

ADAPTATION TO THE THERMAL CLIMATE OF NORTH AMERICA BY THE PITCHER-PLANT MOSQUITO, *WYEOMYIA SMITHII*

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Abstract. We are concerned with the climatic factors that affect the adaptive radiation of species into and within the temperate zone. As one progresses northward, there is a decrease in summer temperatures and a concomitant increase in the duration and intensity of winter cold. For the pitcher-plant mosquito, *Wyeomyia smithii*, it has been shown previously that (1) range expansion and evolution has proceeded from south to north, and (2) either realistically hot summer temperatures or a simulated 7-mo winter reduce fitness by 60% or more in two New Jersey (40° N) populations. Herein, we compare the relative fitness of nine populations of *W. smithii* from 30° to 50° N when exposed to these same stressful summer temperatures and simulated winter. We determined the survivorship, fecundity, and fertility of five cohorts from each population at each temperature in both environments, and we calculated the cohort replacement rate (R_0) as the product of these three component traits. Survivorship declined with increasing latitude in the summer, but not the winter, environment. Fecundity was not correlated with latitude in either environment. Fertility declined with increasing latitude in both environments. R_0 was not correlated with latitude in either environment. Hence, the interpretation of the adaptive responses to climate can depend critically on the trait being examined. Because R_0 is a composite index of fitness that includes any trade-offs among its constituent traits, we place greater reliance on its noncorrelation with latitude. Therefore, we conclude that, despite their clear impact on fitness, summer heat and winter cold have had little impact on the adaptive evolution of *W. smithii* to the climatic gradient of North America. The decline in summer heat and increase in the duration and intensity of winter cold as one moves north also impose a latitudinal gradient in the length of the favorable growing season. Many plants and animals use daylength to cue the seasonal events in their life cycles. We then argue that the timing of seasonal development mediated by photoperiodic response constitutes the most immediate adaptation of populations to novel temperate climates, and that adaptation of thermal responsiveness to summer heat or winter cold takes place over a longer time scale or taxonomic distance.

Key words: *carnivorous plants; diapause; fitness evolution; heat stress tolerance; overwintering; pitcher-plant mosquito; thermal adaptation; Wyeomyia smithii.*

INTRODUCTION

Herein, we consider adaptation to summer heat and winter cold by natural populations of the pitcher-plant mosquito, *Wyeomyia smithii*, along a geographic gradient in central and eastern North America. The major climatic gradient of this region is one of temperature, rather than rainfall (NOAA 1968, MacArthur 1972). With increasing latitude, there is a decrease in summer heat and an increase in the duration and intensity of winter cold. Not surprisingly, the distribution or activities of many animals correlate closely with environmental temperature (Hopkins 1938, Andrewartha and Birch 1954, Pielou 1979, Brown and Gibson 1983, Root 1988, Beaupre 1995, Brown 1995). When confronted with temperatures that approach the limits of tolerance, animals may avoid or mitigate thermal ex-

igencies through behavioral or ecological means (Haufe and Burgess 1956, Muul 1965, Porter et al. 1973, Kingsolver 1983a, b, Masaki 1986, Huey and Bennett 1987, Lynch 1992, Costanzo et al. 1995, Mousseau and Roff 1995, Johnston and Bennett 1996), as well as by confronting them directly through immediate or evolved modifications in physiological response. For animals expanding their ranges into or within the temperate zone, the evolution of physiological responses might include modifications of thermal tolerance and performance breadth (Huey and Kingsolver 1993, Costanzo et al. 1995, Gilchrist 1995), enzymatic activity (Hochachka and Somero 1984, Somero 1992), respiratory pigment loading curves (Reese and Mangum 1994, DiPrisco and Giardina 1996), or cold hardiness (Lee and Denlinger 1985, 1991, Coulson and Bale 1992, Leather et al. 1993). Regardless of the specific mechanism, such modifications should be considered as adaptive only if they can be shown to have a positive effect on fitness. Thus, if northern and southern pop-

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ulations do not differ in fitness when encountering a northern or a southern thermal environment, then it cannot be argued that differences between these populations in performance breadth, thermal tolerance, supercooling, cold hardiness, or any other individual thermal response are adaptive.

In this paper, we examine the contribution of summer heat and winter cold to differential fitness among populations of the mosquito, *Wyeomyia smithii*, that has a broad geographic range in temperate North America. Throughout its range, *W. smithii* oviposits into and completes their entire preadult development only within the water-filled leaves of the purple pitcher plant, *Sarracenia purpurea*. Larvae overwinter in the leaves of their host in a diapause that is initiated, maintained, and terminated by photoperiod. The median or critical photoperiod is closely correlated with the latitude and altitude of origin ($R^2 = 0.96$), or length of the local freeze-free period (Bradshaw 1976).

