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Chapter 3

Evolution of Phenology and Demography in the Pitcher-Plant Mosquito, *Wyeomyia smithii*

William E. Bradshaw and Christina M. Holzapfel

3.1 Introduction

Life-histories may be separated into groups of traits that covary and function together, generally relating to growth and development, reproduction, dormancy and migration (Tauber and Tauber 1981; Tauber et al. 1986; Dingle 1986). Growth, development and reproduction are generally associated with continuous life-cycles and contribute directly to demographies. Dormancy and migration generally disrupt or delay continuous life-cycles. Since a genotype's age at first reproduction and generation time have a large impact on its fitness (Cole 1954; Lewontin 1965; Stearns 1976), developmental or reproductive delays might initially appear to be maladaptive (but see Murphy 1968; Livdahl 1979; Taylor 1980). Organisms in nature do not, however, live in continuous conditions but rather in seasonal environments that are punctuated with periods of unfavourable food, temperature, and/or drought. Dormancy and migration provide "escape in time and space" (Slobodkin 1961) and are thus crucial to long-term fitness despite the concomitant life-cycle delays.

In this paper, we are concerned with evolutionary coordination between two main groups of traits that we define as follows:

Demographic traits are those traits involved with direct development, growth, survivorship and reproduction.

Phenological traits are those traits involved with migration and dormancy. They are usually invoked seasonally by specific direct (temperature, moisture, food) or indirect (photoperiod) cues (Lees 1955; Danilevskii 1965; Beck 1980, Tauber et al. 1986; Danks 1987).

We envision the distinction between these two types of traits to be fundamental to insect life-cycle strategies. Demographic and phenological traits constitute the major determinants of fitness under favourable and unfavourable conditions, respectively. No insect life-cycle in a seasonal environment can be complete

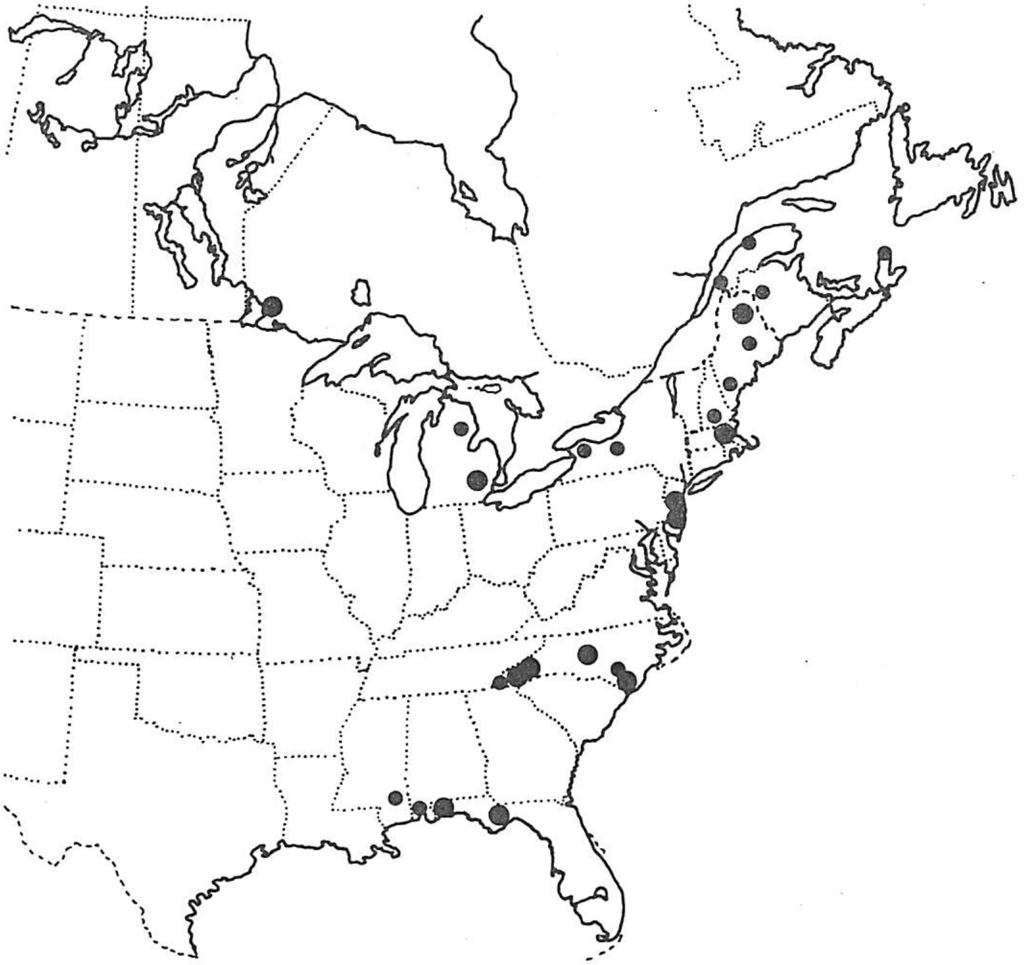

Fig. 3.1. Source of populations considered in this paper. *Small circles*, localities contributing to phenological traits; *large circles*, localities contributing to demographic traits.

without tactics to deal with both conditions and without the ability to convert from one to the other. The value of inducible life-cycle conversions is clear: normal growth, development and reproduction may proceed according to one strategy while conditions are favourable, diverting to another strategy when conditions deteriorate or are likely to do so. Since different kinds of selective force are acting upon each of these two groups of traits, normal growth, development and reproduction should be free from constraints imposed by dormancy and migration, i.e. the two groups of traits should be genetically uncorrelated and free to evolve independently (Dingle et al. 1977; Hegmann and Dingle 1982; Dingle 1986; Palmer and Dingle 1986; Lande 1982; Taylor 1986).

Indices of fitness, such as the finite or instantaneous rates of increase, are assembled from components and subcomponents. Variation in the underlying components may be either coordinated or compensatory. Coordinated variation in causal components results in variation of the composite index of fitness. Compensatory variation results in little or no variation of the composite index but may reflect adapted trade-offs among the causal components. In this chapter, we

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address two questions concerning evolution of the pitcher-plant mosquito, *Wyeomyia smithii*, in eastern North America (Fig. 3.1).

1. Do phenological and demographic traits evolve independently in response to geographical patterns of climate and density-dependent development?
2. Is geographical variation in fitness composed of correlated or independent components and subcomponents?

To answer these questions, we shall examine geographical variation in phenological and demographic traits. These patterns of variation reveal how life-cycle traits have undergone evolutionary divergence during the geographical radiation of *W. smithii*; they do not, however, show whether this divergence has been facilitated, constrained or unaffected by the underlying genetic correlations.

3.2 Life-Cycle of *Wyeomyia smithii*

Wyeomyia smithii develops (Fig. 3.2) only in the water-filled leaves of pitcher plants in eastern North America. The eggs are dropped on to the water by hovering females and hatch directly without any hatching stimulus. Long days sustain direct development but short days induce a larval diapause in the fourth instar at southern latitudes (≤36° N) or in the third instar at more northern latitudes or higher altitudes (Smith and Brust 1971; Evans and Brust 1972; Bradshaw and Lounibos 1972, 1977). Continuous short days maintain diapause. Larvae respond to photoperiod throughout the winter and into the spring, even after prolonged chilling in nature, so that photoperiod is also probably responsible for the termination of diapause (Evans and Brust 1972; Lounibos and Bradshaw 1975). In the north, diapausing third instars may terminate diapause and enter a second, stable fourth instar diapause that acts as a buffer against unpredictable vernal weather (Lounibos and Bradshaw 1975). Females of all populations obligatorily lay their first batch of eggs without a blood meal (Bradshaw and Lounibos 1977). Northern females never take blood meals but continue to produce repeated, small batches of eggs; southern females require a blood meal for the production of their second and subsequent batches (Bradshaw and Lounibos 1977; Bradshaw and Holzapfel 1983; Bradshaw 1980; O'Meara and Lounibos 1981; O'Meara et al. 1981).

