

Reproductive value in a complex life cycle: heat tolerance of the pitcher-plant mosquito, *Wyeomyia smithii*

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Abstract

Because mortality accumulates with age, Fisher proposed that the strength of selection acting on survival should increase from birth up to the age of first reproduction. Hamilton later theorized that the strength of selection acting on survival should not change from birth to age at first reproduction. As organisms in nature do not live in uniform environments but, rather, experience periodic stress, we hypothesized that resistance to environmental stress should increase (Fisher) or remain constant (Hamilton) from birth to age at first reproduction. Using the pitcher-plant mosquito, *Wyeomyia smithii*, we imposed heat stress by simulating the passage of a warm-weather front at different preadult and adult stages. Contrary to either Fisher or Hamilton, stress tolerance declined from embryos to larvae to pupae to adults. Consequently, reproductive value appears to have been of little consequence in the evolution of stage-specific tolerance of heat stress in *W. smithii*.

Introduction

The age-specific changes of an individual's reproductive value (its expected contribution to the ancestry of future generations) have long been a topic of interest (Fisher, 1930; Roff, 2002). According to Fisher (1930, p. 27), as each stage of the life cycle experiences some mortality, a surviving individual's expected contribution to future generations, its reproductive value, should increase with age and, 'the direct action of Natural Selection must be in proportion to this contribution.' Consequently, selection acting on survivorship should increase from birth to age at first reproduction. According to Hamilton (1966), the sensitivity of fitness (Malthusian parameter, r) to age-specific changes in the probability of survivorship should not change from birth to age at first reproduction, but should decline thereafter. Hamilton noted first that the probability of surviving until the age at first reproduction equals the cumulative probability of surviving through each previous age and second that the contribution to fitness by improved survivorship in any prereproductive

stage is not discounted by its specific fecundity. Consequently, any increment in survivorship should have an equal effect on survivorship to age at first reproduction, and hence fitness, regardless of the specific prereproductive age at which it occurs. Despite subsequent theoretical considerations of the relationship between reproductive value and fitness (Schaffer, 1979, 1981; Caswell, 1980, 1982a,b; Ricklefs, 1981; Yodzis, 1981; Charlesworth, 2000) empirical examples have focused on documenting age-related differences in the fitness of individuals and tend to be confounded by microhabitat differences between life stages (Landwer, 1994; Newton & Rothery, 1997; Dudycha & Tessier, 1999; Brommer *et al.*, 2000; Ghalambor & Martin, 2000; Robertson & Rendell, 2001). In this study, we ask whether stages with higher reproductive values are more resistant to environmental stress than are stages with lower reproductive value, in the mosquito, *Wyeomyia smithii*. *Wyeomyia smithii* completes its entire preadult development in a single microhabitat, the water-filled leaves of the purple pitcher plant, *Sarracenia purpurea*.

Wyeomyia smithii has a typical complex life cycle of a holometabolous insect. Complex life cycles are those that involve two or more distinctive phases. In many amphibians and in most holometabolous insects, metamorphosis is accompanied not only by a shifting emphasis from

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growth and development to reproduction, but also by a change in habitat, in trophic affiliation, and in the ability behaviourally to avoid ecological exigencies. This separation of the life cycle into distinctive phases allows for the independent optimization of different components of fitness at different stages of the life cycle (Istock, 1967; Wilbur, 1980; Moran, 1994), including tolerance of thermal stress (Tucić, 1979; Krebs & Loeschke, 1995a,b; Loeschke & Krebs, 1996; Hercus *et al.*, 2000). The life cycle of *W. smithii* is cleanly divided into preadult and adult habitats. The eggs, larvae, and pupae are all restricted to bodies of water that rarely contain more than 50 mL of water and wherein extreme daily temperatures can exceed 40 °C as far north as northern Wisconsin (46°N) (Bradshaw *et al.*, 2004). The adults emerge from the leaves and live a mobile existence in the bogs, swamps, and savannas where pitcher plants grow. Exposure for 10 generations of *W. smithii* to chronic, life-long heat stress sufficient to drive half the experimental populations to extinction results in increased tolerance of acute heat stress in both larvae and adults (Armbruster *et al.*, 1999). Hence, both larvae and adults possess the genetic capacity to evolve increased tolerance to acute heat stress. We therefore use *W. smithii* to test predictions from life-history theory and from the theory of complex life cycles. First, we predict that within the preadult portion of the life cycle, heat tolerance should increase from embryos to larvae to pupae (Fisher) or remain uniform from embryos to larvae to pupae (Hamilton). Second, we predict that heat tolerance of the captive preadult stages should be greater than heat tolerance of the mobile adults. As a measure of performance, we used the per capita expectation of future offspring by maintaining cohorts from the time of oviposition, through exposure to a simulated warm weather front at different stages of the life cycle, and subsequently, to the hatch of their offspring.

