all populations. In the southern year, all populations were multivoltine. Tallies of pupal and egg production during the spring, summer, and fall of the southern year are shown in Figure 7. We define as "spring" the period during which the initial cohort of 240 larvae developed and produced eggs (Fig. 7A,B). Because egg production lagged pupal production by about a week, spring occurred during weeks 1–10 for pupae and weeks 1–11 for egg production. Summer was defined as the period from spring through the switch to short days for pupal production on day 183 during week 27 and through week 28 for egg production. Fall was defined as the remainder of the 36 weeks during which eggs were produced. Pupal production (Fig. 7A) was high for all populations in the spring and early summer, declined in the northern but not southern or midlatitude populations in the late summer, and declined in all populations in the fall. Egg production (Fig. 7B) was high for all populations in the spring and declined in the midsummer when temperatures (Fig. 7C) were highest. Egg production rose in the late summer among southern and midlatitude

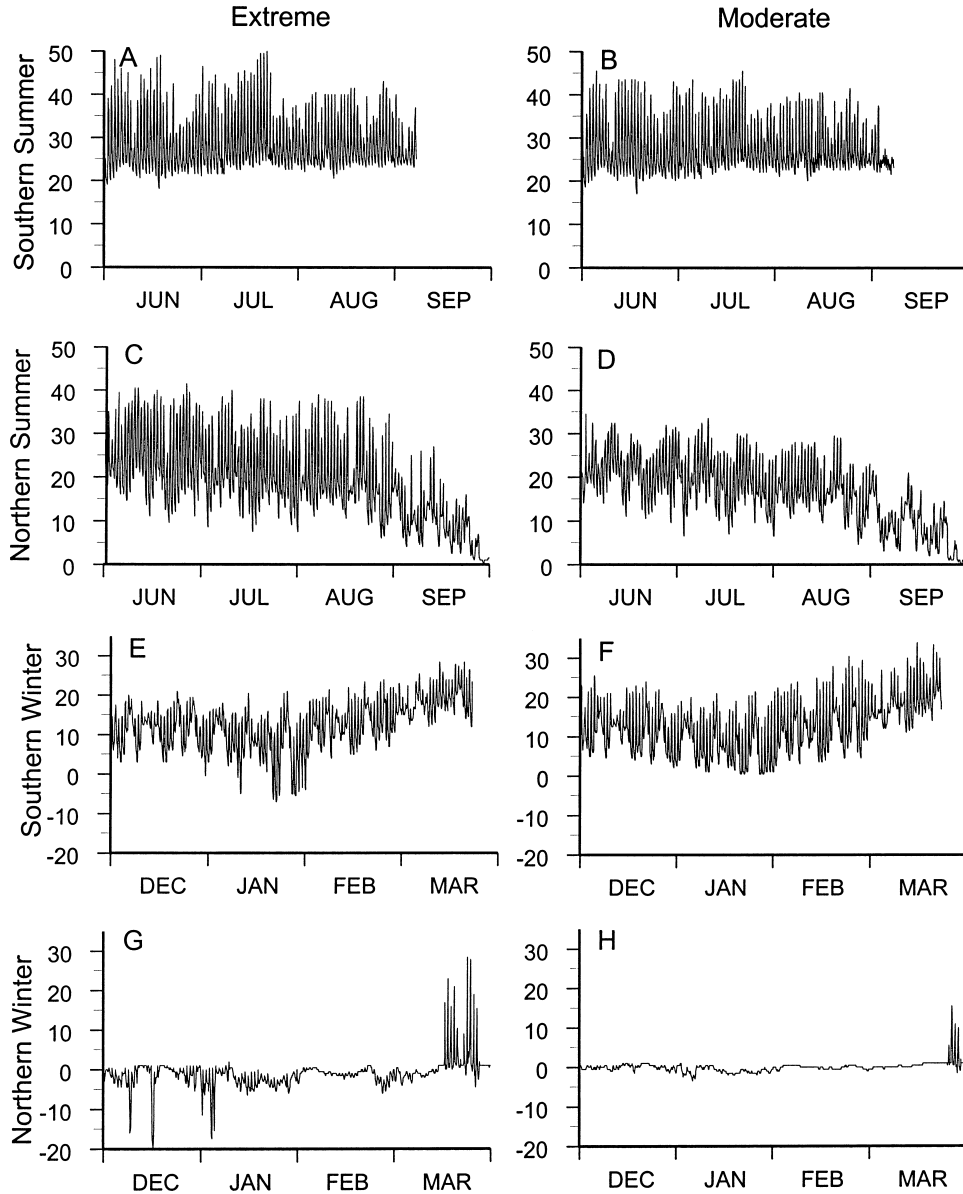


FIG. 4. Pitcher-plant leaf-water temperatures recorded every two hours during summer and winter in a southern pine savannah (Florida, 30°N) and in a northern bog (Wisconsin, 46°N). The “Extreme” plots show the leaves with the highest summer heat or lowest winter cold. The “Moderate” plots show leaves with the coolest summer maxima and the warmest winter minima. The other leaves at each locality were intermediate in their thermal ranges.

but not northern populations. Egg production of all populations declined in the fall.

