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FITNESS CONSEQUENCES OF HIBERNAL DIAPAUSE IN THE PITCHER-PLANT MOSQUITO, *WYEOMYIA SMITHII*

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Abstract. The mosquito, *Wyeomyia smithii*, develops only within the water-filled leaves of the purple pitcher plant, *Sarracenia purpurea*, where it overwinters in larval diapause. We reared uniform-aged cohorts in the leaves of intact plants in three environments: near-optimal summer conditions, intentionally stressful summer conditions, and a 23-wk simulated winter. Overwintering *W. smithii* suffered a 60% loss in fitness (R_0), comparable to the 68% loss in fitness imposed by the summer stress. Overwintering cohorts lost fitness through reduced survivorship, fecundity, fertility, adult longevity, and mass-specific fecundity. We argue that very real trade-offs occur between summer performance and winter performance among temperate insects in general. Survivorship and reproduction of the overwintering generation should therefore be an important source of selection shaping not only the seasonal expression of dormancy, but also the evolution of summer life-history syndromes as well.

Key words: diapause, fitness consequences; life history in a seasonal environment; overwintering; phenology; photoperiodism and seasonal development; pitcher-plant mosquito; seasonality; stress tolerance; trade-offs, life history; *Wyeomyia smithii*.

INTRODUCTION

At temperate latitudes, insects exploit the favorable seasons with active development and reproduction and avoid or endure the unfavorable season through dormancy (diapause) or migration, which provide “escape in time and space” (Slobodkin 1963). The concept of escape implies that insects effectively can avoid the exigencies of the unfavorable season and, when favorable conditions return, resume normal development. Yet, we know that migration involves not only risks to survivorship, but also allocation of resources to wings, flight muscles, and even thoracic space that otherwise might go to reproduction (Southwood 1977, Dingle 1984, Solbreck et al. 1990). Similarly, diapause may (or may not) involve allocation of resources to cold-hardiness or somatic maintenance and thus incur costs to survivorship and/or reproductive performance. In the few cases where it has been examined, post-diapause individuals usually exhibit reduced fecundity relative to non-diapausing individuals; however, in some insects that feed while in diapause, females can actually exhibit increased fecundity or can live longer as adults than non-diapausing females and compensate for energy loss during diapause by post-diapause feeding (Tauber et al. 1986:63–64, 266–271, Danks 1987:37–

44, Leather et al. 1993:148–173, Ishihara and Shimada 1995, Chang et al. 1996).

While previous studies have considered the effects of dormancy on single traits such as survivorship and fecundity that are clearly related to fitness, no one to our knowledge has yet considered how performance of individual traits relates to other potentially compensating traits or to more inclusive composite indices of fitness. Herein we consider the cost of diapause in terms of all of the components of a composite index of fitness, the overwintering cohort replacement rate (R_0) in the pitcher-plant mosquito, *Wyeomyia smithii* (Coq.). We compare fitness and its components among three environments: near-optimal summer conditions (“optimal”), conditions intentionally designed to impose severe environmental stress (“stressful”), and a 22-wk simulated winter (“winter”). We have specifically designed our experiments to mimic as closely as possible the natural environment likely to be encountered by *W. smithii* in nature, and we show that there is a substantive cost of overwintering in diapause. We then relate our findings to theoretical models for the optimal timing of diapause and to tradeoffs in life histories.

Wyeomyia smithii is distributed in North America from the Gulf of Mexico to Labrador and northeastern Saskatchewan (30–54° N). Throughout its range, this mosquito completes its preadult development only in the water-filled leaves of its carnivorous host, the pur-

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ple pitcher plant, *Sarracenia purpurea* L. The leaves of *S. purpurea* persist through the winter and *W. smithii* overwinter in these leaves in a larval diapause that is initiated, maintained, and terminated by photoperiod (Bradshaw and Lounibos 1977).

METHODS

Collection

We collected the mosquitoes (*Wyeomyia smithii*) in October 1993, as diapausing larvae from two localities ("MM" and "PB" of earlier publications from this laboratory, e.g., Bradshaw and Lounibos 1977, Bradshaw and Holzapfel 1992) at 40° N in the New Jersey Pine Barrens. The two localities were separated by 15 km. Within each locality, we identified three clusters of pitcher plants, 100–300 m apart. We collected 1500–2000 larvae from within each cluster and maintained them as separate populations. We reared each population on long days to adulthood and placed their offspring (F_1) on short days to induce diapause. After all populations were synchronized in diapause, we returned them to long days to induce development, reared them to adults, and used their offspring (F_2) in experiments.

