J. evol. Biol. 6: 707–723 (1993)

1010-061X/93/050707-17 $ 1.50 + 0.20/0

Genetic coordination of demography and phenology in the pitcher-plant mosquito, *Wyeomyia smithii*

Jeffrey J. Hard[1], William E. Bradshaw[2] and Christina M. Holzapfel

Department of Biology, University of Oregon, Eugene OR 97403, U.S.A.

Key words: Life-history; epistasis; photoperiod; seasonality; biogeography.

Abstract

Demographic and phenological traits compose the basic elements of an insect's life-history strategy in a seasonal environment. An insect's long-term fitness depends on its ability to exploit favorable conditions, to avoid unfavorable conditions, and to convert from one life style to the other. For the pitcher-plant mosquito, *Wyeomyia smithii*, we show that genetic variation exists in both development time (a demographic trait) and critical photoperiod (a phenological trait) in six populations spanning much of the species' geographical range. During the northward range expansion of *W. smithii* in North America, these traits have evolved independently under strong directional and stabilizing selection. The correlated response in critical photoperiod to divergent selection on development time reveals significantly positive genetic correlations in five populations and a negative correlation in one population. The positive correlations form a genetically coordinated phenotype: faster developing individuals use a shorter photoperiodic switch point and are able to exploit the late favorable season; slower developing individuals use a longer photoperiodic switch point and are able to avoid extending development into the unfavorable season. This genetic coordination of demography and phenology has not, however, prevented their independent evolution. We propose that evolutionary flexibility in *W. smithii* may arise in part from the reorganization of their genetic architecture following repeated founder events during their northward invasion of North America.

[1] Current address: National Marine Fisheries Service, Northwest Fisheries Science Center, Coastal Zone & Estuarine Studies Division, 2725 Montlake Blvd. E., Seattle, WA 98112, USA.

[2] Author for correspondence.

Introduction

For insects in seasonal environments, 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). Demographic traits such as growth, development, and reproduction are generally associated with continuous life cycles and contribute directly to ecological success. Phenological traits such as dormancy and migration generally disrupt or delay continuous life cycles, and 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). Since a genotype's age at first reproduction and generation time have a large impact on 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 unfavorable food, temperature, and/or drought. Dormancy and migration provide "escape in time and space" (Slobodkin, 1961) and thus are crucial to long-term fitness despite the concomitant life-cycle delays (Bradshaw, 1986b).

We believe the distinction between demographic and phenological traits to be fundamental to insect life-history strategies in seasonal environments. Under such circumstances, no life-cycle strategy can be complete without demographic tactics to exploit favorable conditions and without phenological tactics to detect and avoid unfavorable conditions through migration or diapause. The value of inducible life-cycle conversions is clear: normal growth, development, and reproduction may proceed while conditions are favorable for these activities but are discontinued when conditions deteriorate or are likely to do so.

To investigate the evolutionary coordination of demography and phenology, we describe genetic variation and covariation in demographic and phenological life-history traits in the pitcher-plant mosquito, *Wyeomyia smithii* (Coq.). We focus on two representative traits defined as follows: (1) Development time is the time required for direct development from oviposition to adult emergence. We have chosen development time as a representative demographic trait because of its large impact on age of first reproduction, mean generation time, and, consequently, fitness under conditions favoring direct development. (2) Photoperiodic response is the use of daylength to switch from a program of direct development to one of diapause (dormancy). The critical photoperiod of an individual is the daylength below which it enters or maintains diapause and above which it continues or resumes direct development, and the critical photoperiod of a population is the mean of its individual critical photoperiods. We have chosen critical photoperiod as a representative phenological trait because of its widespread occurrence among insects (Beck, 1980; Saunders, 1982; Tauber et al., 1986), its pivotal role in the conversion between direct development and diapause, and its central importance in theoretical models of the optimal timing of diapause (Taylor, 1980, 1985, 1986a, b, c). Response to photoperiod depends not only on the critical photoperiod,

but also on the depth or firmness with which diapause is established. Like critical photoperiod, depth of diapause varies over the range of *W. smithii* (Bradshaw and Lounibos, 1977) and is positively genetically correlated with critical photoperiod (Campbell and Bradshaw, 1992). Thus, more northern populations enter diapause earlier and establish a deeper diapause relative to more southern populations.