These results show that in the southern thermal year, pupal and egg production of all populations declined during the hottest portion of the summer, increased again during late summer in southern and midlatitude populations, but never recovered in northern populations.

Variation in the Photic Year

These experiments were designed to determine the effects of varying the photoperiodic regimen on the fitness of mosquito populations from southern, intermediate, and northern latitudes during a uniform thermal year. Two-way ANOVA of R_y showed significant effects of latitude by photoperiod

interaction (Table 2g). The relative fitness [$R_y(\text{natural photoperiod}) \div R_y(\text{fixed photoperiods})$] (Fig. 8A) of midlatitude populations did not differ from 1.0, but the relative fitness of both southern and northern populations was less than 1.0. Southern populations experienced a 74% loss in fitness in the natural as compared with the fixed photoperiod and northern populations experienced an 88% loss of fitness.

To determine the cause of this loss in fitness of northern and southern populations, we examined larval abundance at the end of the previous growing season, survivorship over winter, survivorship to adult emergence the following spring, fecundity of emerging females, and viability of their resulting embryos (Fig. 8B–F). In no case did midlatitude populations differ from the expected 1:1 ratio of natural:fixed photope-

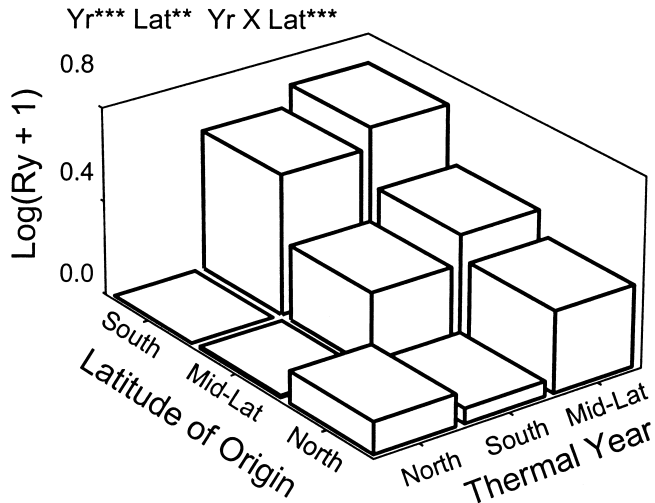


FIG. 5. Fitness (Ry) of *Wyeomyia smithii* during varying thermal years with fixed photoperiods. The results of two-way ANOVA (Table 2a) are shown at the top left of the plot. ** $P < 0.01$; *** $P < 0.001$.

riods. Because we were seeking the cause of significantly reduced fitness in the southern and northern populations separately, we applied the sequential Bonferroni (Rice 1989) to the five correlates of fitness separately for the southern and northern populations. Southern populations experienced a lower larval abundance in the fall in the natural than fixed

photoperiods; neither winter survivorship, spring survivorship, female fecundity, nor embryonic viability was significantly lower in natural than fixed photoperiods. Among northern populations none of the individual fitness correlates was significantly lower in the natural than fixed photoperiods.

Because a lower abundance of diapausing larvae at the onset of winter appeared as a potential cause of reduced fitness in natural as opposed to fixed photoperiods, we examined the patterns of development and reproduction the previous summer with natural day lengths (Fig. 9). In southern populations, rates of pupation continued into the fall (days 150–190) but oviposition ceased after day 155. In midlatitude populations, rates of pupation and oviposition declined in parallel during the late summer. In northern populations, rates of pupation and oviposition remained low throughout the summer and fall.