Conditions common to all experiments

We run all of our experiments in the mosquito's natural habitat, the water-filled leaves of intact pitcher plants. In each environmental treatment, 40 larvae were reared from day of hatch in 30 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- (L:D = 17:7) or short-day (L:D = 8:16) photoperiod that included 0.5 h dim "twilight" at each end of the photophase.

Upon the start of pupation, leaves were checked 3 times per week and the pupae removed, weighed, and transferred to adult-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 *Sarracenia purpurea* for oviposition. The bottom of the cage was covered with adsorbent paper and this paper was soaked with distilled water 3 times per week. These cages were checked 3 times per week for adult eclosion, adult death, and eggs. The eggs were removed, placed in 75 mL distilled water, and the number of first-instar larvae hatching over a 10-d period was recorded. Fer-

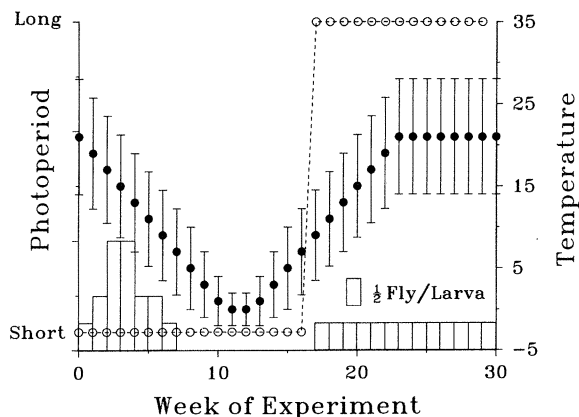


FIG. 1. Simulated winter. The solid circles and error bars show the mean and amplitude of daily temperature (°C) for each week of the experiment. The open circles show the seasonal imposition of short days (L:D = 8:16) that induce and maintain diapause and long days (L:D = 17:7) that terminate diapause and promote the resumption of development. The open vertical bars show the feeding regimen of whole adult *Drosophila*.

tility was then calculated as percentage hatch = [(number of larvae hatching from a cohort) ÷ (number of eggs produced by the cohort)]. Finally, we calculated cohort replacement rate,

$$R_0 = (\% \text{ larvae eclosing as adults}) \\ \times (\text{eggs per eclosed female}) \times (\% \text{ hatch}).$$

Simulated environments

Each of the environmental treatments was run in a different controlled-environment room. Near-optimal summer conditions are based on field observations. 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. We fed each cohort 50 flies on the day of hatch, and 200, 100, and 50 flies at successive weekly intervals, for a total of 10 flies per larva. 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 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. The thermoperiod ranged from 16 to 36°C each day (mean: 26°C), providing hotter temperatures for 10 h/d than the maximum daily temperature under optimal conditions. The relative humidity ranged from 60 to 80% (mean: 70%) each day with minimum humidity corresponding to maximum temperature. Each cohort was fed 3/4 the ration of food provided under optimal conditions: 38 flies on the day of hatch, and 150, 75,

and 38 flies at successive weekly intervals, for a total of 7.5 flies per larva.

To simulate winter 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 weekly to a "midwinter" low of 0°C with a daily fluctuation of 4°C (Fig. 1). These temperatures were no colder than overwintering larvae would encounter in New Jersey. They were cold enough to freeze the water but not the larvae in the leaves. 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 (week 15) at which time the photoperiod was increased to long days. Each cohort was fed 20 flies on the day of hatch and 40, 80, 80, 40, 40, and 20 flies at successive weekly intervals during the simulated autumn (Fig. 1). Larvae received no food from late fall in week 8 when mean daily temperature fell to 7°C until early spring in week 17 when mean daily temperature rose again to 9°C. Starting in week 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 week 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 cages maintained under "optimal" environmental conditions.

Statistical analysis

Our experimental design included 10 cohorts from each of three populations within each of two nearby localities. Preliminary nested ANOVA showed no significant effect of locality or population within locality on R_0 or any of its components. We therefore subjected R_0 and its component traits to Model II ANOVA with the three environmental conditions as fixed effects and the six populations as random effects, testing for significance of the environmental effects by

$$F_{2,10} = MS_{\text{Environment}} / MS_{\text{Population} \times \text{Environment}}$$

When significance ($P < 0.05$) was indicated by the ANOVA, we determined differences between individual means using Ryan's Q (Day and Quinn 1989).