To compare the life histories of populations along a latitudinal gradient that parallels the species' evolutionary pathway (Hard et al., 1993), we sampled three southern (ancestral) populations of *W. smithii* known to differ markedly from three northern (derived) populations in morphology, behavior, physiology, and density at the locality of origin (Bradshaw and Lounibos, 1977; Bradshaw and Holzapfel, 1986, 1990). For each population, we then: (1) imposed six generations of divergent selection on development time by establishing two selected lines, one for fast development and one for slow development, and maintained an unselected control line; (2) estimated the heritability of development time from the direct response to selection; and (3) measured the correlated response of critical photoperiod and depth of diapause to direct selection on development time.

Materials and methods

Wyeomyia smithii

Wyeomyia smithii is distributed in eastern North America from the Gulf of Mexico (30° N) to north-central Manitoba (54° N). Throughout its range, *W. smithii* completes its preadult development year round only in water-filled leaves of its carnivorous host, the purple pitcher plant, *Sarracenia purpurea* L. (Sarraceniaceae). The mosquitoes overwinter in the leaves in a larval diapause initiated, maintained, and terminated by photoperiod (Bradshaw and Lounibos, 1977). In unchilled larvae, the critical photoperiod is the same for the initiation and termination of diapause (Bradshaw and Lounibos, 1972); we take advantage of this relationship in designing the experiments in this paper. Geographical, physiological, morphological, and behavioral evidence indicates that *W. smithii* has evolved from south to north in North America (Istock and Weisburg, 1987; Bradshaw and Holzapfel, 1990) along a gradient of increasing seasonal harshness. Along this gradient, the photoperiodic response of *W. smithii* to seasonal change in daylength increases linearly with latitude and altitude ($R^2 = 0.96$, Bradshaw, 1976; Bradshaw and Lounibos, 1977). Thus, the photoperiodic response of *W. smithii* reflects seasonal adaptation along its evolutionary pathway in North America.

Selection and estimation of responses

Establishment of experimental lines. – Approximately 2000 larval *W. smithii* were collected from the 1987–1988 overwintering generation at each of six localities in eastern North America from 30–49° N (Tab. 1). Collections were made when 100%

Table 1. Origin of populations, percent cumultive inbreeding, and mean effective population size (N_e) in lines selected for fast (F) or slow (S) development or maintained as unselected controls (C) over 14 generations.

Pop[a]	Ref[b]	°N Lat	°W Lon	m Elev	Cumulative Inbreeding[c]			Mean N_e		
					F	S	C	F	S	C
FL1	WI	30.1	85.0	10	4.4	5.2	3.4	89	72	145
FL2	CR	30.7	86.6	40	5.0	7.2	3.4	77	62	137
NC	GS	34.2	78.3	20	5.5	5.5	3.5	77	62	139
MA	FV	42.5	71.5	60	5.0	8.4	3.6	97	51	132
ME	KC	46.2	68.3	250	5.7	5.8	5.1	66	83	91
ON	DL	49.6	94.1	370	6.7	6.2	4.2	86	90	116

[a] Postal code abbreviations of states or provinces; FL2 lies 300 km west of FL1

[b] Cross references to Bradshaw and Lounibos (1977) and Bradshaw and Holzapfel (1990)

[c] Actual cumulative inbreeding during each generation of selection plus 0.8 due to inbreeding during the non-selected generations with $N_e > 250$.

of the population and, hence, all of the population's genotypes were represented by the diapausing larvae in pitcher-plant leaves. Ovipositing *W. smithii* in nature deposit only a few eggs in each pitcher (Bradshaw, 1983) and, to sample as wide a range of genomes as possible, we collected larvae from over 100 leaves, selecting primarily those few leaves on each plant that contained the most larvae.