DISCUSSION

Variation in the Thermal Year

Fitness in the southern thermal year declined with latitude of origin and fitness in the northern thermal year increased with latitude of origin (Fig. 6A). The thermal conditions we imposed are normally experienced by southern and northern populations in nature (Fig. 4). We therefore conclude that there has been thermal adaptation in *W. smithii*. This adaptation is one of relative thermal tolerance rather than thermal specialization because both northern and southern popula-

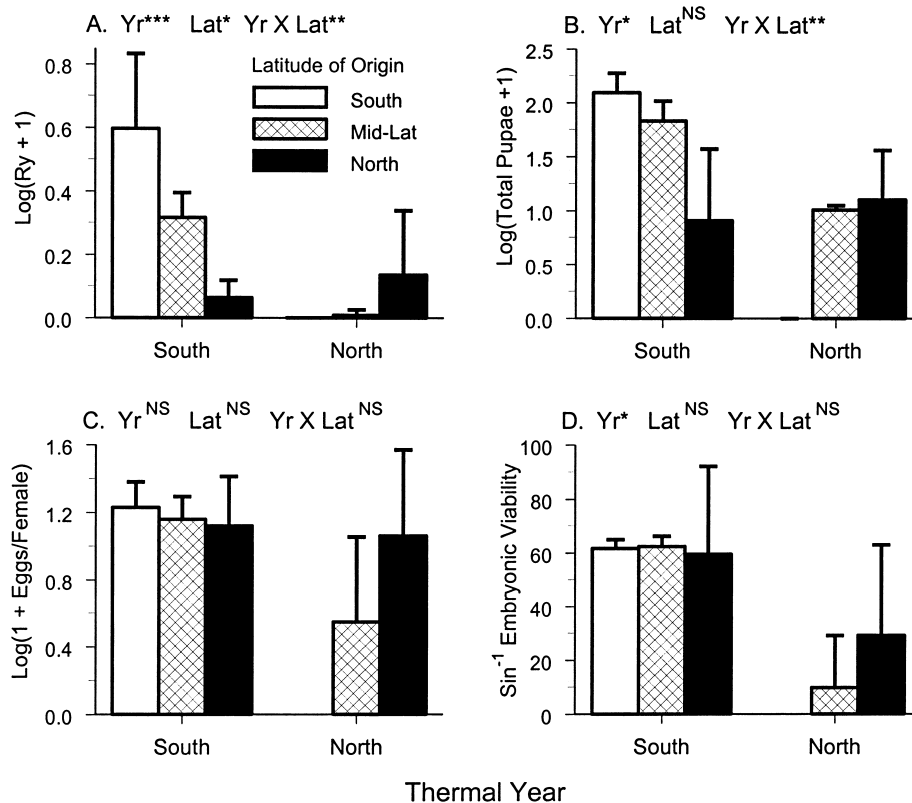


FIG. 6. Fitness (A), total number of adults emerging in the spring (B), average female fecundity (C), and embryonic viability (D) of southern, northern, and midlatitude populations in the southern and northern thermal years. Bars show means ± 2 SE. The results of two-way ANOVA (Table 2b–e) are shown at the top of each plot. ^{NS}, $P \geq 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 2. Results of ANOVA (Type III sums of squares)¹ for performance among southern, midlatitude, and northern populations experiencing (a) southern, midlatitude, and northern thermal years, (b–f) southern and northern thermal years only, or (g) midlatitude thermal year only but with natural or fixed photoperiods.

	df	MS	F	P
(a) Log(Ry + 1) (Fig. 5)				
Thermal year	2	0.5564	37.38	<0.001
Latitude of origin	2	0.1869	8.12	0.010
Env × Ori	4	0.1201	8.07	<0.001
Ori × Pop	9	0.0230		
Error	17	0.0149		
(b) Log(Ry + 1) (Fig. 6A)				
Thermal year	1	0.4633	25.25	<0.001
Latitude of origin	2	0.0826	4.77	0.039
Env × Ori	2	0.2247	12.24	0.003
Ori × Pop	9	0.0173		
Residual	9	0.0184		
(c) Log(total pupae from overwintering generation + 1) (Fig. 6B)				
Thermal year	1	2.587	7.69	0.022
Latitude of origin	2	0.337	0.45	0.653
Env × Ori	2	4.655	13.84	0.002
Ori × Pop	9	0.754		
Error	9	0.336		
(d) Log(eggs per eclosed female + 1) (Fig. 6C)²				
Thermal year	1	0.3806	2.33	0.188
Latitude of origin	2	0.0492	0.35	0.714
Env × Ori	1	0.2626	1.61	0.289
Ori × Pop	9	0.1404		
Error	5	0.1636		
(e) Sin⁻¹(√embryonic viability) (Fig. 6D)²				
Thermal year	1	3,920	8.38	0.044
Latitude of origin	2	58	0.136	0.875
Env × Ori	1	732	1.56	0.315
Ori × Pop	9	423		
Error	4	468		
(f) Log(net female reproductive success + 1)²				
Thermal year	1	0.0096	0.01	0.926
Latitude of origin	2	0.5425	1.91	0.203
Env × Ori	1	0.6295	0.63	0.572
Ori × Pop	9	0.2839		
Error	5	1.0068		
(g) Log(Ry + 1) (Fig. 8)				
Photoperiodic regimen	1	0.2133	16.94	0.003
Latitude of origin	2	0.1835	5.33	0.030
Phot × Ori	2	0.1140	9.05	0.009
Ori × Pop	9	0.0345		
Error	8	0.0126		

¹ Origin was tested as $F = MS(\text{Origin})/MS(\text{Ori} \times \text{Pop})$; other effects were tested as $F = MS(\text{effect})/MS(\text{Error})$.