Wyeomyia is a large neotropical genus with two subtropical species and only one temperate species, *W. smithii*, that extends from the Gulf of Mexico into Canada (Lane 1953, Stone et al. 1959, Darsie and Ward 1981). Biogeographical, physiological, and morphological evidence all indicate that *W. smithii* has invaded North America from South America, and that its range expansion in North America has proceeded from south to north (Ross 1964, Bradshaw and Lounibos 1977, Istock and Weisburg 1987, Bradshaw and Holzapfel 1990). By examining populations over a latitudinal and altitudinal climatic gradient, we are, therefore, examining the performance of a formerly tropical species along its historical, adaptive trajectory into and within a temperate climate. *Wyeomyia smithii* are aquatic as larvae, and pitcher plants occur in humid bogs, swamps, and wet pine savannahs where the weak-flying adults remain close to the emergent vegetation. Consequently, geographically variable climate for *W. smithii* is primarily a function of temperature, and their immediate thermal environment is closely correlated with local temperature (Evans 1971, Bradshaw 1980a). We know of few other organisms that live in so consistent a microhabitat over such wide geographic distances; and, at the same time, live in a microhabitat that so closely reflects local ambient temperature.

Winter cold and summer heat affect fitness within populations of *W. smithii*. Overwintering reduces survivorship in nature (Evans 1971, Bradshaw and Holzapfel 1986), and thermal and hydric stress may limit fitness during the summer (Kingsolver 1979). Under simulated natural conditions in the laboratory, the imposition of realistic, hot summer temperatures reduced fitness (cohort replacement rate, R_0) by 68% and a 7-mo simulated winter reduced fitness by 60% in two New Jersey populations (Bradshaw et al. 1998). Both summer heat and winter cold are, therefore, potentially strong selective forces shaping the thermal responses of these mosquitoes over the thermal gradient of North

America. Herein, we examine the relative degree to which nine populations of *W. smithii* from a broad geographic range (Fig. 1) have adapted to these conditions.

If summer heat and winter cold have been important selective forces in the adaptive evolution of *W. smithii* in North America, then we expect performance under these two conditions to diverge with increasing latitude due to decreasing tolerance of high summer temperature, due to increased overwintering ability, or both. We agree with Huey and Berrigan (1996) that one needs to quantify the presumed benefit of a response "explicitly in the currency of fitness" (Bradshaw 1980b, Bradshaw and Holzapfel 1983, 1989). Effects of the environment on any one measure of performance such as survivorship, fecundity, or fertility may have compensating effects on the others, and cancel each other out when they are combined into a more inclusive measure of performance or index of fitness. As a composite index of fitness during the growing season, we have used Laughlin's (1965) capacity for increase (Bradshaw 1980b, Bradshaw and Holzapfel 1983, 1989, 1990, 1996, Armbruster et al. 1997): $r_c = \ln R_0 / \tau \approx \ln(\text{survivorship} \times \text{fecundity} \times \text{fertility}) / (\text{mean generation time})$.

For organisms that overwinter in diapause, development ceases for an indefinite period of time. Under these circumstances, fitness becomes less directly related to mean generation time and more directly related to survivorship, fecundity, and fertility. The currency of fitness common to both summer and winter generations is then R_0 which we use as our composite index of fitness in this study. We evaluate performance of R_0 and its components (survivorship, fecundity, and fertility) in the same stressful summer and winter environments as previously (Bradshaw et al. 1998) and, for each, test two explicit hypotheses:

H1: Performance in stressful summer or overwintering environments

$H_{1,0}$ is not significantly correlated with latitude of origin.

$H_{1,A}$ is significantly correlated with latitude of origin.

H2: Regression of performance on latitude

$H_{2,0}$ is the same in both environments.

$H_{2,A}$ diverges; i.e., there is a significant latitude-by-environment effect.

METHODS

Variation in leaf, local, and geographic temperatures

To record leaf temperatures, we used thermistor probes from a Rustrak 2133 dual-track temperature recorder (Gulton Industries, Manchester, New Hampshire, USA) housed in a weathertight enclosure. We recorded temperature in a wet pine savannah 7 km