Over its range, *W. smithii* encounters a predictable climatic gradient. In the south, mild winters are followed by long growing seasons, while further north, harsher winters are followed by progressively shorter growing seasons. At the southern end of its range, *W. smithii* may be able to complete up to five generations per year at 30° N (Bradshaw and Holzapfel 1983), declining to two or, rarely, three at 42.5° N (Judd 1959; Istock 1978; Istock et al. 1976), one to two at 45.5° N (Kingsolver 1979), and only one at 49–54° N (Evans 1971). In the south (30° N), generations overlap and blend into each other continuously; in the north (≥42.5° N) generations are relatively discrete.

Resources for developing larvae consist of prey captured by their host leaf.

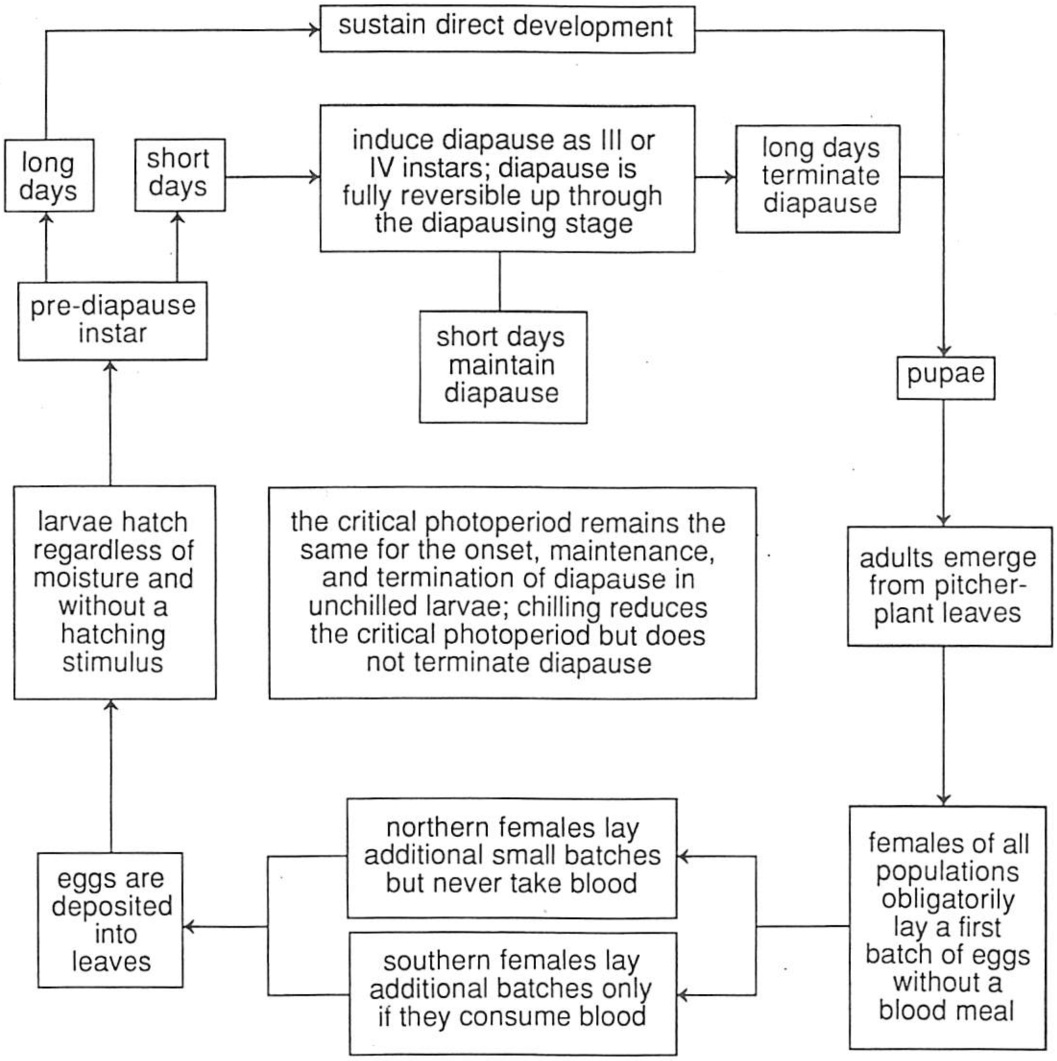

Fig. 3.2. Life-cycle of *Wyeomyia smithii*.

Although prey rapidly disarticulate in the leaf, the more heavily chitinized head capsules of victims remain intact and are readily identified as such among the "gut" contents of leaves. Resources per individual *W. smithii* are then measured as larvae per head capsule of prey captured by the host leaf. Along the Gulf of Mexico at the southern end of their range, pupation success of *W. smithii* is limited by larval density (equals *W. smithii* per head capsule of prey: Bradshaw and Holzapfel 1986). Overwintering *W. smithii* begin to eclose as adults 2 weeks before the first leaves open and the first summer generation saturates available leaves; leaves remain saturated for the duration of the growing season (Bradshaw and Holzapfel 1986). Density also determines the age structure of the overwintering population and, subsequently, its pupation success in the spring. Thus, southern *W. smithii* saturate their environment year-round, including the winter, when even diapause does not provide escape in time or space (Bradshaw and Holzapfel 1986). Density-dependent constraints to development then decrease

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with increasing latitude or altitude (Istock et al. 1976; Bradshaw and Holzapfel 1986). At 42.5° N, *W. smithii* enjoy release from density-dependent constraints during most of the growing season but go through density-dependent bottlenecks in the spring and fall (Istock et al. 1976). The effect of density on vernal development continues to decrease with increasing latitude and, at some northern and high-elevation localities, there is no significant effect of density even on vernal development (Bradshaw and Holzapfel 1986). *Wyeomia smithii* therefore encounter a geographical gradient of density-dependent selection that can be visualized most easily by the crowding of *W. smithii* per head capsule of prey over its entire range from the Gulf of Mexico (30° N) to north-central Canada (Manitoba, 54° N) (Fig. 3.3).

