

GEOGRAPHIC VARIATION IN TOLERANCE OF TRANSIENT THERMAL STRESS IN THE MOSQUITO *WYEOMYIA SMITHII*

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Abstract. Environmental temperature can modify not only rates of ectotherm growth, development, and reproduction, but also, at the extremes, temperature can limit survivorship and ultimately fitness. We studied ectotherm populations from a latitudinal gradient to understand how alterations in the thermal environment (e.g., rapid climate change) may affect the persistence of populations experiencing stress-induced mortality. Populations of the mosquito, *Wyeomyia smithii*, from 30–50° N in North America were subjected to heat and cold stress based on observed field temperatures during the simulated passage of a typical 4–5 d warm- or cold-weather front. Cold but not heat tolerance corresponded to latitude of origin, reflecting previously observed patterns in year-long fitness. Both heat and cold stress resulted in an average of 44% mortality relative to unstressed controls but did not result in a significant loss of fitness (R_0) at the population level. We conclude that individuals most likely to survive during periods of stress are also the individuals most likely to make the greatest contribution to the next generation in the absence of stress. Since individual survivorship must, at some level, become limiting to population viability, these results imply that apparently viable populations may be pushed rapidly to extinction by a small incremental in environmental stress.

Key words: biogeography; climate-change implications; cold stress; cold tolerance; effective population size; fitness; heat stress; heat tolerance; latitudinal variation; pitcher-plant mosquito.

INTRODUCTION

Environmental temperature can modify not only rates of ectotherm growth, development, and reproduction (Sinervo and Adolph 1994, Gilchrist and Huey 2001, Irwin and Lee 2003), but also, at the extremes, temperature can limit survivorship and, ultimately, fitness (Huey and Kingsolver 1989, Lenski and Bennett 1993, Huey and Berrigan 2001). Over latitudinal and altitudinal gradients one therefore expects to find increasing cold tolerance as one proceeds northward or upward and increasing heat tolerance southward or downward. This expectation is borne out by examples both between species (Brattstrom 1968, Parsons 1981, Hochachka and Somero 1984, Huey and Bennett 1987, Gibert et al. 2001) and between populations within species (Dahlgaard et al. 2001, Hoffmann et al. 2001, 2002, Bradshaw et al. 2004). The question then remains as to the population consequences of individual mortality as a component of fitness. First, since survivor-

ship is a direct component of fitness, fitness would be expected to decline with increasing individual mortality. Second, there may be latent adverse effects of thermal environment on subsequent stages of the life cycle (Tauber et al. 1986, Danks 1987, Leather et al. 1993, Bradshaw et al. 1998, Zani et al. 2005) or even future generations (Huey et al. 1995, Chang et al. 1996, Crill et al. 1996, Magiafoglou and Hoffmann 2003), so that fitness is affected disproportionately to the direct effects of thermal stress on immediate survivorship. Third, there may be latent compensating effects of thermal stress in subsequent stages of the life cycle so that fitness is little affected by individual mortality (Bradshaw et al. 2000; see also Irwin and Lee 2000, 2003). Herein, we examine the fitness consequences of heat- and cold-induced mortality of the pitcher-plant mosquito, *Wyeomyia smithii*, from different latitudes.

Wyeomyia smithii completes its pre-adult development only within the water-filled leaves of the purple pitcher plant, *Sarracenia purpurea*. The range of the mosquito follows that of its host plant from the Gulf Coast to Canada. Populations complete one (north) to many (south) generations per year but all populations overwinter in a larval diapause that is initiated, maintained, and terminated by photoperiod (Bradshaw and Lounibos 1977). The eggs, larvae, and pupae are captive within leaf water that rarely exceeds 50 mL in volume and exhibits large daily and seasonal fluctuations in temperature.

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Leaves in a Florida savannah (31° N) experience a highly fluctuating thermal environment in both summer and winter. Summer maxima occasionally exceed 50°C and regularly exceed 41°C; winter minima occasionally fall to -7°C, but in parts of a savannah, never fall below 0°C. Leaves in a northern Wisconsin (46° N) bog experience a highly fluctuating thermal environment in the summer and a relatively stable thermal environment in the winter. Summer maxima only occasionally exceed 41°C; winter minima beneath the snow maintain steady temperatures below 0°C, occasionally fall below -10°C, but, in most parts of the bog, minima rarely fall below -3°C (Bradshaw et al. 2004). In this paper we report on experiments in which we exposed populations of *W. smithii* from 30–50° N to transient heat or cold stress based on observed field temperatures and designed to simulate the passage of a warm- or cold-weather front passing over a given locality. We determined the fitness consequences of heat- or cold-induced mortality imposed on larvae and pupae. We discuss the implications of our results in the context of the persistence of populations experiencing stress-induced mortality.