² Degrees of freedom decline in these ANOVAs because fecundity, embryonic viability, and net female reproductive success are undefined in populations that produced no adults and embryonic viability is undefined in populations that produced no eggs.

tions realize greater fitness in the foreign midlatitude thermal year than they do in their native thermal years (Fig. 5).

We previously concluded that there has not been thermal adaptation in *W. smithii* (Bradshaw et al. 2000). We based this previous conclusion on relative fitness achieved by eight populations collected from over the same range as in the present study and exposed for only one generation either to a southern summer or to a midlatitude winter. These conditions were sufficiently stressful to impose a 68% and 60%

TABLE 3. A posteriori comparison of Ry among southern, midlatitude, and northern thermal years.

Thermal Year	Mean Ry ± SE	Difference from Midlat Ry	Crit Val ¹
South	0.326 ± 0.076	0.179	0.107
Midlat	0.505 ± 0.022		
North	0.048 ± 0.036	0.457	0.130

¹ Critical values from Ryan's Q.

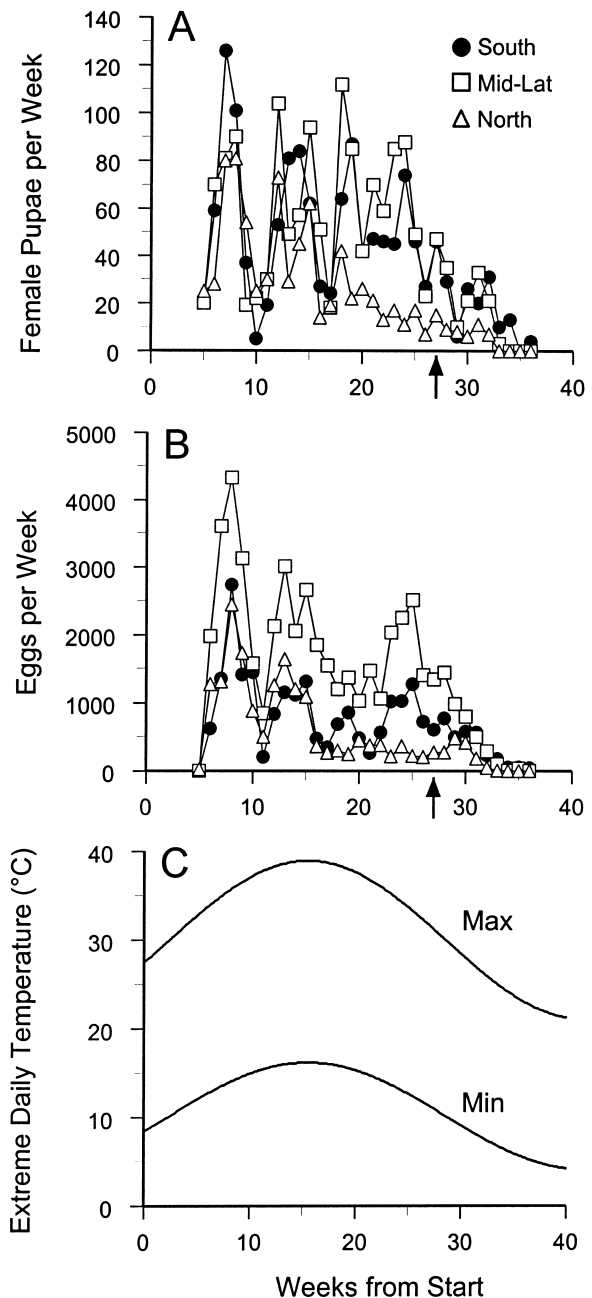


FIG. 7. Rates of pupal (A) and egg (B) production during the southern thermal year (C) showing daily maxima and minima (Max, Min). Arrows show the switch to short days during week 27.