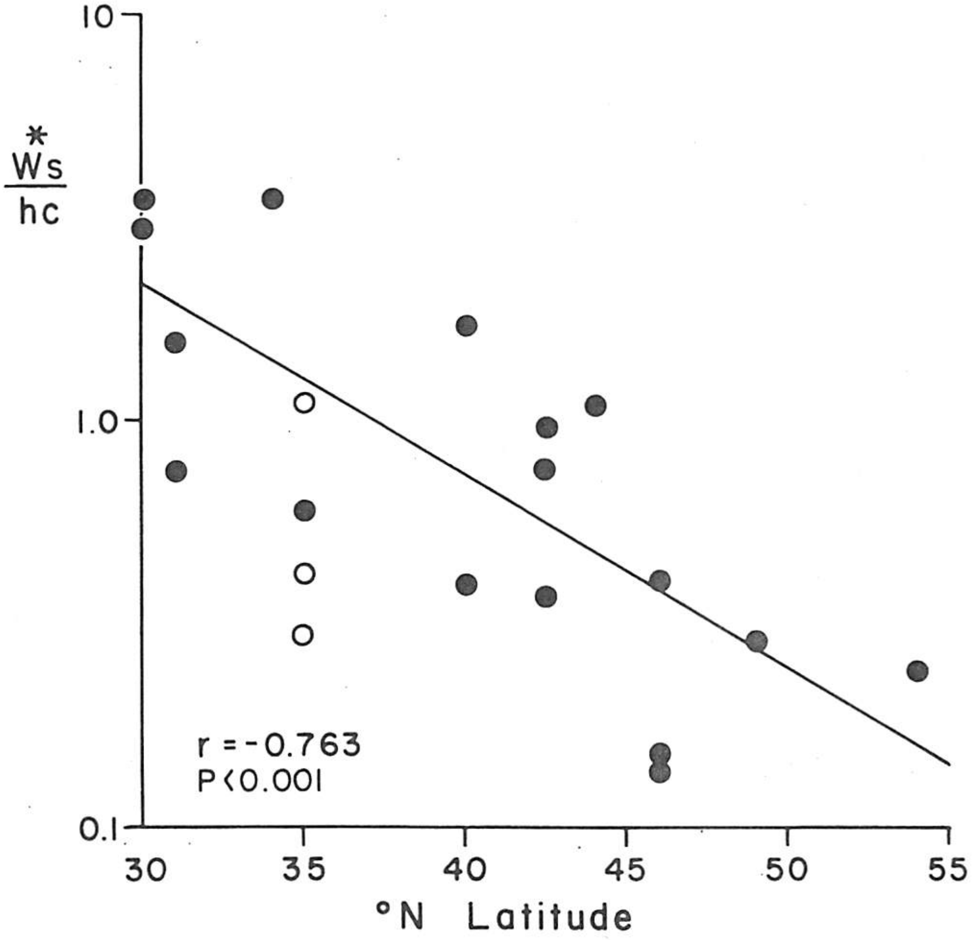


Fig. 3.3. Resource availability to the average larva over the range of *W. smithii*. The regression was run on the latitudinal gradient (●); the mean of three censuses at 900 m at 35° N (○) intercepts the regression line at 43° N. Resource availability is measured as mean crowding of *W. smithii* per head capsule ($\overset{*}{Ws}$/hc) of prey in the overwintering generation when 100% of the population is present as larvae in the leaves. (From Bradshaw and Holzapfel 1986.)

3.3 Life-Cycle Traits and Geography

On the basis of present-day distribution, physiology, morphology and behaviour, we conclude that the evolution of *W. smithii* has proceeded from south to north.

1. *Present day distribution*: the genus has over 50 tropical and subtropical species (Stone et al. 1959) and one temperate species, *W. smithii*, that extends further north than any other species in the genus or even the whole tribe (Sabethini).
2. *Diapause*: southern populations diapause only in the fourth instar; northern populations diapause as third instars but may enter a second, stable, fourth-instar diapause in the spring (Lounibos and Bradshaw 1975).
3. *Anal papillae*: southern populations possess four long anal papillae as in most mosquitoes; northern populations have but two short anal papillae (Bradshaw and Lounibos 1977).
4. *Blood feeding*: southern populations require a blood meal for the second and subsequent ovarian cycles; northern populations mature repeated egg batches without blood meals (highly unusual among mosquitoes) and are not known to bite under any circumstances (Bradshaw 1983, 1986a; O'Meara et al. 1981; O'Meara and Lounibos 1981).

Life-cycle traits of *W. smithii* should then reflect its evolution along the geographical gradient of climate and density-dependent development.

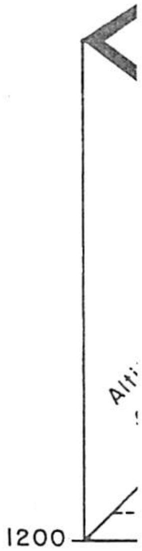
3.3.1 Predictions

Phenological traits, including critical photoperiod, stage of diapause and depth of diapause should respond to the regular geographical change in growing season and seasonal harshness, showing greater conservatism in the north than in the south. Demographic traits, including capacity for increase, its components and their subcomponents should respond to the regular geographical change in density (Fig. 3.3) as well as climate. Theories of r- and K-selection or of stage-specific mortality both predict that northern populations should exhibit lower density-tolerance and higher productivity than southern populations (Stearns 1976; Mueller and Ayala 1981; Mueller 1988). These predictions are reinforced both by the decrease in seasonal harshness (Roughgarden 1971) and by the increase in the growing season (King and Anderson 1971) with the concomitant increase in resource tracking (Bradshaw and Holzapfel 1983) at southern relative to northern latitudes.

3.3.2 Observed Phenological Traits

Critical Photoperiod

The critical or median photoperiod is the switch-point between long- and short-day responses, i.e. the number of hours of light per day that initiates or maintains 50% diapause in a population. In *W. smithii*, the critical photoperiod is tightly



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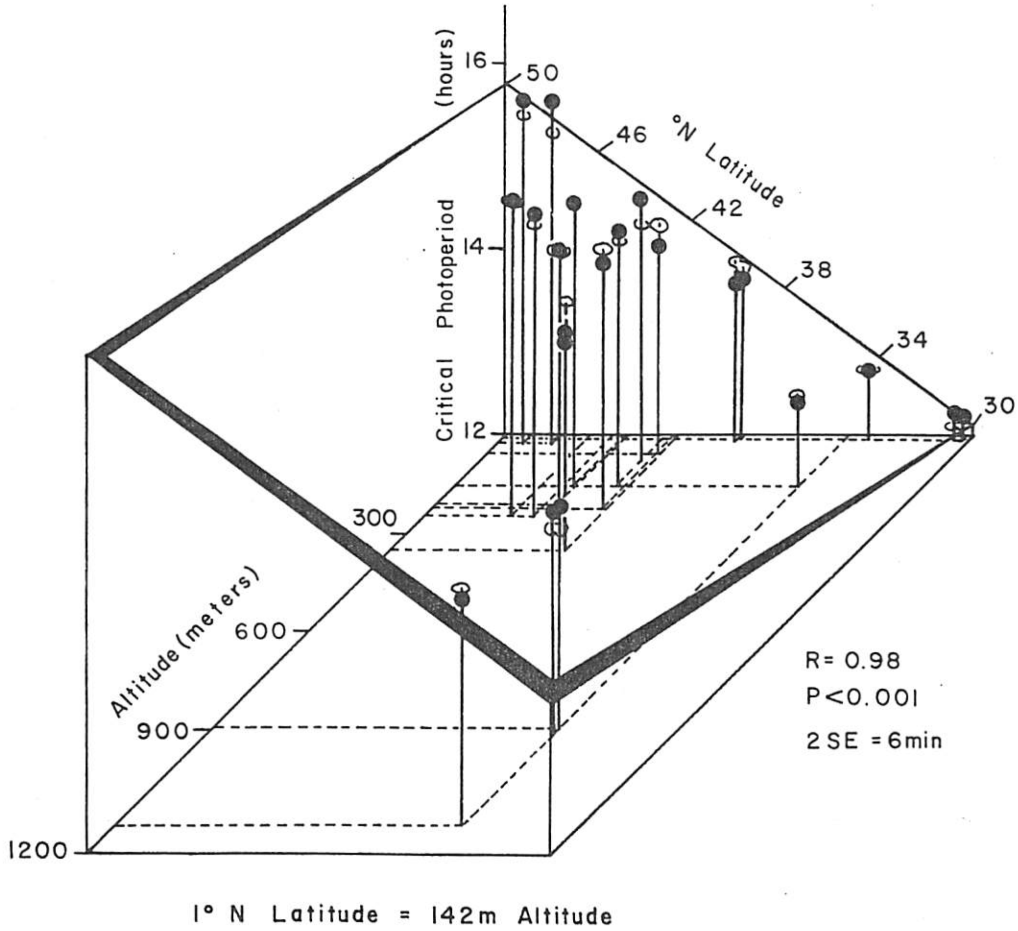


Fig. 3.4. Relationship between critical photoperiod, altitude and latitude. Circles on the regression plane indicate where the projections of the data points cross that plane. (From Bradshaw 1976. Reprinted by permission from *Nature* vol. 263, pp. 384–386, copyright © 1976 Macmillan Magazines Ltd.)

correlated with latitude and altitude of origin (Fig. 3.4). Photoperiodic response of *W. smithii* thus tracks the climatic gradient of eastern North America very closely (Bradshaw 1976; Bradshaw and Lounibos 1977).