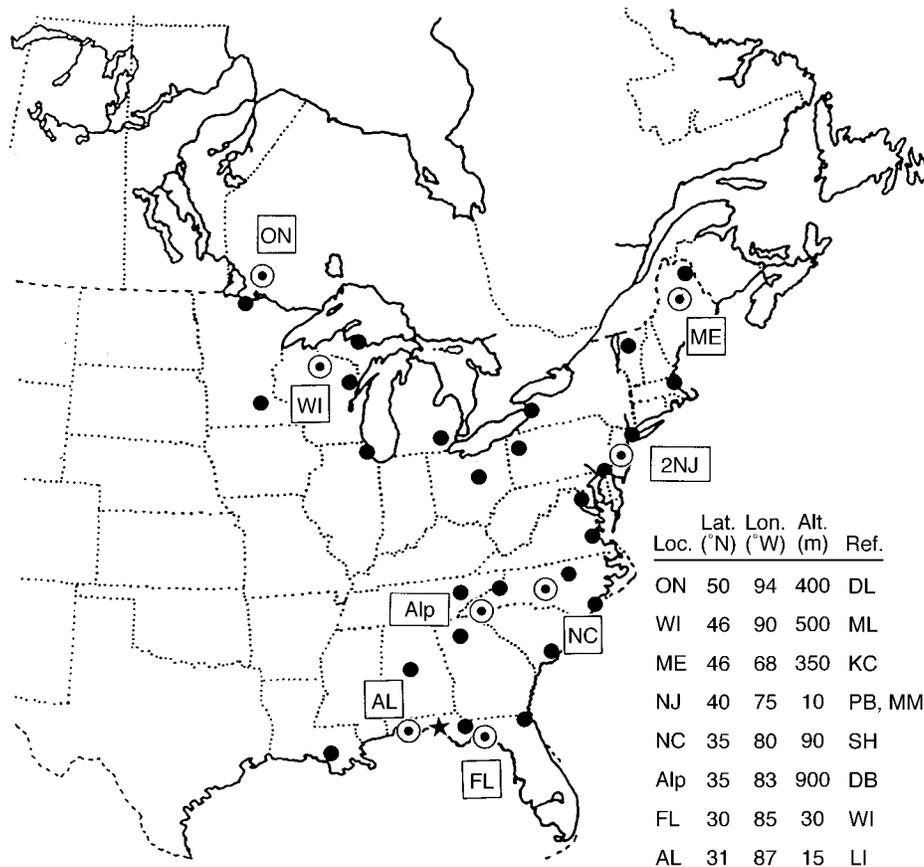


FIG. 1. Localities of origin of mosquito populations (⊙), weather stations (●), and the locality at which leaf temperatures were recorded (★). "Alp" refers to the alpine (900 m) locality in North Carolina. The inset provides the latitude (°N), longitude (°W), and altitude (m) of each locality, as well as a reference (Ref.) that identifies populations from specific localities used in previous studies from this laboratory. The two New Jersey localities are 15 km apart at the same altitude.

northwest of Holt, Florida, (★, Fig. 1) in two leaves of *S. purpurea*: one leaf was in deep, continual shade, the other was exposed to continual full sunlight; hereafter, shade and sun leaves, respectively. Temperature recordings started on 28 October 1975, and continued through 22 May 1976. The recorder was powered by a 12-V marine battery that was changed five times during this period, providing six periods of recording of about 2 wk each. We obtained air temperatures from the National Climate Center (Federal Building, Asheville, North Carolina 28801 USA) for the corresponding period from the Milton (Florida) Experimental Station, about 21 km from the Holt locality. To determine the relationship between leaf and weather station temperatures, we performed second-order polynomial regression of leaf maxima and minima on air maxima and minima for the current and previous days using Proc GLM in SAS (SAS 1985).

As an estimate of summer heat or winter cold at each locality, we used the Climatic Atlas of the United States (NOAA 1968) to determine the mean July maxima and mean January minima over a 30-yr period at 26 weather stations surrounding our source populations of *W. smi-*

thii (Fig. 1). We used partial regression (SAS 1985, Proc REG) to correlate these seasonal climatic extremes with the latitude, longitude, and altitude of the weather station. Then, using the significant terms of the regression, adjusted the latitudes of our localities of mosquito origin to take into account the estimated longitudinal or altitudinal effects on the seasonal climatic extremes at those localities.

Collection and maintenance

Approximately 2000 *W. smithii* larvae were collected from each of nine localities in eastern North America (Fig. 1). Collections were made in the spring of 1993 before adult emergence so that 100% of the genotypes were still available for sampling as larvae in the pitcher-plant leaves. Samples were taken from 50 (lowland AL–NJ) to 200 (northern and alpine) plants over 100 to 500 m distance within each population. Samples were maintained on ice, transported to the laboratory, and reared through 5–7 generations before the start of experiments, as described in Hard et al. (1992).

In the generation prior to the experiments, the larvae were synchronized in diapause on short days, and then

transferred to "optimal" long-day conditions. The resulting adults were maintained in large 7.5-L cages and allowed to mate en masse. Eggs of the experimental generation were collected three times per week, and first instars were removed daily. Experimental cohorts were established on the day of hatch and all experimental cohorts were started within a 48-hr period.

Conditions common to all experiments

We ran all of our experiments in the mosquito's natural habitat, the water-filled leaves of intact pitcher plants. In each environmental treatment, cohorts of 30 larvae each were reared from day of hatch in 23 mL distilled water in separate leaves on intact pitcher plants. To simulate natural prey capture by the host plant, experimental leaves were provided with rations of individually counted, freeze dried, adult *Drosophila melanogaster*. Plants were placed in terraria in controlled-environment rooms programmed to simulate natural temperature cycles with smooth, sine-wave thermoperiods that lagged the photoperiod by 3 h. The photic environment was programmed for long-day (L:D = 17:7) or short-day (L:D = 8:16) photoperiod with an additional 0.5 h dim "twilight" at each end of the photophase.

Upon the start of pupation, leaves were checked three times per week and the pupae transferred to adult 0.95 L cohort cages. Each cage was provided with pesticide-free raisins for adult nutrition, an open jar with 50 mL distilled water for pupae and adult eclosion, and a single 10–20 mL cut leaf of *S. purpurea* for oviposition. The bottom of the cage was covered with absorbent paper; this paper was soaked with distilled water three times per week. These cages were checked three times per week for adult eclosion, adult death, and eggs. The eggs were removed, placed in 75 mL distilled water, and the number of larvae hatching over a 10-d period was recorded. Fertility was then calculated as percent hatch = (number of larvae hatching from a cohort)/(number of eggs produced by the cohort). Finally, we calculated cohort replacement rate: $R_0 = (\text{percent larvae eclosing as adults}) \times (\text{eggs per eclosed female}) \times (\text{percent hatch})$.