RESULTS AND DISCUSSION

Performance under optimal conditions provides a measure of the fitness potential of *Wyeomyia smithii*. Performance under stressful conditions provides a measure of the loss of fitness when *W. smithii* experience

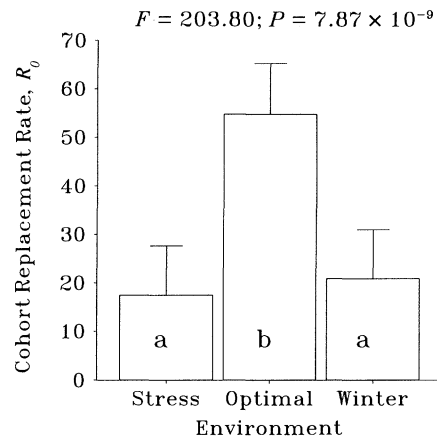


FIG. 2. Cohort replacement rate, R_0 , of *Wyeomyia smithii* experiencing optimal, stressful, and winter environments. F values from the ANOVA are listed with $df = 2, 10$ and the associated probability at the top of the graph. Error bars show mean + 2 SE; bars with the same lower-case letters in them do not differ ($P > 0.05$) in a posteriori multiple-comparison tests (Ryan's Q).

an environment that was intentionally designed to impose severe stress. The impact of overwintering on fitness can then be evaluated by comparing performance of *W. smithii* under winter conditions with performance under optimal and stressful conditions. The cohort replacement rate (R_0) was higher under optimal conditions than under stressful or overwintering conditions (Fig. 2). R_0 did not differ between the stressful and the overwintering conditions. These results show that overwintering *W. smithii* suffer a 60% loss in fitness, comparable to the 68% loss in fitness imposed by the stressful conditions.

Reduced R_0 reflected similar declines in performance in each of its constituent life-history traits (Fig. 3). Relative to optimal conditions, both overwintered and stressed cohorts experienced lower survivorship to adulthood (Fig. 3A), lower female fecundity (Fig. 3B), and lower egg fertility (Fig. 3C). Since fecundity of *W. smithii* scales with female size (Bradshaw and Holzapfel 1992), reduced fecundity might be due in part to reduced female mass (Fig. 3D). However, overwintered and stressed females also suffered reduced adult survivorship (Fig. 3F) and reduced mass-specific fecundity (Fig. 3E). The lower survivorship and fecundity under stressful conditions could be ascribed to the high temperature and low humidity encountered directly by adult females. Overwintered females, however, pupated during the late thermal spring during week 22 or later (Fig. 1) so that adult females encountered the same temperature and humidity conditions as did adult females in the optimal environment. Lower mass-specific fecundity and longevity of the