To determine the genetic basis of critical photoperiod, we subjected laboratory-reared diapausing larvae from Florida (30° N) and Maine (46° N) to naturally increasing photoperiods. We used astronomic time switches (street-lamp timers) that program a sine-function change in photophase over a 365-day period. In our experiments, we used the near-linear increase in photophase (2–3 min increment per day) that occurs in the vicinity of the vernal equinox. At each cumulative 15 min increment in photoperiod, we removed all pupae and allowed the adults from each specific, cumulative 15 min increment to mass swarm in an isolated cage. We reared the resulting F_1 progeny on short days, induced diapause, and then subjected them to the same naturally increasing photoperiod as their parents had experienced. From the offspring–parent regression of median (critical) photoperiod (Fig. 3.5, left), we concluded that a substantial amount of genetic variation underlies photoperiodic response within populations of *W. smithii*. However, because of the experimental setup, differences in photoperiod of pupation also

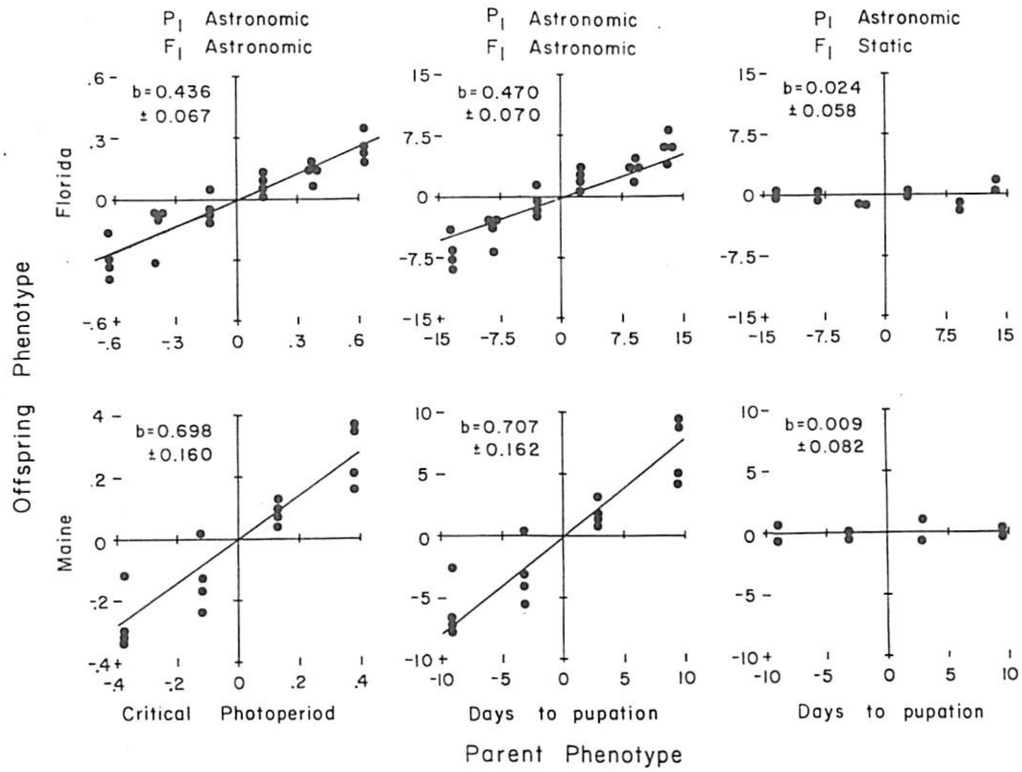
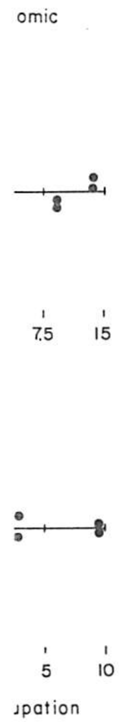


Fig. 3.5. Heritability of critical photoperiod within populations from Florida and Maine. *Left*: critical photoperiod expressed as deviations (in h) from parent mean critical photoperiod (*x*-axis) and from offspring mean critical photoperiod (*y*-axis). *Middle* and *right*: mean days to pupation expressed as deviations (in days) from parent mean days to pupation (*x*-axis) and from offspring mean days to pupation (*y*-axis). In both the left and middle figures, diapausing parents (P_1) and diapausing offspring (F_1) were exposed to naturally increasing photoperiods that incremented 2–3 min per day. In the right-hand figures, diapausing parents (P_1) were exposed to naturally increasing photoperiods that incremented 2–3 min per day and their diapausing offspring (F_1) were exposed to static long days. Slopes ±95% confidence limits are shown in the upper left of each graph.

reflect differences in time to develop from the onset of experimental conditions. The offspring–parent regression of days to pupation (Fig. 3.5, middle) indicates a heritability similar to that of critical photoperiod. To see whether differences in response to the astronomic clock may have reflected variation in post-diapause development time rather than in photoperiodic response, we exposed samples of F_1 larvae to static, unambiguous long days (Fig. 3.5, right). In this case, we found no correlation between parental and offspring development times. Thus the correlation between offspring and parent development time (Fig. 3.5, middle) reflects the underlying correlation between their critical photoperiods (Fig. 3.5, left) and not inadvertent selection for post-diapause development time. Consequently, within populations of *W. smithii*, variation in photoperiodic response has a large heritable component. Analysis of covariance revealed heterogeneity of slopes for critical photoperiod (Fig. 3.5, left; $F_{1,36} = 12.04$, $P<0.01$), indicating that genetic variation for critical photoperiod is significantly higher in the northern than in the southern population. We therefore conclude that heritable variation for photoperiod response has not been diminished by long-term



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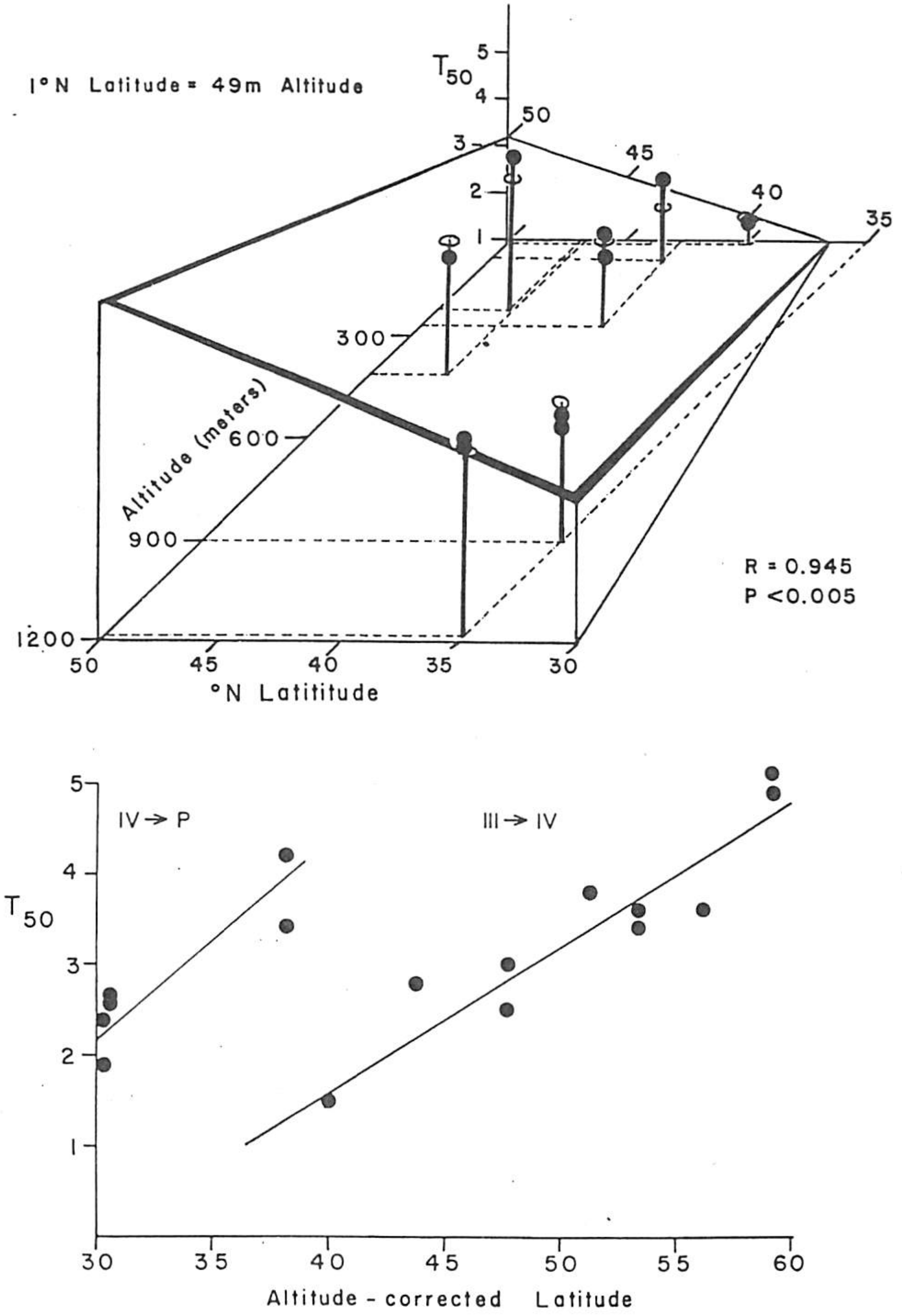