MATERIALS AND METHODS

Collection and maintenance

We collected *Wyeomyia smithii* from three geographic regions: a southern region along the Gulf of Mexico in Florida and Alabama (30–31° N), an intermediate region from Maryland and New Jersey (38–40° N), and a northern region (46–50° N) from Newfoundland to Ontario (Fig. 1). These localities correspond to localities CI, CR, LI, WI; HV, MM, NP, PB; and DL, GM, KC, ML, respectively, of previous publications from this laboratory (e.g., Bradshaw et al. 1998, 2000, 2004). These populations spanned 95% of *W. smithii*'s longitudinal range in the southern and intermediate regions and over 2500 km in the northern region. Animals were synchronized by rearing larvae on diapause-promoting, short-day photoperiod (L:D = 8:16) at 21 ± 0.5°C. To create the experimental generation, >2000 larvae per population were reared on diapause-terminating long days (L:D = 18:6) at fluctuating thermoperiod ranging from 15° to 30°C. Larvae were fed ad libitum a 4:1 by volume suspension of ground guinea-pig chow and freeze-dried brine shrimp every 5–7 d. Adults were maintained in a 3.8-L cage and provided with a leaf of *S. purpurea* for oviposition and pesticide-free raisins as a carbohydrate source. Throughout their range, *W. smithii* produce at least the initial large clutch of eggs without blood feeding.

Experimental conditions

Duration of exposure to stress.—In eastern North America, a typical weather front requires 3–7 days to transit a given locality (Landsberg et al. 1959). To mimic the occurrence of extreme weather events, we there-

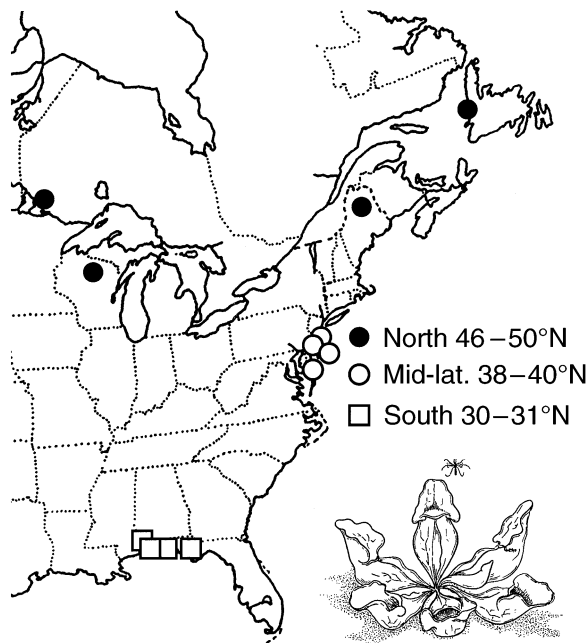


FIG. 1. Map of eastern North America, showing collecting localities, and *Wyeomyia smithii* about to oviposit in *Sarracenia purpurea*.

fore imposed thermal stress for a four-day period. This simulated the transient nature of extreme conditions that might be imposed by a passing weather front.

Fitness and tolerance.—Fitness was measured as R_0 = per capita expectation of future offspring. We used R_0 rather than r_c (capacity for increase = $R_0 \div$ mean generation time) because (1) R_0 serves as a more consistent index of fitness when comparing treatment effects where generation times differ by weeks (heat stress) vs. months (cold stress) (Bradshaw et al. 1998, 2000) and (2) it is not subject to the direct effect of temperature on generation time (Huey and Berrigan 2001). We used the simple concept of tolerance (e.g., Shelford 1913:303) as the “relative capacity of an organism to grow or thrive when subjected to an unfavorable environmental factor” (*Merriam-Webster Online Dictionary*)⁴ to calculate tolerance = (performance [survivorship or R_0] under stressful conditions) \div (performance under control conditions). Tolerance >1 indicates thermal adaptation; tolerance = 1 indicates full accommodation to stress; tolerance <1 indicates susceptibility to stress.