Simulated environments

Experiments were initiated concurrently in separate controlled-environment rooms. All environments reflected natural temperatures that we have observed in the field. For optimal summer conditions, we provided a thermoperiod with a mean of 21°C that fluctuated from 13° to 29°C each day at a constant 80% relative humidity (RH). We have used this regimen to simulate near optimal conditions over the last decade in our studies of life history evolution in *W. smithii*.

Stressful summer conditions were designed to impose broad physiological stress over the whole life cycle of the mosquito, and consisted of increased larval and adult temperature, low adult humidity, and reduced

larval food (Bradshaw et al. 1998). The thermoperiod ranged from 16° to 36°C each day (mean 26°C), providing a daily maximum in the highest range of those we have observed in the field (Fig. 2). Humidity ranged from 60% to 80% RH (mean 70% RH) each day with minimum humidity corresponding to maximum temperature. Each cohort was fed three-fourths the ration of food provided under optimal conditions. To simulate the timing of prey capture by pitcher-plant leaves in the field (Bradshaw and Holzapfel 1983), experimental cohorts were fed 29 flies on the day of hatch, and 108, 56, and 29 flies at successive weekly intervals for a total of 7.4 flies per larva. Performance (R_0 , survivorship, fecundity, and fertility) was determined for five cohorts of 30 larvae each from each population.

To simulate winter (Bradshaw et al. 1998), the controlled-environment room was programmed to provide a smooth, sine-wave daily thermoperiod that commenced at the optimal summer conditions with a mean of 21°C and a daily fluctuation from 13° to 29°C during the first week of the experiment. Thereafter, both the daily mean and fluctuation in temperature were reduced by ~2°C/wk to a "midwinter" low of 0°C with a daily fluctuation of 4°C during wks 11 and 12. These conditions were cold enough to freeze the water, but not the larvae, in the leaves. They were colder than winter conditions we observed in Florida (Fig. 2), but were not as cold as those observed in Manitoba (Evans 1971). After 2 wk at 0°C, the daily mean and fluctuation were increased by the reverse amount each week until the return of optimal summer temperatures. From the day of hatch, larvae received a diapause-inducing, short-day photoperiod until mean daily temperature rose to 5°C (wk 15), at which time the photoperiod was increased to long days. Each cohort was fed 15 flies on the day of hatch and 30, 60, 60, 30, 30, and 15 flies at successive weekly intervals during the simulated autumn. Larvae received no food from late fall in wk 8, when mean daily temperature fell to 7°C, until early spring in wk 17 when mean daily temperature rose again to 9°C. Starting in wk 17, each cohort was censused weekly and received a ration of one fly per two mosquito larvae, rounded up to the nearest whole fly. Pupation commenced during wk 22 so that larvae received a total of 11–14 flies per larva during the combined fall, winter, and spring. Pupae were transferred to adult cohort cages maintained under optimal environmental conditions. Initially, five cohorts were set up for each population. During the 3-mo winter, four of the 45 leaves senesced and leaked their contents, leaving four cohorts from the Florida population, two from the low elevation North Carolina population, and five from the remaining seven populations.

RESULTS

Temperatures in Florida pitcher-plant leaves fluctuated 15–25°C/d and ranged from a winter low minimum of –4°C to a summer maximum of 38°C (Fig. 2A, B).