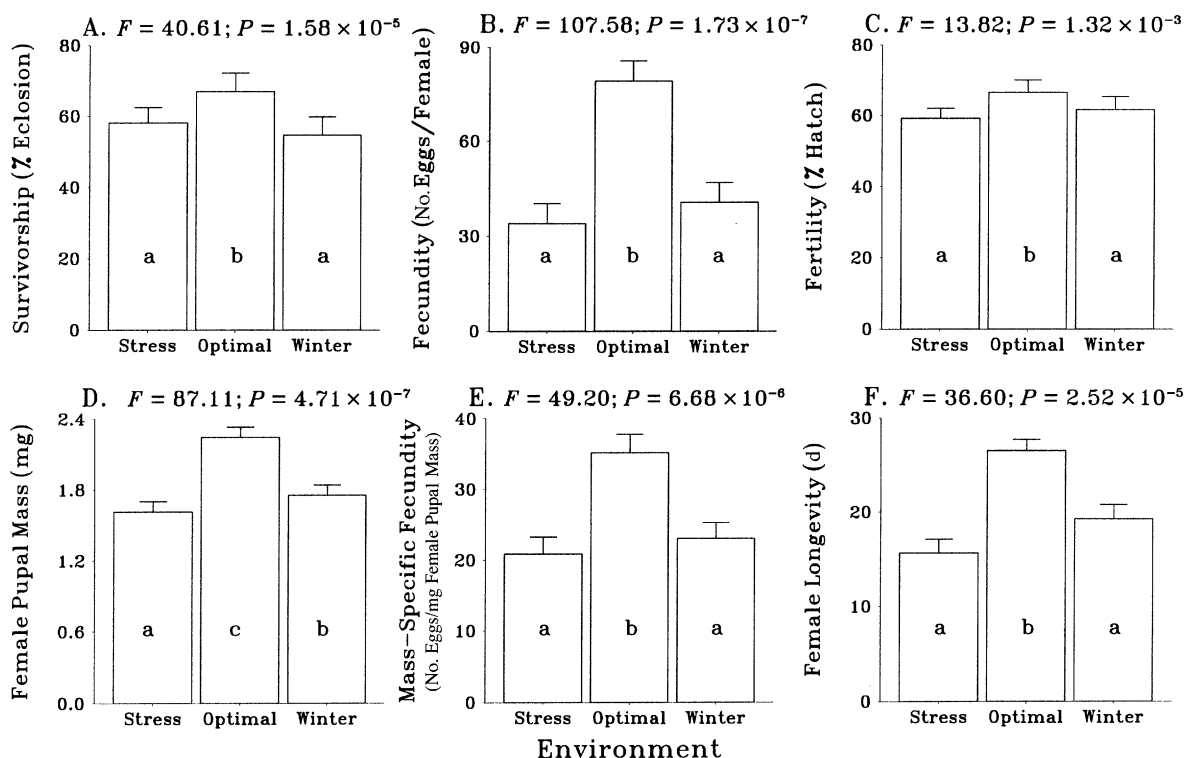


FIG. 3. Components of fitness contributing to R_0 . Conventions are as in Fig. 2.

overwintered females therefore reflected the latent effects of overwintering rather than the direct effects of the adult environment. In sum, diapause and development of *W. smithii* under simulated overwintering conditions extracted a cost of reduced performance in every aspect of survivorship and fecundity and, consequently, in the more inclusive, composite index of fitness, R_0 itself.

Although *W. smithii* feed and can grow while in diapause (Bradshaw and Lounibos 1972), prey capture by the host leaf limits resources of developing larvae (Istock et al. 1976, Bradshaw and Holzapfel 1986). Prey capture is restricted to younger leaf ages so that *W. smithii* effectively overwinter on a fixed resource (Bradshaw 1983, Bradshaw and Holzapfel 1986). The reduced performance of overwintering cohorts (Figs. 2 and 3) may then reflect allocation of available resources to somatic maintenance during diapause and during autumnal and vernal development. This allocation away from future survivorship and reproduction represents a very real cost of overwintering. Diapause does not represent an escape from an unfavorable season but rather, as in migration, diapause represents an investment in an alternative lifestyle with its own benefits, costs, and tradeoffs.

A series of models by Taylor (1986a, b) showed a

relatively robust optimal time to switch from continuous development to hibernal diapause but also showed that the fitness function surrounding the optimum was asymmetric. The cost of developing too late in the season is the risk of death and a fitness of zero; the cost of diapausing too early is a quantitative loss in reproductive potential. The fitness function surrounding the timing of diapause is then more truncated on the winter than summer side of the optimum. In our experiments, the overwintering cohorts actually received more food than the cohorts under optimal conditions but realized lower nutrition-dependent components of fitness such as pupal mass and lifetime fecundity (Moeur and Istock 1980, Bradshaw and Holzapfel 1992). This result may be due to the utilization of resources by diapausing larvae prior to and during winter (Leather et al. 1993: 166–170). In our experiments, as in nature (Istock et al. 1975, Lounibos and Bradshaw 1975), *W. smithii* enter diapause at the end of the summer while it is still warm. Hence, post-diapause fecundity may depend on whether females enter diapause earlier while it is still warmer, or later when it is cooler. If so, selection would favor a later entry into diapause, resulting in a more symmetric fitness function around the optimal time to enter diapause than portrayed in Taylor's models.

Among insects in general, there appear to be trade-

offs between the components of fitness during the summer and components of fitness during the winter. Pest species that had evolved in temperate seasonal climates lose or show reduced expression of diapause-related traits after long-term culture in aseasonal laboratory environments (Tauber et al. 1986: 231–236). Analogously, temperate, migratory milkweed bugs, *Oncopeltus fasciatus*, maintain a positive genetic correlation among wing length, fecundity, and flight propensity but this correlation structure breaks down in non-migratory, tropical populations (Dingle et al. 1988). Finally, in *Drosophila pseudoobscura* the frequency of specific chromosomal inversions cycles annually, and high-elevation populations exhibit a higher mean frequency of the “winter” inversion than do the low-elevation populations (Dobzhansky 1948). These observations suggest that maintenance of the physiological and developmental machinery for overwintering at temperate latitudes imposes a cost to maximizing performance during the summer.

We have shown that hibernal diapause constitutes a severe stress to *W. smithii* and we have argued that very real trade-offs occur between summer performance and winter performance among temperate insects in general. Survivorship and reproduction of the overwintering generation should therefore be an important source of selection, shaping not only seasonal expression of dormancy and migration but also the evolution of summer life-history syndromes as well.