Heat stress.—From each of the 12 stock populations, we started 16 cohorts of 35 larvae/cohort (eight control and eight experimental) under diapause-averting long days (L:D = 18:6) and a near-optimal daily sine-wave thermoperiod that fluctuated from 15° to 30°C and lagged the light cycle by 2 h. Control cohorts remained under these conditions for their entire lifetimes. Ex-

⁴ (<http://www.m-w.com>)

perimental cohorts were maintained under these conditions until the first larva in a cohort pupated. At that time, the larvae and pupae were transferred for four days to insulated boxes that increased mid-afternoon temperatures over a 4-h period. We chose to increase mid-afternoon temperatures only because stressful temperatures occur during the hottest portion of the day, not over all 24 hours (Bradshaw 1980, Bradshaw et al. 2000). Preliminary experiments indicated that 41°C is near the upper lethal limit for this species. Therefore, mid-afternoon temperatures in the insulated boxes were raised to a maximum of 41°C on each of the four days of thermal stress. Additional details for the imposition of heat stress are provided in Zani et al. (2005). Surviving larvae and pupae were removed from the boxes after the heat treatment on the 4th day, and remained at the near-optimal temperature cycle for the remainder of their lives. Pupae from all cohorts were pooled into a single adult cage for each population so that survivorship to adulthood and the sex of the emerging adults could be determined from the pupal exuviae. Adults were allowed to mate en masse. Eggs were collected 3 times per week and number of hatched eggs counted after five days. For each population, we calculated survivorship = (total number of adults eclosing) ÷ (number of larvae and pupae in the population on the day the first larva in each cohort had pupated), and R_0 = (total number of F_1 that hatched) ÷ (total number of larvae + pupae in the population on the day the first larva in each cohort had pupated).

Cold stress.—We started 16 cohorts of 35 larvae/cohort from each of the 12 populations (except for one southern population [CI], for which only six cohorts were available). Cohorts were reared from day of hatch under diapause-inducing short days (L:D = 11:13) and a daily sine-wave thermoperiod that lagged the light cycle by 4 h and fluctuated from 10° to 27°C at the beginning of the acclimatization. To simulate natural conditions during the fall, the daily maxima and minima decreased over a three-month period following a smooth sine function until nightly temperatures reached a minimum of 0°C (daily maximum at this time was 9°C). During this period larvae were fed until the mean daily temperature dropped below 12°C, at which point feeding ceased in order to allow animals to clear their guts of possible ice-nucleating agents.

Once the daily minimum temperature reached 0°C, the 16 cohorts were assigned to one of two treatments using a six-sided die, resulting in 8 cohorts totaling 280 larvae per treatment. The first treatment served as the control where larvae experienced a constant 5°C; the second treatment imposed cold stress at a constant -3°C where the water but not the mosquitoes froze. The temperature for cold stress was chosen based on field observations (Paterson 1971) and pilot experiments confirming that this temperature was indeed near the lower lethal limit for this species. Following a day for the water in the cold-treatment dishes to equilibrate

to the sub-zero conditions, these cohorts were seeded with an ice crystal to ensure the water surrounding the larvae froze. All treatments were exposed to these conditions for four additional days and nights. Columns of dishes and dishes within those columns were rotated daily within each treatment to ensure that each dish experienced any variation of temperature within the 5°C or within the -3°C incubator. Following the cold treatment, dishes were transferred to the 5°C incubator (already containing controls) for five days. Both cold-treated and control larvae were then transferred to a 10°C incubator (L:D = 10:14) for four weeks of acclimation. Following this acclimation, animals were reared and pupae, adults, and eggs maintained on diapause-terminating long days (L:D = 18:6) with a fluctuating thermoperiod ranging from 15° to 30°C. For each population, we calculated survivorship = (total number of adults eclosing) ÷ (number of larvae in the population on the day experimental larvae were transferred to -3°C), and R_0 = (total number of F_1 that hatched) ÷ (total number of larvae in the population on the day experimental larvae were transferred to -3°C).

Analyses.—To improve normality, survivorship was arcsine square-root transformed and the per capita replacement rate was calculated as $\log(R_0 + 1)$ to account for zeros. Statistical analyses were conducted using SuperANOVA (Abacus Concepts 1989). When ANOVA indicated significant differences among means, a posteriori contrasts (comparisons of means) were used to test for differences between specific means and corrected for multiple comparisons using sequential Bonferroni.