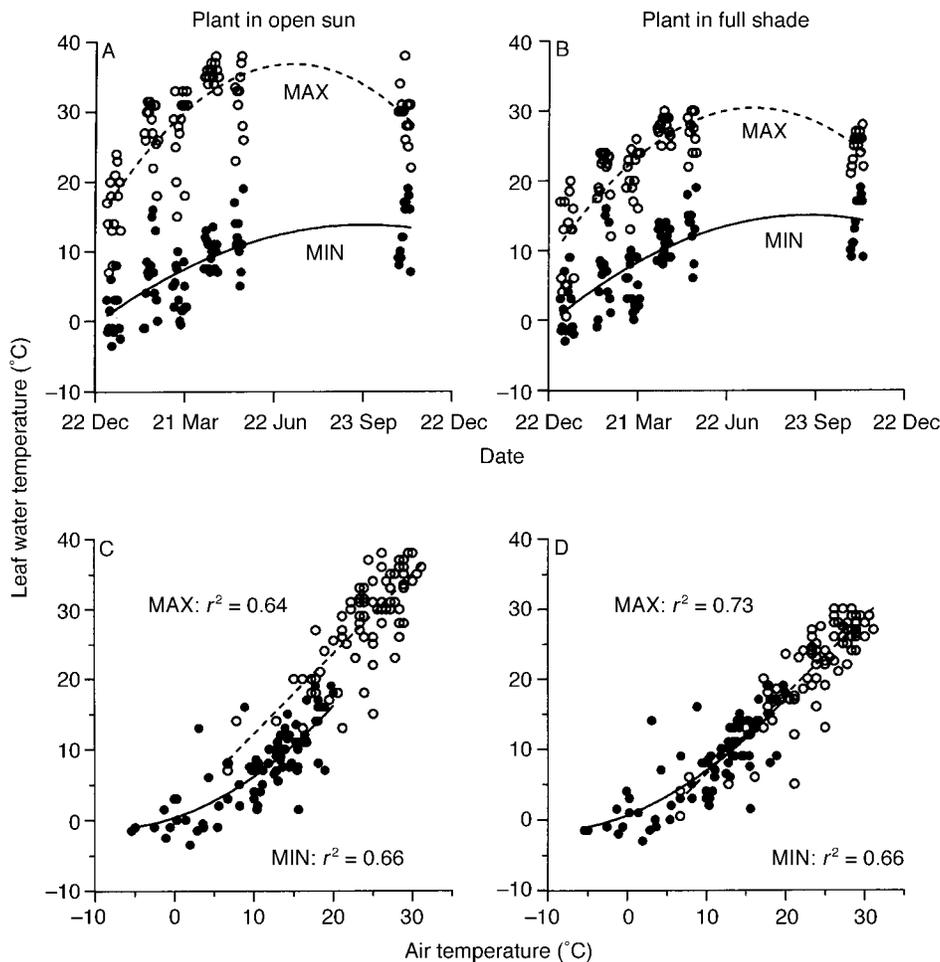


FIG. 2. Maximum and minimum daily leaf temperatures on pitcher plants in the open sun (A, C) or in the full shade (B, D) in a wet pine savannah near Holt, Florida (★, Fig. 1). (A, B) Seasonal march of temperature in sun and shade leaves. The curves show second-order polynomial regression of leaf temperatures on date to illustrate seasonal trends. (C, D) Relationship between leaf water maximum and minimum daily temperatures at the Holt, Florida, locality, and air maximum and minimum daily temperatures at the Milton, Florida, Experimental Station, 21 km away. Coefficients of determination are shown for linear regression of leaf maximum on air maximum for the current day, and for second-order polynomial regression of leaf minimum on the geometric mean air minimum for the current and previous days. All regressions are very highly significant ($P < 0.001$).

Maximum daily temperatures in sun leaves averaged (± 1 SE) $6.08 \pm 0.37^\circ\text{C}$ warmer than in the shade leaves; minimum daily temperatures in sun leaves also averaged $0.73 \pm 0.08^\circ\text{C}$ cooler than in the shade leaves. Consequently, the more exposed sun leaves experienced more extreme maxima and minima than did the shade leaves.

Polynomial regression of leaf on the current and previous day's air temperature was linear for leaf maxima and curvilinear for leaf minima, with a strong influence of the previous day's temperature on the latter but not the former. Figure 2C, D shows linear regression of maximum leaf temperature on maximum air temperature during the current day, and second-order polynomial regression of minimum leaf temperature on the geometric mean minimum air temperatures during the

current and previous days. The regressions were all very highly significant, and the coefficients of determination ranged from 64% to 73%. Our goal here was not to model causality of leaf temperatures as in Kingsolver (1979), but simply to show that leaf maxima and minima reflect prevailing air temperatures. Consequently, we used mean daily July maxima and mean daily January minima (NOAA 1968) to estimate the long-term climatic extremes likely to have been encountered by *W. smithii* at their localities of origin.

Mean July maximum was significantly correlated with latitude (partial $r^2 = 0.762$, $P < 0.001$), but with neither longitude (partial $r^2 = 0.027$, $P = 0.100$) nor altitude (partial $r^2 = 0.023$, $P = 0.117$):

$$\text{July maximum } (^\circ\text{C}) = 45.56 - 0.410 (^\circ\text{N latitude}). \quad (1)$$

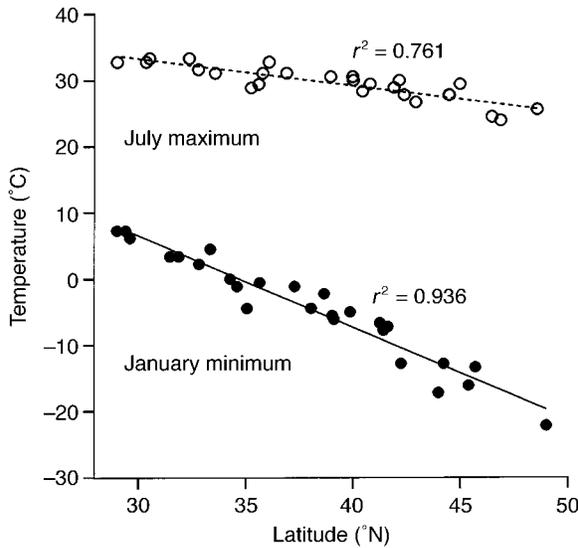


FIG. 3. Relationship between mean daily maximum air temperature in July and untransformed latitude, and between mean daily minimum air temperature in January and equivalent latitude (Eq. 3) over a 30-yr period for 26 weather stations surrounding pitcher-plant localities (Fig. 1).