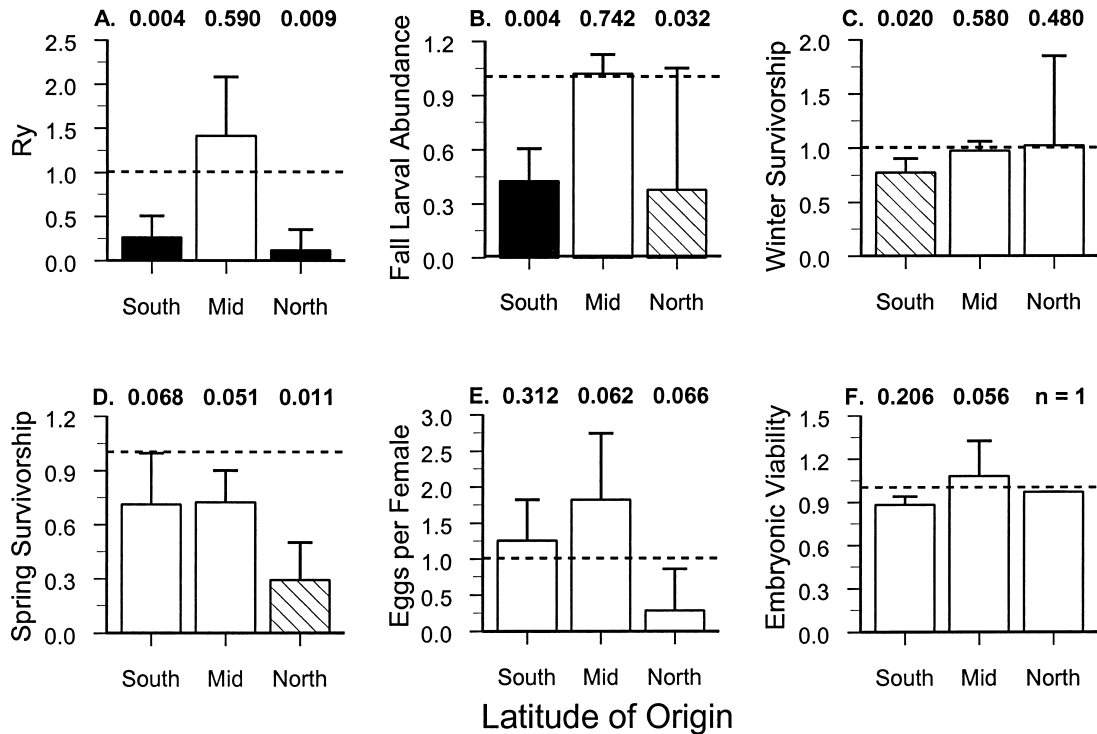


FIG. 8. Relative fitness (mean + 2 SE) and its correlates expressed as the ratio of performance in the natural:fixed photoperiods during a benign midlatitude thermal year. A ratio of 1.0 (dashed line) indicates no effect of photoperiodic regimen; a ratio less than 1.0 indicates a loss of fitness in the natural relative to the fixed photoperiod years. The numbers at the top of each plot show the P -values for t -tests testing whether the relative performance of southern and northern populations is < 1.0 (one-tailed) or whether the relative performance of the midlatitude populations is different from 1.0 (two-tailed). Open bars indicate nonsignificance ($P \geq 0.05$); in B–F, black bars indicate significance ($P < 0.05$) after sequential Bonferroni; bars with diagonal lines indicate nonsignificance after sequential Bonferroni.

loss of fitness, respectively, compared to optimal conditions (Bradshaw et al. 1998). However, we observed no latitude of origin by thermal-environment interaction. Southern populations did no better in the southern summer than did northern populations; northern populations did no better in the midlatitude winter than did the southern populations. There are several reasons for the disparity between the previous and present results. In the previous study, the northern thermal year was not as extreme and, in the southern thermal year, fitness was evaluated only over a single generation. In our present northern year the overwintering population remained at -3°C for several months (Fig. 3), not $0 \pm 2^{\circ}\text{C}$ for two weeks as in the previous study. The lower temperature for the longer period was a closer approximation to actual winter leaf temperatures in the north (Fig. 4G,H), and resulted in greatly reduced to zero winter survivorship (Fig. 6B) as compared with our earlier study where none of the populations went extinct.

Our present southern year allowed for multivoltinism, as would be normal at 30°N (Bradshaw 1983) and imposed a higher maximum temperature of 38°C versus 36°C than in the previous study. The major loss of fitness in the southern year was due to the decline in pupal and, consequentially, egg production over the long, hot summer (Fig. 7A,B). This decline in fitness accumulated over several generations. Upon the return of cooler temperatures in the late summer, southern and midlatitude but not northern populations increased pupal and egg production.