Fig. 3.6. Depth of diapause as measured by T_{50}, the number of long days required to terminate diapause in 50% of the sample population. *Upper panel*: regression of T_{50} on latitude and altitude for third instar diapausing populations produced an altitude–latitude conversion factor of 49m/°N; this conversion factor was then used to correct for altitude of fourth-instar diapausing populations. *Lower panel*: comparative T_{50} for third-(III) and fourth-(IV) instar diapause plotted on a common axis of altitude-corrected latitude. P, puparium. (From Bradshaw and Lounibos 1977.)

directional selection for increasing critical photoperiod during the northward adaptive radiation of *W. smithii*.

Depth of Diapause

The depth or intensity of diapause can be measured by the number of long days required to terminate that diapause. This number is determined by exposing diapausing larvae to various numbers of long days (0, 1, 2 . . . *n*) and then returning them to short days. Depth of diapause is then measured as T_{50}, the number of pulsed long days required to terminate diapause in 50% of the population. Depth of diapause in *W. smithii* generally increases with latitude (Fig. 3.6). but, superficially, appears irregular. At the southern edge of its range, *W. smithii* enters a shallow, fourth-instar diapause and the depth of this diapause increases with latitude. In northern populations, however, diapause occurs in the third instar. Within third-instar diapausing populations, diapause is again shallow among the southernmost populations and increases with latitude or altitude. The shift from a deep to a shallow diapause at 35–40° N is accompanied by a concomitant shift from a fourth- to a third-instar diapause. At the same time, the ability to enter a second, fourth-instar diapause is preserved among northern populations (Lounibos and Bradshaw 1975; Bradshaw and Lounibos 1977). This pattern, then, reflects a continuum of progressive conservatism: at each increment in latitude or altitude, irrevocable commitment to adult emergence becomes increasingly remote. As with critical photoperiod, depth of diapause tracks the climatic gradient of North America very closely.

3.3.3 Observed Demographic Traits

To assess the effects of climate and density-dependent selection on demographic traits, we collected populations of *W. smithii* from 12 localities (Fig. 3.1) representing six geographical zones along a latitudinal and altitudinal gradient. After rearing these populations for at least two laboratory generations to eliminate field effects, we reared them at varying densities under controlled but near natural conditions of light, temperature and feeding schedule in the leaves of intact pitcher plants (Bradshaw 1986a). For each locality and density (10, 20, 40 or 60 per leaf) at a constant food level (200 *Drosophila melanogaster*), we determined Laughlin's (1965) capacity for increase:

$$\begin{aligned} r_c &= \ln\text{(replacement rate)/(mean generation time)} \\ &= \ln R_o/T \end{aligned}$$

where the components of r_c are:

$$\begin{aligned} R_o &= \Sigma E_x/n_o \\ &= \text{[eclosion success] [sex ratio] [fecundity]} \\ &= \text{[\% eclosion] [\% females eclosing] [eggs/eclosed female]} \end{aligned}$$

$$\begin{aligned} T &= \Sigma(xE_x)/\Sigma E_x \\ &= \text{[development time] + [maturation time]} \\ &= \text{[days to median eclosion] + [days from median eclosion to median egg]} \end{aligned}$$

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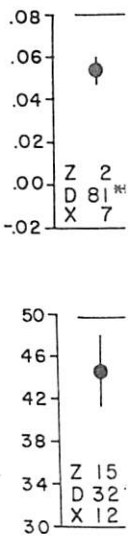

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ΣE_x = number of eggs oviposited by a uniform aged cohort at time x, the number of days since oviposition of the cohort

n_o = initial cohort size

We then performed 2-way analysis of variance (ANOVA) on each demographic trait with treatments geographical zone of origin (n=6) and density in the laboratory (n=4). Each cell in the ANOVA included two replicates (=independent localities) so that we could test for zone×density interaction.

Zone Effects

Neither r_c nor its components, R_o and T, differed among zones (Fig. 3.7). All three traits responded dramatically to density in the laboratory but there was no significant zone×density interaction. Thus, r_c, R_o, and T did reflect proximal effects of density but not longer-term evolution along a geographical gradient of climate and density-dependent selection. As we have already pointed out (Bradshaw and Holzapfel 1989) and will not belabour here, the theory of r- and

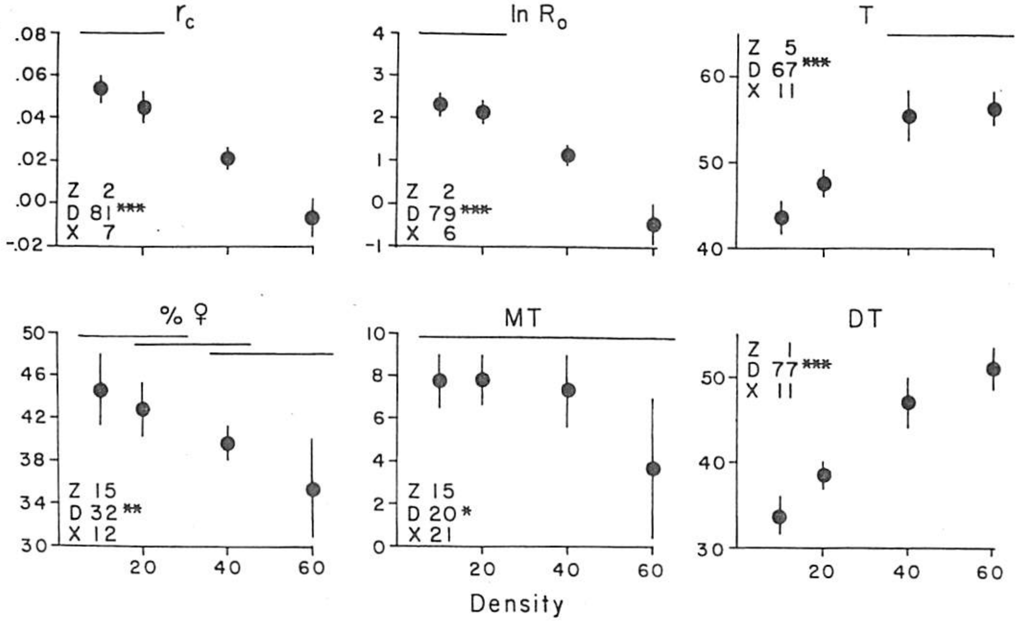

Fig. 3.7. Effect of density in the laboratory and geographical zone of origin on mean (±2 SE) capacity for increase (r_c), its components replacement rate (ln R_o) and mean generation time (T), and its subcomponents sex ratio (% female, arcsine transformed), development time (DT), and maturation time (MT). Within each graph is given reduction in total sum of squares ascribable to zone of origin (Z), density in the laboratory (D), and their interaction (X) from 2-way ANOVA. $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; otherwise, not significant. *Horizontal lines* at the top of each graph extend over means that are not significantly different ($P>0.05$) from Duncan's multiple range test. (r_c, R_o and T from Bradshaw and Holzapfel 1989; © 1989 by The University of Chicago. 0003–0147/89/3306–0007 $02.00. All rights reserved)

K-selection fails completely to predict the pattern of demographic traits in *W. smithii*.