RESULTS

Survivorship.—Average tolerance to stress was <1.0 for both heat (difference from 1.0 = 0.44 ± 0.05 (mean ± 2 SE); $t = 9.80$; $P < 0.001$) and cold (0.44 ± 0.18 ; $t = 4.88$; $P < 0.001$) treatments, i.e., both the heat and the cold treatments imposed a significant amount of stress, each resulting in 44% loss of survivorship. Tolerance was significantly affected by the geographic region of origin as well as the region \times temperature interaction, but not the main effect of temperature (Table 1A, Fig. 2A). Considered separately, heat tolerance was not affected by region of origin (Table 1B) while cold tolerance was (Table 1C). Southern populations were less tolerant of cold stress than either the mid-latitude or northern populations; there was no difference in cold tolerance between mid-latitude and northern populations (Table 1D).

In addition to expressing survivorship relative to the controls, we also compared survivorship of the controls separately. While survivorship of the controls was different between the temperature treatments it was not affected by region of origin or the treatment \times region interaction (Table 1E). This result underscores the need

TABLE 1. Results of ANOVAs for *Wyeomyia smithii* tolerance of heat (H) vs. cold (C) stress among three geographic regions (south, mid-latitude, north), with population (Pop) nested within region (R) or population nested within region and stress treatment (HC) as the error term.

ANOVA	df	MS	F	P
A) Tolerance as measured by survivorship (Fig. 2A)				
Region	2	0.194	11.09	<0.001
Heat vs. cold	1	<0.001	0.003	0.956
R × HC	2	0.324	18.55	<0.001
Pop(R × HC)	18	0.017		
B) Heat tolerance as measured by survivorship				
Region	2	0.015	0.57	0.586
Pop(R)	9	0.026		
C) Cold tolerance as measured by survivorship				
Region	2	0.502	57.16	<0.001
Pop(R)	9	0.009		
D) Cold tolerance as measured by survivorship: a posteriori contrasts				
South vs. mid-lat.	1	0.681	77.44	<0.001
South vs. North	1	0.820	93.29	<0.001
Mid-lat. vs. North	1	0.006	0.74	0.413
E) Survivorship among controls only				
Region	2	27.4	0.60	0.560
Heat vs. cold	1	1388.8	30.40	<0.001
R × HC	2	21.1	0.46	0.639
Pop(R × HC)	18	45.7		
F) Tolerance as measured by fitness, R_0 (Fig. 2B)				
Region	2	<0.001	0.002	0.998
Heat vs. cold	1	0.023	0.170	0.690
R × HC	2	0.219	1.58	0.234
Pop(R × HC)	18	0.135		
G) R_0 among controls only				
Region	2	0.067	4.75	0.022
Heat vs. cold	1	2.472	176.56	<0.001
R × HC	2	0.182	12.88	<0.001
Pop(R × HC)	18	0.014		

to incorporate the controls in our index of tolerance as we did.

Fitness (R_0).—Average tolerance did not differ from 1.0 for both heat (difference from 1.0 = 0.09 ± 0.11 [mean ± 2 SE]; $t = 1.55$; $P = 0.148$) and cold (-0.02 ± 0.25 ; $t = 0.17$; $P = 0.865$), i.e., neither the heat nor the cold treatments significantly affected fitness. Tolerance as measured by R_0 was not significantly affected by the geographic region of origin, temperature treatment, or their interaction (Table 1F, Fig. 2B).

In addition to expressing R_0 relative to the controls, we also compared R_0 of the controls separately. Fitness of the controls differed among regions of origin, between temperature treatments, and treatment \times region interaction (Table 1G). This result is consistent with our previous results (Bradshaw et al. 2004) and also underscores the need to incorporate the controls in our index of tolerance as we did.

R_0 vs. survivorship.—For both heat and cold treatments, tolerance as measured by R_0 was greater than tolerance as measured by survivorship (t test for paired comparisons with $df = 11$, difference [mean ± 2 SE]: heat = 0.35 ± 0.07 ; $t = 9.91$; $P < 0.001$; cold = 0.42 ± 0.30 ; $t = 2.77$; $P = 0.018$).

DISCUSSION

Among species of temperate ectotherms, upper thermal limits generally vary less over latitudinal gradients than do lower thermal limits (Vannier 1994, Lutterschmidt and Hutchison 1997, Goto and Kimura 1998, Gaston and Chown 1999, Addo-Bediako et al. 2000). Similarly in *Wyeomyia smithii*, latitude of origin had a greater effect on cold than heat tolerance as measured by survivorship (Fig. 2A). One reason for this differential tolerance to heat and cold stress across latitudinal gradients may be that at temperate latitudes, mid-summer maxima vary less with latitude than do mid-winter minima (Bradshaw et al. 2004). Consequently, it is to be expected that with increasing latitude, animals would gain cold tolerance faster than they would lose heat tolerance. In an earlier study with these same populations of *W. smithii*, Bradshaw et al. (2004) found geographic differences in both heat and cold tolerance. A simulated northern winter was lethal to southern but not intermediate or northern populations in the single overwintering generation. A simulated southern summer imposed a greater loss of fitness in northern than in intermediate or southern populations; however, sev-