We therefore conclude that thermal adaptations among populations having evolved over a climatic gradient in nature may become apparent as a result of thermal stress only when populations are stressed to the brink of extinction as in the northern thermal year or only when the adverse effects of temperature can accumulate over several generations as in the southern thermal year.

Variation in the Photic Year

The midlatitude thermal year provided a benign thermal environment as compared with the northern or southern thermal years (Fig. 5). In this benign thermal environment, relative fitness in the natural midlatitude photoperiod was lower in both the southern and northern populations and did not differ from 1.0 in the midlatitude populations (Fig. 8A). Reduction in fitness among southern populations was due to a low number of larvae actually overwintering in diapause (Fig. 8B) which, in turn was due to continued autumnal pupation (Fig. 9A) that resulted in few or no eggs (Fig. 9B). We interpret these results to mean that southern populations, with shorter critical photoperiods (Bradshaw and Holzapfel 2001) continued to develop into the fall at temperatures below those at which adults could survive and reproduce. The genetically programmed response to day length in southern populations prevented their timely entry into diapause and resulted in their 74% loss of fitness in the thermally benign, midlatitude photic year.

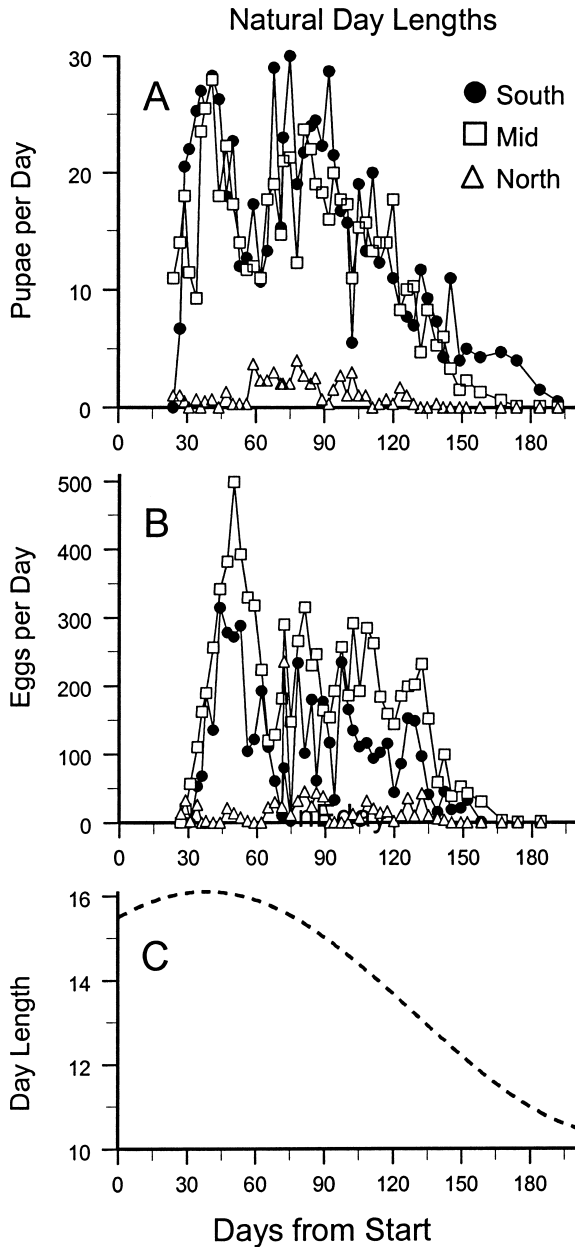


FIG. 9. Rates of pupal (A) and egg (B) production during the benign midlatitude thermal year with the corresponding gradual, midlatitude change in day length (C).

Reduction in fitness among northern populations appeared due to a concatenation of factors that were not individually significant: low fall abundance, low spring survivorship, and low female fecundity (Fig. 8B,D,E). Pupation rate and, consequently, egg production was low among northern as compared with southern and midlatitude populations throughout the previous summer (Fig. 9). The longest days of summer stimulated development in only a few individuals. We interpret these results to mean that northern populations, with longer critical photoperiods, entered diapause during the summer and, consequently, had to survive both the remaining warm season as well as winter on resources they had sequestered during the early and midsummer. Resources the following spring were

not sufficient to sustain development in many of the individuals that survived winter. The genetically programmed response to day length in northern populations forced them to endure a warm-season diapause, thereby depleting their resources, and resulted in their 88% loss of fitness in the thermally benign, midlatitude photic year.

This loss of fitness resulted from "transplanting" populations over only 6–10° of latitude; we expect the loss in fitness would have been even more extreme had we transplanted them over the full 16–20° range of latitude, as we did with the thermal environment.