Material and methods

We collected approximately 2000 *W. smithii* larvae from the New Jersey Pine Barrens (PB: 40°N, 74°W, 10 m elevation) and reared them through six generations prior to the start of the experiments, as described in Hard *et al.* (1992).

Our basic approach was to maintain a large outbreeding colony of mosquitoes in a 3.8 L cage in order to generate the experimental cohorts of different stages for simultaneous treatment. All mosquitoes were reared from day of oviposition in 150 × 25 mm dishes with 50–75 mL distilled water 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 4 h. At the start of the experiment, cohorts of 30–35 equal-aged individuals were drawn from the outbreeding colony. Cohorts of eggs were assigned to control ($n = 4$) or treatment

($n = 3$) groups by rolling dice. Cohorts of eggs, larvae ($n = 3 \times 4$ instars), or pupae ($n = 3$) were transferred to 100 × 25 mm dishes with 35 mL of distilled water and exposed to heat stress. Cohorts of adults ($n = 3$) were placed in 0.95 L adult cages and exposed to heat stress. As stressful temperatures in pitcher-plant leaves occur during the hottest portion of the day, not over all 24 h (Bradshaw, 1980; Bradshaw *et al.*, 2000), experimental cohorts were subjected to increased mid-afternoon temperatures (highs ~40 °C). Controls were maintained at previous conditions (highs ~30 °C; see Fig. 1). These conditions were continued for four consecutive days to simulate the passage of a warm weather front, which, in eastern North America, normally requires 3–7 days to transit a given locality (Landsberg *et al.*, 1959).

Elevated experimental temperatures were achieved by using insulated boxes (20.5 L inside volume, able to contain 20 100 × 25 mm dishes) placed in the same room as the controls. Each box was equipped with 7.5 × 12.5 cm windows in each side and the top to transmit light and received air from an external blower to maintain ambient room temperature within the box. At the start of the each day's heat treatment, the external blowers were turned off and an internal blower (to circulate air and minimize hot spots within the box) plus a 25 W heater was turned on. Warming up and cooling down each required over an hour and maximum temperature was regulated at 40.5 ± 1.5 °C (± 1 SD) by a thermostat within each box. The daily maximum temperature within the boxes occurred at the same time of day as maximum temperature within the room.

Dishes were assigned a random initial position by rolling dice and each day rotated through the four experimental boxes in alternating columns (i.e. front, back) and shifting positions in the stack (e.g. 1, 3, 5, 7 or 2, 4, 6, 8). This rotation compensated for possible variations in temperature within and among the replicate experimental chambers. When the number of experimental cohorts was less than the capacity of the

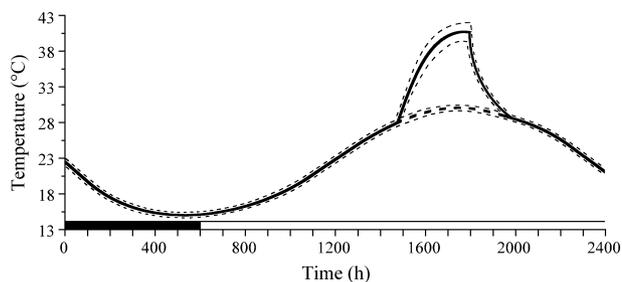


Fig. 1 Thermal conditions. Daily thermoperiod (± 1 SD; thin dashed lines) experienced by heat-treated (thick solid line) and control (thick dashed line) cohorts. Dark box along horizontal axis indicates 6 h night.

box, the unused dishes were filled with 35 mL distilled water to provide a consistent buffer against heating and cooling among boxes, stacks within a box, and treatment times. The top and the bottom dishes of each column were not used, so as to provide an additional buffer against temperature fluctuations. For the box with adult cages, dishes with 35 mL of distilled water were placed above and beneath each adult cage to maintain a constant thermal mass in each box. After 4 days of treatment, surviving control and experimental animals were returned to the larger dishes with food and remained at the near-optimal temperature cycle (Fig. 1) for the remainder of their lives. All pupae were placed into 0.95 L adult cages so that survivorship to adulthood and the sex of the emerging adults could be determined from the pupal exuviae. Adults within each cohort were allowed to mate en masse, except for the controls, in which adults from all four replicates were allowed to mate en masse. Eggs were collected three times per week and hatching was counted 5 days thereafter. For each cohort, the per capita expectation of future offspring (R_0) was calculated as the number of offspring eventually produced by each cohort per individual present at the beginning of the heat treatment in that cohort. R_0 was chosen as an index of fitness as it integrates across the life cycle to incorporate both the immediate and latent effects of heat stress. It is important to consider the composite index of fitness as well as its components as qualitative results may differ among such indices (Fisher *et al.*, 1990; Bradshaw *et al.*, 2000).