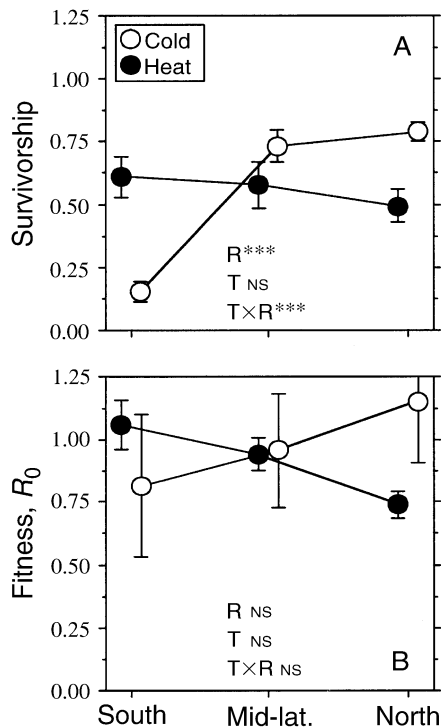


FIG. 2. Regional variation in heat and cold tolerance. Regional mean (± 1 SE) of *Wyeomyia smithii* populations is shown for ratio of the response to the thermal stress treatment relative to the respective control from southern, mid-latitude, and northern regions (Fig. 1): (A) survivorship to pupation; (B) per capita expectation of future offspring (R_0). Results of the Table 1 ANOVA are shown: R, region; T, treatment (heat, cold); T \times R, treatment by region interaction. Levels of significance are: *** $P < 0.001$; NS, $P > 0.05$.

eral generations in the southern summer were required before the adverse effects of heat stress became apparent on rates of pupal and egg production in northern populations. The results in Fig. 2 are in accord with these prior results.

Herein, for each temperature treatment, tolerance as measured by R_0 (per capita expectation of offspring) was greater than tolerance as measured by survivorship. Despite a 44% decline in survivorship in both treatments, fitness as measured by R_0 was not affected by thermal stress in either treatment. It would therefore appear that the individuals most likely to survive during periods of stress are also the individuals most likely to make the greatest contribution to the next generation in the absence of such stress. While we do not have direct evidence that survivors have higher reproductive output than those that died, variation in reproductive success among individuals in a population can reduce effective population size to a fraction of the census population size (Roff 1997: Table 8.4). Our results indicate that, all other ecological factors being equal, transient heat or cold stress having a large effect on

the census population size may be having a minimal impact on the effective population size.

Our results should not be biased by the reduced oviposition or embryonic survivorship at higher adult densities. First, at high cage densities females will continue to seek out and oviposit in the youngest available leaf, regardless of the number of eggs already in that leaf (Bradshaw 1983). Second, at a fixed level of per capita larval resources the number of hatching first instars per eclosed female is not significantly correlated with adult density (W. E. Bradshaw and C. M. Holzapfel, *personal observations*: 10–30 adults per 0.95-L cage; $r = +0.34$, $n = 15$ replicates, $P = 0.213$). Had there been density-dependent oviposition or embryonic viability, the correlation should have been negative, not nonsignificant and positive. If anything, an Allee effect should have biased our experiments against the result we obtained.

As survivorship approaches zero, it necessarily becomes limiting to fitness, regardless of subsequent potential fecundity and embryonic viability. During simulated winters among populations from 30–50° N, minimal temperatures of $0 \pm 2^\circ\text{C}$ for two weeks revealed no geographic variation in either survivorship or R_0 in *W. smithii* (Bradshaw et al. 2000), but minimal temperatures of -3°C for several months resulted in 100% mortality and extinction of southern but not mid-latitude or northern populations (Bradshaw et al. 2004). Transient minimum temperatures of -3°C for five days showed an intermediate pattern: relative to the untreated controls, southern populations exposed to the cold treatment suffered an average of 85% mortality but no significant decline in fitness (Fig. 2). From these observations, we conclude that apparently viable populations achieving positive fitness when confronted with thermal stress may nonetheless be teetering on the edge of extinction. The implication is that when confronted with climate change, populations may make a rapid transition from viability to extinction over a few degrees of temperature change.

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