Mean January minimum was significantly correlated with latitude (partial $r^2 = 0.914$, $P < 0.001$) and longitude (partial $r^2 = 0.023$, $P = 0.009$), but not altitude (partial $r^2 = 0.002$, $P = 0.446$):

$$\text{January minimum (}^\circ\text{C)} = 64.30 - 1.382(^\circ\text{N latitude}) - 0.181(^\circ\text{W longitude)}. \quad (2)$$

Eq. 2 provides a correction factor of $0.181/1.382 = 0.131$ for converting units of longitude to units of equivalent latitude, resulting in equivalent latitudes

that range from 41.2° to 61.9°N . Subtracting 11.8°N from each of these values gives a longitudinally corrected latitude:

$$\text{Equivalent latitude} = ^\circ\text{N latitude} + 0.131(^\circ\text{W longitude}) - 11.8. \quad (3)$$

This equivalent latitude correlates linearly with January minimum and also approximates the uncorrected latitudinal range that correlates linearly with July maximum (Fig. 3). Consequently, we used untransformed latitudes as a covariate for performance in the summer stressful environment, and equivalent latitudes (Eq. 3) as a covariate for performance in the overwintering environment.

We used a two-step approach to the analysis of R_0 and its components. First, to test whether evolution of summer stress tolerance or winter tolerance were possible, we performed two-way ANOVA with population and environments as fixed effects (Table 1). R_0 , fecundity, and fertility, but not survivorship, differed between environments. This result is trivial since there is no a priori reason to expect heat stress to have an identical effect on fitness as overwintering. More importantly, R_0 and each of its components differed among populations, and there was a consistent, significant population by environment effect. These results show, respectively, that evolution of summer stress tolerance or overwintering tolerance is possible, and that independent evolution to either environment is possible.

Second, to test whether evolved differences in summer stress tolerance or overwintering ability were related to geography, we performed analysis of covariance (ANCOVA) with latitude as the covariate and environment as a fixed effect (Table 1). For the summer

TABLE 1. Cohort replacement rate ($R_0 = \ln[R_0 + 1]$) and its components; survivorship (SUR = percentage adult eclosion, arcsine square-root transformed), fecundity (FEC = eggs per eclosed female, square-root transformed), and fertility (FER = percentage hatch, arcsine square-root transformed) in stressful summer and overwintering environments.

Dependent variable	Two-way ANOVA				ANCOVA			
	POP ($F_{8,68}$)	ENV ($F_{1,68}$)	P × E ($F_{8,68}$)	R^2 †	LAT ($F_{1,82}$)	ENV ($F_{1,82}$)	L × E ($F_{1,82}$)	R^2 †
R_0	3.56	21.86	3.65	56	1.42	0.20	0.03	16
P	0.002	***	0.001		0.237	0.653	0.863	
SUR	3.14	0.18	2.60	41	13.64	6.89	6.30	20
P	0.004	0.674	0.015		***	0.010	0.014	
FEC	3.86	15.32	3.48	54	0.71	1.25	0.32	12
P	***	***	0.002		0.403	0.267	0.573	
FER‡	5.09	19.62	2.16	56	16.98	0.54	2.12	33
P	****	***	0.043		***	0.465	0.149	

Notes: Sources of variation are population (POP), environment (ENV), and their interaction (P × E) for the two-way ANOVA, and latitude (LAT), environment, and their interaction (L × E) for ANCOVA. Exact probabilities are shown unless: *** $P < 0.001$ or **** $P < 0.0001$. Significant ($P < 0.05$) values of F are shown in boldface.

† Percentage reduction in total sum of squares due to all independent variables combined (model R^2); the minimum percent reduction in total sum of squares for a significant effect of LAT, ENV, or L × E alone is 4.6% and 4.9% for 82 and 76 error degrees of freedom, respectively.

‡ Error degrees of freedom are 62 for ANOVA and 76 for ANCOVA because fertility is undefined in the cohorts with zero fecundity.