Among the subcomponents of fitness (Fig. 3.7), sex ratio, development time and maturation time did not vary among zones or reflect significant zone×density interaction. Fecundity (Fig. 3.8) varied significantly among zones of origin and among densities in the laboratory but did not exhibit any zone×density interaction. Duncan's multiple range test revealed that fecundity declined steadily with increasing density (Fig. 3.8) but failed to identify a significant difference between any two zones (Fig. 3.8). Inspection of mean fecundities revealed no consistent geographical pattern: far southern populations did not

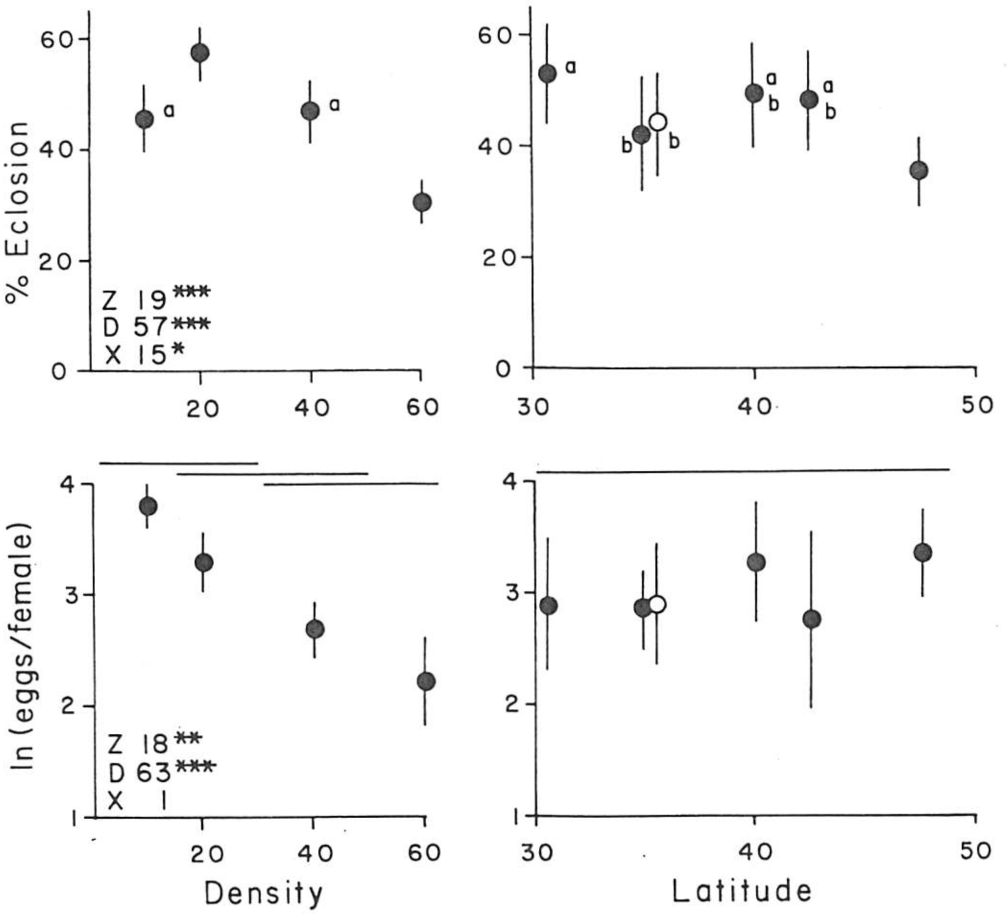

Fig. 3.8. Effect on (*top*) eclosion success (mean arcsine transformed percentage eclosion ±2SE) and (*bottom*) fecundity (mean ln (eggs per ecdysed female) ±2 S.E.M.) of density in the laboratory (*left*) and zone of origin plotted as mean latitude of the two contributing localities (*right*). In both the top and bottom plots, the *open circle* denotes the high elevation zone (900 m at 35° N). *Z*, *D* and *X* as in Fig. 3.7. Means accompanied by the same letter or lying underneath the same horizontal line are not significantly different by Duncan's multiple range test (*P*>0.05). (Fecundity data from Bradshaw 1986a.)

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differ from far northern populations and the lowest and highest fecundities occurred in adjacent zones. Thus, fecundity, like the other traits described above, reflected primarily the proximal effects of density and not longer term evolution along a geographical gradient of climate and density-dependent selection.

Eclosion success (Fig. 3.8) reflected density, geography, and their interaction, with density being the more important component. Eclosion success was lower at 10 larvae per leaf, peaked at 20 larvae per leaf, and then declined at higher densities. A similar Allee effect was observed in laboratory populations of *Aedes aegypti* (Wada, 1965) and *A. sierrensis* (Hard et al. 1989). In the case of *W. smithii*, at a density of 10/leaf, development time is significantly faster (Fig. 3.7) and there is a non-significant tendency towards greater fecundity (Fig. 3.8) than at a density of 20/leaf so that R_o and, consequently, r_c do not decrease at the lowest density (Fig. 3.7). Eclosion success among zones (Fig. 3.8) appeared to decrease with latitude. Neither zone mean ($r=-0.71$; d.f.=3; $P>0.05$) nor locality mean ($r=-0.61$; d.f.=8; $P>0.05$) was significantly correlated with latitude (excluding the high elevation zone or high elevation localities at 35° N) but these correlations might have been significant with a larger sample size. Further, there was no significant correlation between mean eclosion success and density of *W. smithii* per unit resource at the locality of origin (for all localities except one Gulf Coast population for which we do not have an estimate of field density: $r=0.31$; $n=11$; $P>0.05$). Thus, eclosion success, also, reflected primarily the proximal effects of density and only to a lesser extent, if at all, the longer-term evolution along a geographical gradient of climate and density.

Phenotypic variation of demographic traits in response to variable resources vastly exceeds differences due to locality or zone of origin (Figs. 3.7 and 3.8). Decreased resources result in a linear decline of capacity for increase but the reason for this decline varies with resource level. At modest resource limitations, generation time increases but replacement rate remains much the same. At more severe levels of resource limitation, generation time remains relatively stable, while replacement rate continues to decline. Moeur and Istock (1980) also found that moderately reduced resources in *W. smithii* resulted in the prolongation of pre-adult development, especially in the fourth instar, with no resultant decline in pupal size or adult fecundity. At more severe resource reductions (Fig. 3.7), prolongation of development appears to reach a higher asymptote, whereafter net fecundity is reduced. These observations suggest that there are physiological trade-offs between development time and fecundity in *W. smithii* but that the specific trade-off is density dependent.

The low residual variations in demographic traits (Figs. 3.7 and 3.8) show that homeostatic adjustment greatly exceeds genetic differences in response to density. Slobodkin (1968) has proposed that the optimal strategy for organisms evolving in a heterogeneous environment is to "behave in a homeostatic fashion so as to avoid or counteract the effects of introduced (environmental) variation". On the other hand, homeostatic ability is advantageous over a genetically fixed phenotype only when homeostatic adjustment can take place within the magnitude and time constraints of environmental variation (Bradshaw 1976, 1986b). In the case of mosquitoes, adult size and, consequently, ovariole number and blood-meal capacity, are fixed by the time of pupation (Hawley 1985). The prolongation of larval development and the determination of potential adult fecundity are all developmental alternatives available to larvae within the time frame of reduced resources. Consequently, most of the phenotypic variation in demographic traits of *W. smithii* encountering variable density in the laboratory reflects individual homeostasis rather than genetic divergence among populations.

Locality Effects

The above results indicate that r_c, its components and its subcomponents are all refractory to the broader gradient of climate and density-dependent selection experienced by *W. smithii* throughout its range. These results are based on phenotypic differences observed among six geographical zones. The lack of among-zone variation may be due to an impotence of climate- and density-dependent selection to mediate the evolution of demographic traits or to a lack of heritable variation in the traits themselves. However, if there is significant variation among localities regardless of zone, then heritable variation for those traits must exist and phenotypic evolution must have taken place.