To test whether the preadult stages were, in aggregate, more heat resistant than the adults, we calculated a stage-weighted index of R_0 from egg through larval and pupal stages:

$$R_0(\text{ELP}) = \sum R_{0,i}p_i,$$

where $R_{0,i}$ is R_0 achieved after exposure to heat stress at stage i (embryo, instars 1–4, and pupa) and p_i is the density-independent probability of being in the i th stage at the time of a warm-weather front's passage (using data from Bradshaw & Lounibos, 1972): $p_i = (\text{mean duration of the } i\text{th stage})/(\text{total time from oviposition to adult eclosion})$.

To improve normality, survivorship and embryonic viability were arcsine square-root transformed, fecundity was square-root transformed, and R_0 was calculated as $\log_{10}(R_0 + 1)$ to correct for cohorts with zero fitness. Statistical analyses were conducted using SuperANOVA (Abacus Concepts Inc., 1989). When a one-factor ANOVA, with life stage (control, egg, larvae, pupae, or adult) as the factor, indicated significant differences among means, *a posteriori* contrasts (comparison of means) were used to test for differences between specific means using a sequential Bonferroni correction to account for multiple comparisons.

Results

Although we tested for the effects of heat stress on each of the four larval instars separately, there were no significant differences in R_0 , survivorship, fecundity, or embryonic viability between any of the instars ($P > 0.25$). Hence, we combined the instars into one larval stage for subsequent analyses by averaging the mean performance of each of the four larval instars.

R_0

Per capita replacement rate was lower among mosquitoes stressed as pupae or adults than as embryos or larvae ($F_{4,17} = 13.61$; $P < 0.001$; Fig. 2a). In comparison with the control, R_0 of neither embryos nor larvae differed from unstressed animals (embryos: $F_{1,17} = 0.19$, $P = 0.666$; larvae: $F_{1,17} = 2.71$, $P = 0.118$). The stage-weighted R_0 of cohorts experiencing heat stress as embryos, larvae, or pupae (mean \pm SE: $R_0(\text{ELP}) = 5.71 \pm 1.516$) was higher than R_0 of cohorts experiencing the heat stress as adults (0.00 ± 0.000 ; $t = 3.76$; $P < 0.013$). $R_0(\text{ELP})$ was also greater than 1.0 ($t = 3.10$; $P = 0.027$), meaning that, in aggregate, *W. smithii* experiencing the warm-weather front as preadults were still able to maintain positive fitness.

Survivorship

Survivorship tended to decrease with advancing stage of exposure to stress ($F_{3,17} = 15.60$; $P < 0.001$; Fig. 2b). Survivorship was higher in the controls and in cohorts exposed to heat stress as embryos than in cohorts stressed as larvae ($F_{1,17} = 21.57$; $P < 0.001$), but survivorship in stressed larvae was higher than in stressed pupae or adults ($F_{1,17} = 27.81$; $P < 0.001$).

Fecundity

Because the heat treatment was lethal to all adults, we could not determine fecundity or viability of their subsequent embryos. Excluding adults, fecundity did not differ among the controls and mosquitoes stressed as embryos, larvae, or pupae ($F_{3,15} = 0.39$; $P = 0.759$; Fig. 2c).

Embryonic viability

Excluding adults, embryonic viability did not differ among the controls and mosquitoes stressed as embryos or larvae ($F_{2,13} = 0.42$; $P = 0.667$; Fig. 2d), but was lower in mosquitoes stressed as pupae ($F_{1,17} = 15.54$; $P < 0.001$).