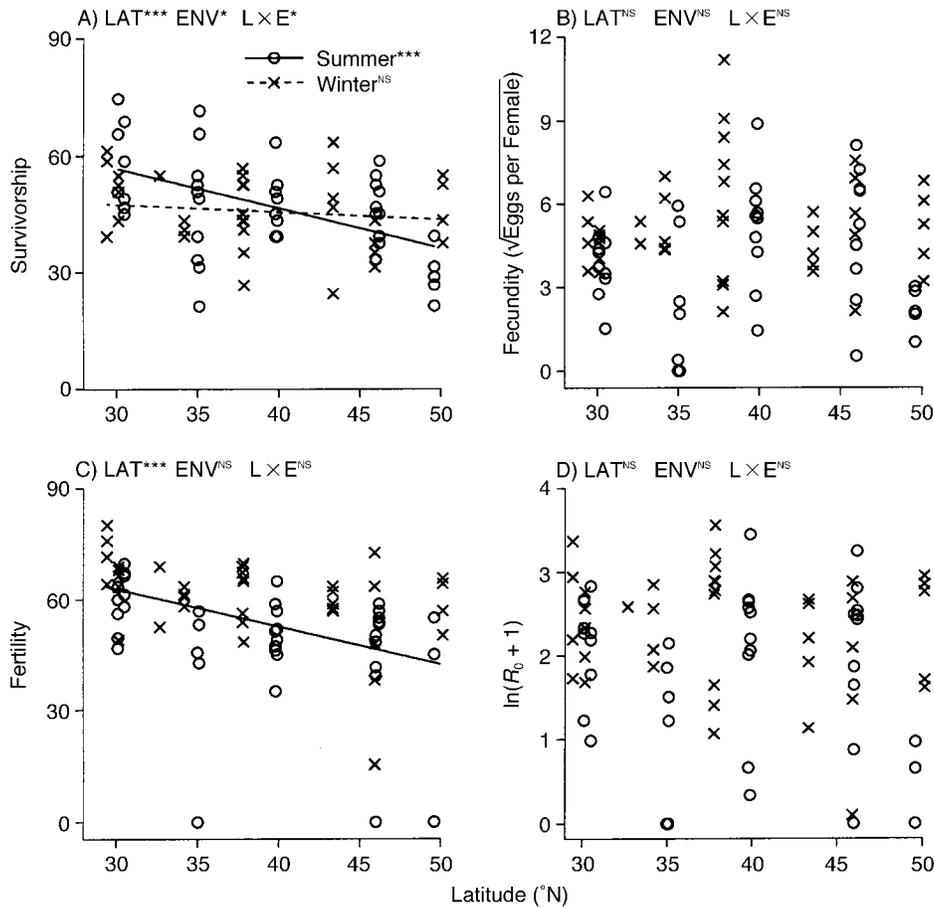


FIG. 4. Latitudinal variation of R_0 and its components in a thermally stressful summer environment (Summer) and an overwintering environment (Winter). The caption at the top of each graph shows the results of ANCOVA from Table 1 with latitude (LAT) as the covariate and environment (ENV, Summer or Winter) as the fixed effect, and with $L \times E$ representing the interaction term. Fertility and survivorship (percentages) are shown on the arcsine(square-root)-transformed scale. Separate regressions are shown for summer and winter survivorship because of the significant $L \times E$ interaction. A common regression is shown for summer and winter fertility (C) since there was no significant effect of ENV or $L \times E$. Significance levels: * $P < 0.05$; *** $P < 0.001$; NS = not significant ($P > 0.05$).

stressful environment, that correlated directly with latitude (Eq. 1), we used the uncorrected latitude of origin; for the winter environment, we used the equivalent latitude (Eq. 3) corrected for the effect of longitude on mean minimum January temperature (Eq. 2).

Survivorship (Fig. 4A) declined with increasing latitude and there was a significant effect of both environment and latitude by environment interaction. A posteriori regression of survivorship on latitude within each environment showed a significant decline in survivorship with increasing latitude under stressful summer ($F_{1,43} = 16.64$, $P < 0.001$), but not stressful winter, environments ($F_{1,39} = 0.85$, $P = 0.361$). Fecundity (Fig. 4B) was not correlated with latitude in either environment. Fertility (Fig. 4C) declined with increasing latitude, but there was no significant effect of environment or environment by latitude interaction. R_0 was not correlated with latitude, and there was no significant effect

of environment or environment by latitude interaction (Fig. 4D).

DISCUSSION

The interpretation of the effects of the thermal environment on the adaptive evolution of *W. smithii* depends upon the measure of fitness examined. All of the significant correlations between mosquito performance and latitude are negative; survivorship in the summer environment (Fig. 4A) and fertility in both the summer and winter environments (Fig. 4C) decline with increasing latitude. One might have concluded that the declines in survivorship and fertility in the summer environment represent the loss of a costly physiological tolerance of summer heat extremes as summer heat declines with increasing latitude (Fig. 3). But, what benefit is gained by reducing this cost? As survivorship declines in the summer environment, there is no con-

comitant increase in winter survivorship (Fig. 4A), and as fertility declines in the summer environment, it also declines, not increases, in the winter environment (Fig. 4C). Average allozyme heterozygosity in *W. smithii* declines with increasing latitude (Armbruster et al. 1998), and one might have concluded that the parallel decline in fertility in both summer and winter environments (Fig. 4C) represents a general decline in homeostasis as a result of lost heterozygosity. But, neither survivorship in the winter environment nor fecundity in either environment is higher in the more heterozygous southern populations. Survivorship, fecundity, and fertility in summer and winter environments do not, therefore, lead consistently to the conclusion either that there are trade-offs between performance in the two environments, or that there are trade-offs among these measures of fitness.

The most robust prediction is not that performance in either environment should be correlated with latitude, but that the difference in performance in the summer and winter environments should diverge as summer heat declines, and, concomitantly, the intensity of winter cold increases. By the use of latitude for mean July maximum and equivalent latitude for mean January minimum (Fig. 3), we have a latitudinal scale over which the regression of performance in the summer and winter environments should diverge, i.e., over which we expect a significant latitude by environment interaction. We observe a significant latitude by environment effect only in survivorship (Fig. 4A). This interaction results from a decline in performance in the summer environment with no concomitant change in performance in the winter environment, and with no discernable benefit in the performance of fecundity or fertility. Selection does not operate on survivorship, fecundity, or fertility in isolation; rather, selection acts by optimizing the combination of these traits that results in the greatest fitness. When survivorship, fecundity, and fertility are combined into a single, composite index of fitness, any correlation with latitude disappears (Fig. 4D). In fact, there is less geographic divergence between summer and winter performance in R_0 (lower L \times E effect in Table 1) than in any of its components. We, therefore, conclude that there is no compelling evidence that summer heat and winter cold have been important selective forces in the adaptive evolution of *W. smithii*.