Maintenance of stock populations and the basic culture regimen were modified from Bradshaw (1986a) and Bradshaw and Holzapfel (1989) and described in Hard et al. (1992). Two controlled-environment rooms were used in this study. The short-day room maintained a constant $21 \pm 0.5°$ C with a diapause-inducing photoperiod of L : D = 8 : 16. The long-day room maintained a sine-wave 24 h thermoperiod fluctuating from 13° C–29° C (mean, 21° C), a relative humidity of 80%, and a long-day photoperiod (17 h light, 7 h dark) leading the thermoperiod by 3 h. A 0.5-h transitory twilight preceded and followed the main photophase. The environment in this room simulated natural midsummer conditions common to all six populations (Bradshaw, 1980; Bradshaw and Phillips, 1980) and provided unambiguous long days to allow uninterrupted development in all six populations (Bradshaw and Lounibos, 1977). All experiments were run in the leaves of intact pitcher plants. To mimic the prey-capture cycle of pitcher-plant leaves in nature (Bradshaw, 1983; Bradshaw and Holzapfel, 1983), each replicate leaf received 25 freeze-dried adult *Drosophila melanogaster* Meigen when mosquito larvae were added, an additional 100 flies after 7 d, 50 flies after 14 d, and 25 flies after 21 d. After the addition of 100 flies, leaf contents were gently stirred with a smooth glass paddle whenever fungal mycelia were visible among decaying flies. The use of live pitcher plants as experimental habitats and the environmental conditions in the controlled-environment room enabled us to simulate conditions in nature, thus minimizing the effect of the novel laboratory environment on estimates of genetic parameters in the newly colonized populations (Stearns, 1976; Gupta and Lewon-

tin, 1982; Service and Rose, 1985; Bell and Koufopanou, 1986; Clark, 1987). To eliminate residual field effects, all populations were cultured with mass mating in the long-day room for two generations without artificial selection before starting selection experiments. Selection was initiated in the F_2 generation.

Selection on development time. – In the long-day room, eggs were collected daily from each of the 12 initial lines (selected and control from each of 6 populations), floated on distilled water in petri dishes, and allowed to hatch. Eggs were checked for hatching at approximately the same time daily. Larvae in a given day's hatch were counted and a fraction then retained for selection (Fig. 1). In this way, larvae founding each succeeding generation were derived from eggs laid throughout the duration of oviposition and thus sampling the broadest range of females practically possible.

Larvae within a selection line were kept in the same terrarium, which was assigned each generation with the use of a random number table, as were the allocation of replicates to individual leaves. Leaves were rinsed thoroughly with distilled water; then, in each development-time replicate, a leaf received 20 freshly hatched larvae in 15 ml distilled water. In the F_2 generation, 200 larvae in 10 leaves were used to establish the control (unselected) line and 600 larvae in 30 leaves to establish the cohort from which the founders of the fast and slow developing lines were to be selected. Thereafter, 200 larvae in 10 leaves were used to found each succeeding control generation and 400 larvae in 20 leaves to found each line selected for fast or slow development.

Beginning 16 d after a leaf initially received freshly hatched larvae, the leaf contents were removed three times weekly with a teflon-tipped 60-ml Saybolt pipet and examined for pupae. Leaves were censused until all larvae had died or pupated or until 90 d had elapsed since the leaf was colonized. Pupae were removed, sexed, and placed in 50 ml distilled water in covered plastic dishes labelled by line, sex, and oviposition date.

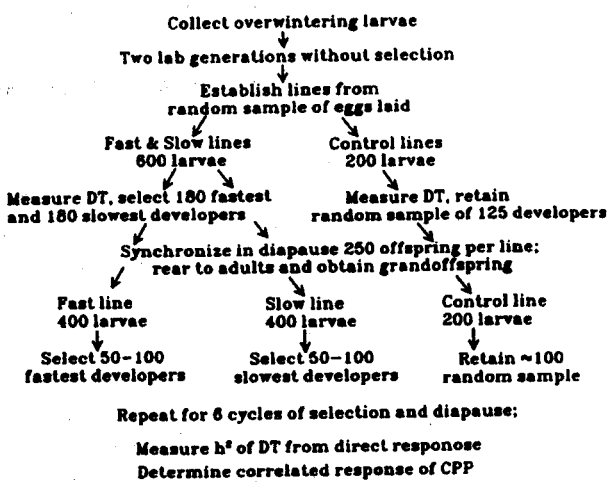

Fig. 1. Design of experiments to measure genetic heritability in development time (DT) and the correlated response of critical photoperiod (CPP) to selection on development time.