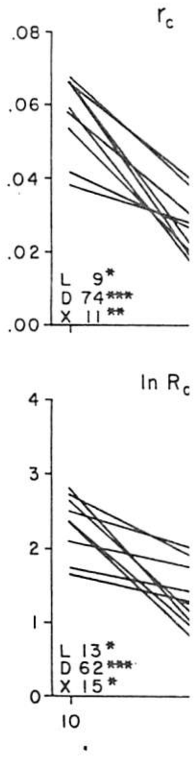
To examine differences among localities requires an estimate of within-locality variation. Consequently, we made a second, independent determination of r_c, its components, and its subcomponents in nine populations of *W. smithii* (two locations each from 30–31° N, 900 m at 36° N, 40° N and 42.5° N and one locality at 49° N) at two densities (10 and 40 per leaf).

This procedure provided replicated norms of reaction across two densities (Fig. 3.9) for capacity for increase, its components, and its subcomponents. Two-way ANOVA then revealed significant locality and/or locality×density effects for capacity for increase, replacement rate, mean generation time, eclosion success and development time. Eggs per ecdysed female, previously found to vary among zones (Fig. 3.8) did not vary significantly among localities (Fig. 3.9), probably because of the lower discriminating power of the latter ANOVA. Sex ratio, although reflecting effects of density did not vary among zones or localities or exhibit zone or locality×density interaction. Maturation time did not reflect effects of zone, locality, density, or their interaction, in agreement with Moeur and Istock (1980), who likewise found no variation within populations for this trait. Taken together, the results in Figs. 3.7–3.9 indicate that evolutionary divergence has taken place in all the above traits except sex ratio and maturation time. Thus, the lack of among-zone variation in r_c, in its components, and in all but two of its subcomponents does not represent a lack of genetic variability underlying the traits but rather a lack of climate or density-dependent development, per se, to select for their evolutionary divergence.

Genetic Divergence

The degree of genetic divergence between two populations may be reflected by the degree of heterosis observed in their hybrids (Endler 1977; Falconer 1981; Dingle et al. 1982).

To examine possible heterotic effects, we performed 13 crosses among the 12 populations of *W. smithii* contributing to Figs. 3.7–3.9. These 13 crosses represented parent populations with median and extreme phenotypes and geographical displacements (Bradshaw and Holzapfel 1989). We considered heterosis to occur if the difference between hybrid and mid-parent phenotypes



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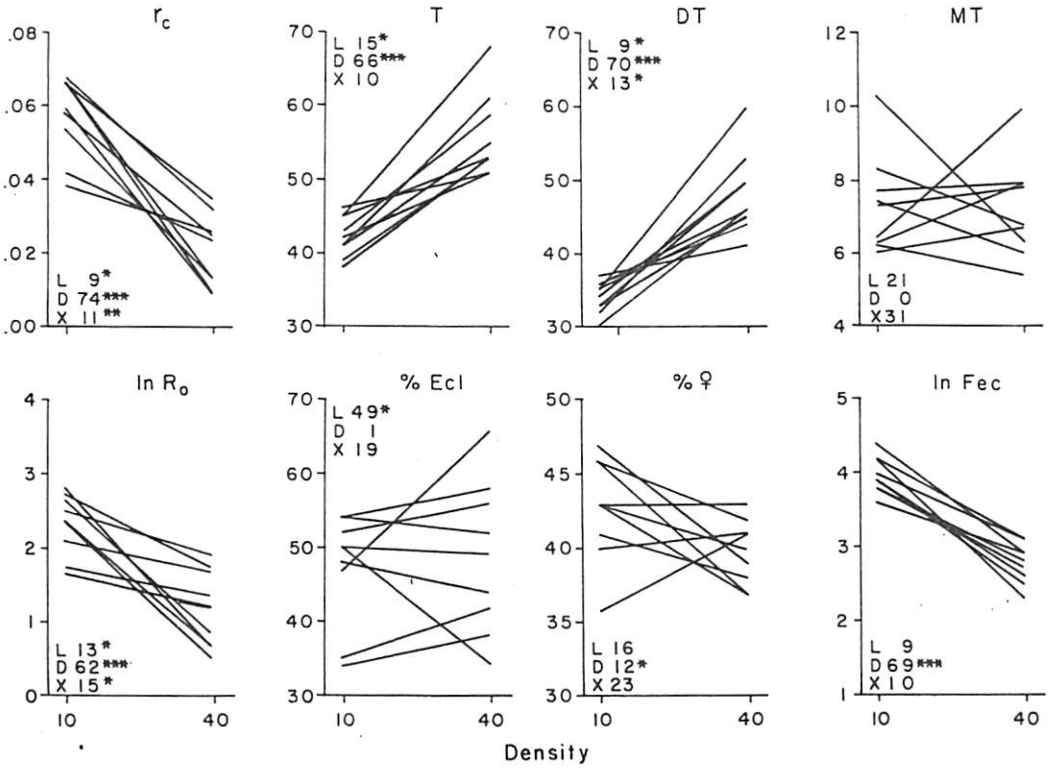

Fig. 3.9. Effect of density on capacity for increase, its components, and its subcomponents for each of nine localities of origin regardless of zone. Trait abbreviations are as in Fig. 3.7 except: % Ecl=arcsine transformed percentage eclosion; ln Fec=ln (eggs/female). Each point represents the mean of two independent determinations in the laboratory for each population from a given locality. Within each graph is provided the percentage reduction in total sum of squares from 2-way ANOVA with treatments locality of origin (*L*), density in the laboratory (*D*), and their interaction (*X*). Significance of each treatment is given by $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; otherwise, not significant. A significant effect of locality indicates a significant difference among localities in the trait indicated; a significant effect of *X* indicates that localities differ in response to density by the trait indicated.

was in the direction of increased fitness (increased r_c) and tested for significant differences between hybrid and mid-parent values by one-way ANOVA.

Fig. 3.10 shows that capacity for increase, its components, and two of its subcomponents (sex ratio and development time) all exhibited significant heterosis but maturation time, fecundity and pupation success did not. The data in Fig. 3.9 provide evidence that all the demographic traits except sex ratio and maturation time have diverged both genetically and phenotypically among populations of *W. smithii*. The data in Fig. 3.10 show that sex ratio has diverged genetically even though this divergence has not resulted in significant phenotypic differences among populations (Fig. 3.9). Conversely, fecundity that varied significantly among populations (Fig. 3.9) did not produce significant heterosis in hybrid populations (Fig. 3.10). Consistently, maturation time did not differ among populations (Fig.3.9) and did not produce significant heterosis in hybrid populations (Fig. 3.10). The demographic traits in *W. smithii* thus illustrate that within the geographical divergence of a single species, substantial phenotypic divergence can occur through substantial, or minimal, non-detectable genetic

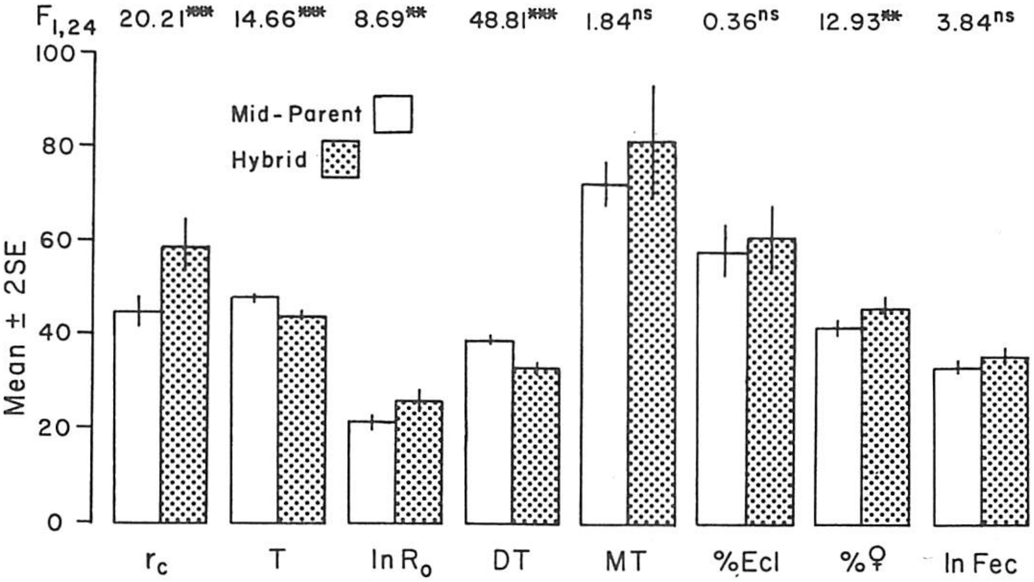

Fig. 3.10. Heterosis of demographic traits observed among hybrids between parent populations of *W. smithii* representing median and extreme phenotypic divergence of traits and of geographical separation. At the top of the figure, $F_{1,24}$ is the *F*-value from one-way ANOVA between hybrid and mid-parent phenotypes for each trait. Trait abbreviations as in Fig. 3.9. Note that heterosis is indicated for a significantly lower hybrid than mid-parent value for *T*, *DT* and *MT*. ^{ns}not significant; $^{**}P<0.01$; $^{***}P<0.001$.

divergence; and significant genetic divergence can occur with minimal, non-detectable phenotypic divergence.