Implications

Life-history evolution has historically emphasized the components and correlates of r , mainly in aseasonal environments (Roff 1992; Stearns 1992). Because summer weather is favorable for growth, development, and reproduction, most of these studies, and especially those with *Drosophila* (Mueller and Ayala 1981; Rose and Charlesworth 1981a,b; Mueller 1988; Service et al. 1988; Service 1989; Partridge and Fowler 1992), assume a world of endless summer. But temperate organisms, including *Drosophila* (Mitrovski and Hoffmann 2001; Hoffmann et al. 2003), do not enjoy endless summer and must cope with winter on an annual basis. Overwintering can affect the subsequent expressions of life-history traits the following spring, generally resulting in reduced performance (Tauber et al. 1986; Danks 1987; Leather et al. 1993; Chang et al. 1996; Bradshaw et al. 1998). Further, adaptation to summer opportunities may conflict with adaptation to winter exigencies. Development time is an important contributor to fitness through its impact on generation time but also in the "fitting" of generations within the available growing season so that seasonality could also affect age and size at reproduction and the number, size and frequency of offspring (Masaki 1967; Istock 1978, 1983; Roff 1980; Nylin et al. 1989; Nylin 1992; Mousseau and Roff 1989; Ayers and Scriber 1994; Mitrovski and Hoffmann 2001; Hoffmann et al. 2003). Chromosomal inversions in *Drosophila* that fluctuate seasonally, show an increase in the winter genotype with either increasing latitude (Rodríguez-Trelles et al. 1996) or altitude (Dobzhansky 1948), and a decrease in the winter genotype with progressive global warming (Rodríguez-Trelles and Rodríguez 1998; Levitan 2003). Pest species that had originally evolved in temperate seasonal climates lose or show reduced photoperiodism or tendency to diapause after long-term culture in an aseasonal lab environment (Hoy 1978; Tauber et al. 1986). In the milkweed bug, *Oncopeltus fasciatus*, temperate populations maintain a positive genetic correlation among wing length, fecundity, and flight propensity (Palmer and Dingle 1986) but this correlation structure breaks down in nonmigratory, tropical populations (Dingle et al. 1988). All of these observations indicate an interdependence between summer and winter performance on the determination of year-long fitness. Yet, photoperiodism and the timing of seasonal development are given but token coverage in broader treatises on evolutionary response to stress (Hoffmann and Parsons 1991), on adaptation to temperature (Johnston and Bennett 1996), on adaptive response to global climate change (Kareiva et al.

1993), or on life-history evolution (Stearns 1992; Roff 1992; for an exception, see Roff's 2002, Ch. 6, discussion of life-history evolution in predictable environments). Photoperiodic response can evolve rapidly in response to a novel climate experienced by introduced species (Hoy 1978; Tauber et al. 1986; Fochs et al. 1994) or during range expansion (Lounibos et al. 2003), in response to altered predator phenology (Hairston and Walton 1986), or in response to rapid climate warming (Bradshaw and Holzapfel 2001).

As environmental temperatures tend towards extremes, organisms can avoid or mitigate accumulating thermal stress through evasive behavior or physiological acclimation. These responses generally represent the reversible modifications of plastic phenotypes. But, photoperiodic response provides a genetically hard-wired anticipatory cue of future events. The evasive behavior (migration) or physiological acclimation (dormancy) is not subject to later revision when the favorable season ends earlier or later than anticipated. Individual mobility or physiological flexibility may enable organisms to find refuges from thermal extremes, but there are no refuges from mis-interpreting photic cues.

Reciprocal transplants across latitudes in nature always confound the effects of the thermal and photic environments on fitness. To our knowledge, no one has heretofore held the thermal year constant while varying the photic year. When we hold the thermal environment constant for a midlatitude year, we show that in a temperate seasonal environment, which is thermally benign for all populations north to south, there is a strong selective advantage to possessing the local, latitudinally specific photoperiodic response. We need to reiterate that it is the length of the growing season, and not local day length, that selects on the optimal time to enter diapause and, consequently, the critical photoperiod. Because global warming is proceeding faster at higher than at lower latitudes (DeGaetano 1996; Hegerl et al. 1996; Easterling et al. 2000), high latitude populations will experience an amelioration of their thermal environment; selection on extreme cold tolerance will be relaxed. At the same time, northern growing seasons will become longer; selection for shorter critical photoperiods will be intensified. We therefore expect to see more rapid evolution of photoperiodic response than of thermal tolerance as a consequence of global warming among northern, temperate ectotherms.