Discussion

The passage of a simulated warm-weather front reduced fitness in *W. smithii* (Fig. 2a), due primarily to the effects

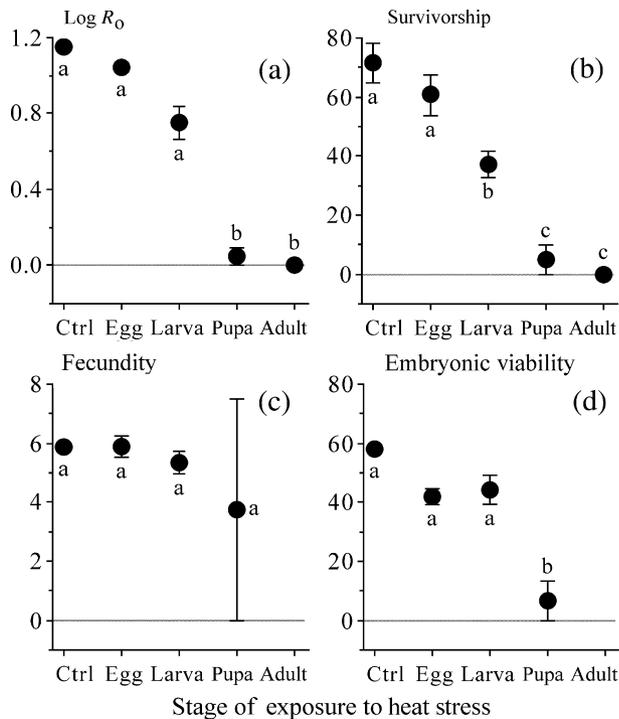


Fig. 2 Average performance (± 1 SE) of replicate *W. smithii* cohorts ($n = 3$ per life stage) after exposure to heat treatment or as unheated control ($n = 4$ cohorts). Average performance of each of four individual larval instars is averaged together as 'larva.' (a) R_0 is $\log(R_0 + 1)$ transformed (adults of control cohorts combined to produce single control cohort); (b) survivorship is arcsine square root transformed (for control, egg, and larva cohorts, survivorship is to pupation; for pupa, survivorship is to adulthood; for adults, survivorship is for 24 h post-heat stress); (c) fecundity is square root transformed; (d) embryonic viability is arcsine square root transformed. Mean values with the same lower case letter do not differ after sequential Bonferroni to correct for multiple comparisons.

of heat on survivorship (Fig. 2b). Heat tolerance declined from embryos to larvae to pupae. Heat stress during the pupal stage with the highest reproductive value not only imposed the highest immediate mortality (Fig. 2b) but also resulted in a subsequent loss of embryonic viability (2D). These results strongly negated our predictions that later preadult stages with a higher reproductive value should be either more resistant (Fisher, 1930) or equally resistant (Hamilton, 1966) to environmental stress. In the unique test with *W. smithii* where the consistency of microhabitat factors out differences in mobility and captivity among stages, reproductive value is of no consequence in determining stage-specific tolerance of heat stress.

Differences in thermal tolerance among stages of complex life cycles follow two nonexclusive patterns. First, thermal tolerance tends to be greater in the life stages that experience thermally more variable than thermally more stable environments. Second, thermal

tolerance tends to be greater in life stages that are sessile or are captive in a restricted habitat than in life stages that are mobile or live in an open habitat. Thermal tolerance in some animals combines both patterns. Both the newts, *Taricha rivularis* and *Notophthalmus viridescens* have aquatic larvae and terrestrial adults but *T. rivularis* develops in small, thermally variable bodies of water while *N. viridescens* develops in larger, thermally stable bodies of water. Thermal tolerance is greater in larval than adult *T. rivularis* (Licht & Brown, 1967) but the reverse holds for *N. viridescens* (Hutchison, 1961). Apart from *T. rivularis*, thermal tolerance among amphibians in general decreases with increasing mobility from embryos to small larvae to large larvae to adults (Atlas, 1935; Schechtman & Olson, 1941; Moore, 1942; Brown, 1967; Herreid & Kinney, 1967; Licht & Brown, 1967; Zweifel, 1968, 1977; Kuramoto, 1978; Duellman & Trueb, 1986: p. 124, 164; Sherman & Levitis, 2003). Thermal tolerance is greater in sessile, intertidal barnacles, *Balanus balanoides*, than in their planktonic larvae (Crisp & Ritz, 1967). Thermal tolerance in *Drosophila buzzatii* that develop in cactus rots is greater in relatively more mobile larvae than in either the immobile eggs or pupae (Krebs & Loeschcke, 1995a,b; Loeschcke & Krebs, 1996). Finally, thermal tolerance is greater, in aggregate, among the captive preadult stages of *W. smithii*, than among the mobile adults (Fig. 2a,b). We therefore conclude that individual mobility and habitat thermal stability have been more important than reproductive value in the evolution of stage-specific thermal tolerance of organisms with complex life cycles.

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