The lack of perceivable adaptation by *W. smithii* to summer heat or winter cold is not unprecedented. In the laboratory, populations of *Drosophila melanogaster* undergoing selection in warm or cool conditions are capable of diverging rapidly in their physiological response to temperature (Huey et al. 1991, Partridge et al. 1995). In nature, by contrast, the clear patterns of evolved differences in thermal responses occur primarily among species or genera (Brattstrom 1968, Parsons 1981, Hochachka and Somero 1984, Huey and Bennett 1987, van Berkum 1986, 1988), and are usually

weak or completely absent among populations within species occupying even a broad climatic range (Hertz et al. 1983, Crowley 1985, Tauber et al. 1987, van Berkum 1988, Lynch 1992, Delpuech et al. 1995). Regardless of whether the lack of variation in thermal response within species is due to low genetic variation or to weak selection, adaptive modification of thermal response appears to be a slowly evolving process in nature.

In addition to the immediate influence of hot summer or cold winter temperatures, the thermal environment at temperate latitudes also includes a strong seasonal component. The decrease in summer temperature and the increase in the duration and intensity of winter cold with increasing latitude also result in a decline in the length of the favorable or growing season. 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 events; developing long enough to maximize the opportunities of summer, but entering dormancy soon enough to avoid the exigencies of winter (Levins 1969, Cohen 1970, Taylor 1980). Many plants and animals use day length to cue the seasonal events in their life histories (Withrow 1959, Anonymous 1960, Aschoff 1965, Menaker 1971, Dingle 1996). Among a wide variety of arthropods, the median or critical photoperiod cueing seasonal development increases with latitude, inducing dormancy at an earlier date at higher latitudes; and, thereby, providing an apparent adaptation to the latitudinal gradient in the length of the growing season. In marked contrast to thermal responses, photoperiodic responses show a clear correlation with latitude within, as well as between, species (Danilevskii 1965, Saunders 1982, Taylor and Spalding 1986, Tauber et al. 1986, Danks 1987), including *W. smithii* (Bradshaw 1976, Bradshaw and Lounibos 1977, Lair et al. 1997).

Within species that show latitudinal variation in both thermal and photoperiodic response, that of photoperiodic response tracks the climatic gradient more closely. Temperate populations of the Asian tiger mosquito, *Aedes albopictus*, are more cold hardy than tropical populations, but temperate populations that do not differ in cold hardiness still show a latitudinal cline in photoperiodic response (Hawley et al. 1987, Fochs et al. 1994, Hanson and Craig 1994, 1995, Hanson 1995). In temperate Japan, diapausing *Drosophila auraria* and *D. triauraria* are more cold tolerant than nondiapausing flies, but there is no clear correlation between cold hardiness and latitude; however, both species show a clear latitudinal cline in photoperiodic response (Kimura 1984, 1988). In addition, at extreme high latitudes or altitudes, where summer warmth is not sufficient to complete a generation in a single season, insects may spread their development over two or more years, diapausing twice or more during an individual lifetime (Corbet 1956, Tauber et al. 1986, Danks 1987, Wipking

and Mengelkoch 1994). These patterns suggest that it is the timing of development and dormancy in concert with the locally changing seasons that presents the most immediate impediment to range expansion into or within the temperate zone, not limitations by summer heat or winter cold. We then propose that the thermal responses, which are often invoked to explain range distributions of species and genera, are actually secondary adaptations that take place after populations are well established and have evolved harmony with the local seasonal cycle.

The study of adaptation often has meant a post hoc rationalization for the adaptive significance of particular structures or physiological processes. The trait is then "demonstrated" to be adaptive by showing parallel variation among several species over spatial or temporal gradients of the putative selective factor (Levins 1968). Such parallel variation is still only correlative and does not necessarily identify causation (Gould and Lewontin 1979). The resulting "adaptive" scenarios derive their appeal from their inherent plausibility, rather than from rigorous testing of a priori hypotheses (Huey and Berrigan 1996). We have provided evidence for a noncorrelation between a composite index of fitness, R_0 , and latitude of origin in both stressful summer and stressful winter environments. We believe that these results exclude summer heat and winter cold as potent forces of selection in the range expansion of *W. smithii* in North America. We have proposed that photoperiodism constitutes the primary adaptive response of populations dispersing into novel seasonal environments. This proposition is supported by the close correlation between photoperiodic response and latitude in *W. smithii*, as well as in a large number of other arthropods. However appealing or plausible, this correlation with latitude does not demonstrate causality. To our knowledge, no study, including any of our own, has tested directly the hypothesis that photoperiodically timed seasonal development, independent of direct adaptation to summer heat or winter cold, has been the essential adaptation that has enabled the range expansion of a population into the habitat where we currently find it.

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