Conclusions

Over their range from the Gulf of Mexico to Canada, *Wyeomyia smithii* exhibit evolved differences in thermal and photoperiodic responses that are adaptive (result in increased fitness) in the thermal and photoperiodic environments of their respective latitudes of origin.

Evolved differences in thermal response are due to differences in thermal tolerance rather than thermal specialization because both northern and southern populations realize greater fitness in a latitudinally intermediate thermal year than in their respective native thermal years. Evolved differences in heat tolerance were expressed when the adverse effects of high temperatures accumulated over several generations; evolved differences in cold tolerance were expressed when the duration and intensity of winter cold forced populations

to the verge of extinction. Thermal tolerances determined over a single generation or under conditions that effect less than a severe loss of fitness are therefore likely to underestimate the extent to which populations may be limited by thermal tolerances.

Evolved differences in photoperiodic response are due to the maladaptive timing of development and dormancy, even in an otherwise thermally benign environment. Southern populations with short critical photoperiods continue to develop later in the year at temperatures in which their adults cannot survive and reproduce. Northern populations with long critical photoperiods enter an early, warm-season diapause and deplete resources that could contribute to overwintering survivorship and to development and reproduction the following spring and summer.

These conclusions have two important implications relating to rapid climate change. First, reciprocal transplants between latitudes are likely to overestimate the effects of climate warming because of the confounding effects of the foreign photoperiodic environment with heat and drought stress. Second, because global warming in the Northern Hemisphere is proceeding faster at northern than at southern latitudes and because this change represents an amelioration of the thermal environment and a concomitant increase in the duration of the growing season, we conclude that there should be more rapid evolution of photoperiodic response than of thermal tolerance as a consequence of global warming among northern, temperate ectotherms.

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APPENDIX

The daily and annual flux in temperatures and the annual flux in photoperiod are generated as smooth cosine functions programmed by a microprocessor (Auto-Matrix Inc., Export, PA):

$$Y = \frac{1}{2}(\text{MAX} + \text{MIN}) - \frac{1}{2}(\text{MAX} - \text{MIN})\text{COS } \theta \quad (1)$$

where θ is in degrees. Using the mean monthly maxima and minima for July and January at 30, 40, and 50°N (Appendix Table 1), daily maximal and minimal air temperatures (°F) are generated from Eq. (1):

$$Y = \text{Max}^{\circ}\text{F} \quad \text{MAX} = \text{Jul Max} \quad \text{MIN} = \text{Jan Max}$$

$$Y = \text{Min}^{\circ}\text{F} \quad \text{MAX} = \text{Jul Min} \quad \text{MIN} = \text{Jan Min}$$

$$\theta = (\text{JD} - 15) * 360/365$$

where JD is the Julian Date in days from January 1. Air temperatures are then converted to daily maximal (Max°C) and minimal (Min°C) leaf temperatures using the regression of leaf (°C) on local airport (°F) temperatures during January–August in north-central Florida on a plant in the open; leaf temperatures spanned –3 to 38°C (Bradshaw et al. 2000):

$$\text{Max}^{\circ}\text{C} = -22.5 + 0.675\text{Max}^{\circ}\text{F} \quad (r^2 = 0.65) \quad (2)$$

$$\text{Min}^{\circ}\text{C} = -13.0 + 0.400\text{Min}^{\circ}\text{F} \quad (r^2 = 0.62) \quad (3)$$

Leaf temperatures ($T^{\circ}\text{C}$) at time t (minutes) during the day are generated from daily maxima (Eq. 2) and minima (Eq. 3) using Eq. (1) so that the thermal day lags the solar day by 3 h (Bradshaw 1980):

$$Y = T_t \quad \text{MAX} = \text{Max}^{\circ}\text{C} \quad \text{MIN} = \text{Min}^{\circ}\text{C}$$

$$\theta = (t - 180) * 360/1440$$

Variable day length in the 40°N thermal year: using the day length from sunrise to sunset + both civil twilights (Bradshaw and Phillips 1980) on the summer and winter solstices at 40°N (Beck 1980), photoperiod (h) on a given day (PPD_D) is generated from Eq. (1): $Y = \text{PPD}_D$ MAX = 16.1 h MIN = 10.3 h $\theta = (\text{JD} + 10) * 360/365$.

TABLE A1. Mean monthly air Temperatures ($^{\circ}\text{F}$) compiled from ESSA (1968).

Latitude	Mean January		Mean July	
	Maximum	Minimum	Maximum	Minimum
30°N	64.6	42.8	91.0	73.0
40°N	43.3	28.4	86.5	67.1
50°N	20.1	2.8	79.5	56.3