Covariation of Traits

The above results still leave open the question as to whether geographical variation in capacity for increase is composed of covarying or independently varying components and subcomponents. The independence of demographic traits from broader geographical influence means that evolutionary trade-offs among the traits themselves should be apparent once the proximal effects of density have been factored out. Since density in the laboratory does have such a dramatic influence on demographic traits (Figs. 3.7–3.9), there is no optimal density at which to look for these trade-offs. Consequently, we examined covariation among r_c, its components, and its subcomponents at each locality by factoring out density effects and then determined correlations between traits among localities. We corrected for the species-wide effects of density by: (a) finding the mean value of each trait at each density across localities; (b) regressing this mean value on density or, in the case of percentage eclosion, on density and ln (density) to account for the curvilinear response of this trait to density; and (c) calculating the mean deviation from regression across densities for each trait at each locality. Thus, the mean trait at each locality was based on four observations (deviations from regression on density). To look at correlated divergences of traits among localities, we made pair-wise correlations (n=12 each) between mean deviation from regression of r_c, its components, and its subcomponents.

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With a sample size of 12, the least significant correlation is $r=0.57$ ($r^2=0.32$) but the averaging of four independent deviations from regression should reduce sampling variance.

Capacity for increase among localities (Fig. 3.11) was very highly correlated with replacement rate among localities but not mean generation time among localities. Further, replacement rate and mean generation time were not themselves correlated and the non-significant correlation was negative. It would thus appear, firstly, that divergence of capacity for increase is due mainly to divergence of replacement rate and not mean generation time. Secondly, there have been no adaptive (genetic) trade-offs between replacement rate and generation time. That is, among localities, an evolved increase in replacement rate has resulted in a concomitant increase in capacity for increase without a compensatory increase in mean generation time. Although all three traits exhibit evolutionary divergence among localities (Fig. 3.9), only replacement rate covaries with capacity for increase and both vary independently of mean generation time. Among the components of replacement rate, only percentage eclosion and fecundity vary among localities while sex ratio does not (Fig. 3.9) and only fecundity covaries with replacement rate (Fig. 3.11). Among the components of mean generation time, development time varies among localities,

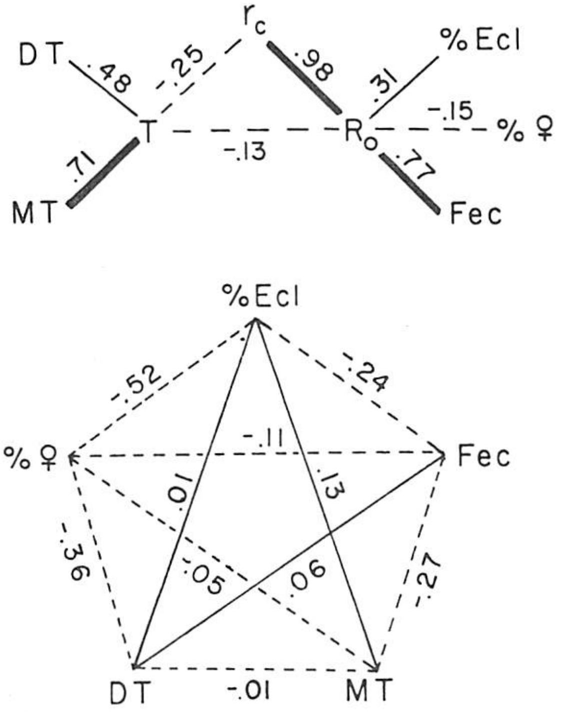

Fig. 3.11. Correlation between r_c and its components, between the components and each of their respective subcomponents, and among all the subcomponents from mean values ($n=4$) of each trait at each of 12 localities. Means represent residuals from regression of each trait on density in the laboratory. *Continuous lines* indicate positive correlations; *broken lines* indicate negative correlations; *heavy lines* indicate significant correlations ($P<0.05$). The correlation coefficient (r) is given on each line. Note that with a sample size of 12, the least significant $r=0.57$. Abbreviations as for Figs. 3.7 and 3.9.

while maturation time does not (Fig. 3.9), but only the latter covaries with mean generation time (Fig. 3.11). Among the five subcomponents of fitness, three vary among localities while two do not (Fig. 3.9) but none of the five covaries with any other (Fig. 3.11).

From these results, we conclude that among geographically diverse populations of *W. smithii*, subcomponents of fitness have generally evolved independently of one another and may or may not covary with their respective component of fitness. Covariation of components with their subcomponents is not predictable from the magnitude of geographical variation of the individual component or subcomponent. Finally, covariation among capacity for increase and either of its components is not predictable from the magnitude of their separate geographical variation and does not reflect any compensatory response or reflect any genetically based trade-off selection.

3.4 Conclusions

We may now answer our original questions. Phenological and demographic traits of *W. smithii* have evolved independently over the geographical range of *W. smithii* in North America from 30–49° N. Phenological traits show strong, consistent trends of progressive conservatism along an eco-climatic gradient. Evolutionary divergence of demographic traits has also taken place but independently of this same gradient. We therefore conclude either that phenological and demographic traits are, as we originally proposed, genetically uncorrelated with each other or that genetic correlations do exist between them but such correlations do not constitute significant barriers to adaptive radiation of this species over geographical distances.

Among demographic traits, there appears to be a coordinated axis of capacity for increase, replacement rate and fecundity. This coordination has not resulted in any substantial compensatory trade-off in other components or subcomponents of fitness. All of the evidence indicates that it is the immediate exigencies of the local microhabitat, not regional differences in climate or density-dependent development, that have selected for divergent demographic traits in *W. smithii*. It is not therefore surprising that capacity for increase, replacement rate and mean generation time all exhibit strong heterosis among hybrids between various populations (Bradshaw and Holzapfel 1989; Istock 1981).

Wyeomyia smithii is a single species of mosquito living within the water-filled leaves of a single species of insectivorous plant over wide geographical distances (Fig. 3.1). We have previously (Bradshaw 1976; Bradshaw and Lounibos 1977; Bradshaw and Holzapfel 1983, 1989) cited this unusual habitat consistency among localities as a good reason for considering life-cycle evolution in *W. smithii*, since geographical variation is unlikely to be confounded by local microhabitat variation. The conclusion we have just made above is therefore surprising, but it does suggest that microhabitat variation may be far more important in shaping the demographies of most other organisms that live in more variable habitats than does *W. smithii* (Bradshaw and Holzapfel 1989).

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vary independently of each other and may covary with, or be independent of, components of fitness that in turn may covary with, or be independent of, the composite index of fitness. It is therefore very dangerous to extrapolate to fitness from any of its components or correlates without determining actual connection (Livdahl and Sugihara 1984; Hard et al. 1989). The lack of more significant covariation among demographic fitness traits (Fig. 3.11) implies that adaptive trade-offs have not been an important component in the evolutionary divergence of *W. smithii* populations, regardless of the underlying genetic correlations within populations. Specialized adaptations such as facultative iteroparity (Bradshaw, 1986a) or high intraspecific competitive ability (Bradshaw and Holzapfel, 1989) may then evolve and dramatically affect realized fitness in a given local habitat without adversely affecting potential fitness over a broad range of habitats.

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