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LITERATURE CITED

- Bradshaw, W. E. 1983. Interaction between the mosquito *Wyeomyia smithii*, the midge, *Metriocnemus knabi*, and their carnivorous host *Sarracenia purpurea*. Pages 161–189 in J. H. Frank and L. P. Lounibos, editors. Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, New Jersey, USA.
- Bradshaw, W. E., and C. M. Holzapfel. 1986. Geography of density-dependent selection in pitcher-plant mosquitoes. Pages 48–65 in F. Taylor and R. Karban, editors. The evolution of insect life cycles. Springer-Verlag, New York, New York, USA.
- Bradshaw, W. E., and C. M. Holzapfel. 1992. Reproductive consequences of density-dependent size variation in the pitcherplant mosquito, *Wyeomyia smithii* (Diptera: Culicidae). *Annals of the Entomological Society of America* **85**: 274–281.
- Bradshaw, W. E., and L. P. Lounibos. 1972. Photoperiodic control of development in the pitcher-plant mosquito, *Wyeomyia smithii*. *Canadian Journal of Zoology* **50**:713–719.
- Bradshaw, W. E., and L. P. Lounibos. 1977. Evolution of dormancy and its photoperiodic control in pitcher-plant mosquitoes. *Evolution* **31**:546–567.
- Chang, Y.-F., M. J. Tauber, and C. A. Tauber. 1996. Reproduction and quality of F₁ offspring in *Chrysoperla carnea*: differential influence of quiescence, artificially-induced diapause, and natural diapause. *Journal of Insect Physiology* **42**:521–528.
- Danks, H. V. 1987. Insect dormancy: an ecological perspective. Biological Survey of Canada (Terrestrial Arthropods), Ottawa, Ontario, Canada.
- Day, R. W., and G. P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. *Ecological Monographs* **59**:433–463.
- Dingle, H. 1984. Behavior, genes, and life histories: Complex adaptations in uncertain environments. Pages 169–194 in P. W. Price, C. H. Slobodkinoff, and W. S. Gaud, editors. A new ecology: novel approaches to interactive systems. Wiley, New York, New York, USA.
- Dingle, H., K. E. Evans, and J. O. Palmer. 1988. Responses to selection among life-history traits in a nonmigratory population of milkweed bugs (*Oncopeltus fasciatus*). *Evolution* **42**:79–92.
- Dobzhansky, T. 1948. Genetics of natural populations. XVIII. Experiments on chromosomes of *Drosophila pseudoobscura* from different geographic regions. *Genetics* **33**: 588–562.
- Ishihara, M., and M. Shimada. 1995. Trade-off in allocation of metabolic reserves: effects of diapause on egg production and adult longevity in a multivoltine bruchid, *Kytorhinus sharpianus*. *Functional Ecology* **9**:618–624.
- Istock, C. A., K. J. Vavra, and H. Zimmer. 1976. Ecology and evolution of the pitcher-plant mosquito. 3. Resource tracking by a natural population. *Evolution* **30**:548–557.
- Istock, C. A., S. S. Wasserman, and H. Zimmer. 1975. Ecology and evolution of the pitcher-plant mosquito. 1. Population dynamics and laboratory responses to food and population density. *Evolution* **29**:296–312.
- Leather, S. R., K. F. A. Walters, and J. S. Bale. 1993. The ecology of insect overwintering. Cambridge University Press, Cambridge, UK.
- Lounibos, L. P., and W. E. Bradshaw. 1975. A second diapause in *Wyeomyia smithii*: seasonal incidence and maintenance by photoperiod. *Canadian Journal of Zoology* **53**: 215–221.
- Moeur, J. E., and C. I. Istock. 1980. Ecology and evolution of the pitcher-plant mosquito. IV. Larval influence over adult reproductive performance and longevity. *Journal of Animal Ecology* **49**:775–792.
- Slobodkin, L. B. 1963. Growth and regulation of animal populations. Holt, Rinehart & Winston, New York, New York, USA.
- Solbreck, C., D. B. Anderson, and J. Förare. 1990. Migration and the coordination of life-cycles as exemplified by Lygaeinae bugs. Pages 197–214 in F. Gilbert, editor. Insect life cycles: genetics, evolution, and coordination. Springer-Verlag, London, UK.
- Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? *Journal of Animal Ecology* **46**:337–365.
- Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. Seasonal adaptations of insects. Oxford University Press, New York, New York, USA.
- Taylor, F. 1986a. The fitness functions associated with diapause induction in arthropods. I. The effects of age structure. *Theoretical Population Biology* **30**:76–92.
- . 1986b. The fitness functions associated with diapause induction in arthropods. II. The effects of fecundity and survivorship on the optimum. *Theoretical Population Biology* **30**:93–110.