Pupal dishes were checked at approximately the same time daily for adult eclosion, when development time (d from oviposition to adulthood) was recorded. Truncation selection was imposed on development time in each population so that the fastest 10–25% of developing individuals founded the fast line in the next generation and a similar proportion of the slowest-developing individuals founded the slow line in the next generation (Fig. 1). Selected adults in a given line were allowed to swarm, mate, and oviposit in a 12-liter adult cage; unselected adults were discarded after measurement of their development times. Adults eclosing after 70 d of age were rare and were not allowed to found new lines in any population; however, adults that eclosed between 70 and 90 d old were included in calculation of selection responses. Because male *W. smithii* develop on average faster than females, selection criteria were imposed independently on males and females in order to equalize as much as possible the intensity of selection on each sex.

Even at low density, development in *W. smithii* is spread over 10 wk. Consequently, generations overlap. To synchronize development within and between all lines, the offspring of each selected generation were placed in petri dishes in the short-day room to induce diapause. After the offspring of the selected generation were synchronized in diapause, that diapause was terminated abruptly by transferring the larvae to the long-day room. Developing larvae were reared to adulthood and their resulting offspring reared in leaves and again subjected to selection. Thus, selection was imposed only every other generation but this technique permitted simultaneous selection by truncation for a temporal character in diverging lines (Fig. 1). This procedure provided three other advantages. First, excess population could be culled by removing a set fraction of the diapausing larvae that resulted from a given day's oviposition, ensuring that each female contributed a fixed fraction to the breeding population. Secondly, emergence times from diapause under unambiguously long days were highly synchronous, thus greatly increasing the probability of random mating with respect to development time before selection was imposed in the next generation. Finally, linkage disequilibrium resulting from selection and assortative mating that occurred within selected lines could be reduced.

Non-selected generations equalled or exceeded 250 individuals; average effective population sizes ($1/N_e = 1/4N_♂ + 1/4N_♀$) in selected lines varied from 51 to 97 (Tab. 1). On two occasions, truncation selection resulted in low effective sizes of 22 (NC population) and 25 (ON population). The cumulative inbreeding coefficients, based on the known number of males and females remaining after selection and assuming a minimum N_e of 250 in the non-selected generations, ranged from 3.4 to 8.4% (Tab. 1). The selected lines should therefore have avoided most stochastic variation in correlated response that can occur in small populations (Gromko et al., 1991).

Direct response of development time to selection. – Development times at any density were approximately lognormally distributed; consequently, the analysis of development time was based on $\log_{10}$ transformation of raw data. In each generation, truncation selection of fast or slow developing individuals was aimed at optimizing the selection differential and maintaining a sufficient breeding popula-

tion to found the next generation. Each generation's selection was based on the performance of the previous generation and the selection differential calculated from the actual performance of each generation.

For each population, the realized heritability of development time was calculated after six generations of divergent selection of this trait from the ratio of total selection response (**R**) to total selection differential (**S**) (Hill, 1972): $h^2_{DT} = \mathbf{R}/\mathbf{S}$. The variance of the heritability was calculated by: $\sigma^2(h^2_{DT}) = \sigma^2(\mathbf{R})/\mathbf{S}^2$, where $\sigma^2(\mathbf{R})$, the variance in response was estimated from

$$\sigma^2(\mathbf{R}) \simeq 2(f_f + f_s)h^2_{DT}\sigma^2_z + (1/M_f + 1/M_s)\sigma^2_z$$

where f_f and f_s are the inbreeding coefficients in the fast- and slow-selected lines, respectively, at the end of six generations of selection, σ^2_z is the phenotypic variance of development time, and M_f and M_s are the sample sizes for development time in the respective selected lines in the final generations (for the ith selected line, $1/M_i = [M_{i(mo)} + M_{i(fa)}]/[4M_{i(mo)}M_{i(fa)}]$, where mo refers to mothers and fa refers to fathers). Because selection differentials differed between both generations and sexes, the inbreeding coefficients for each selected line were calculated by

$$2f \simeq \sum \{1/[4N_{mo}(k)] + 1/[4N_{fa}(k)]\}$$

where $N_{mo}(k)$ and $N_{fa}(k)$ are the numbers of mothers and fathers selected in generation k (M. Lynch and B. Walsh, unpubl. MS).

Correlated response of critical photoperiod under changing photoperiods. – The correlated responses of critical photoperiod and depth of diapause were determined for each population and selected line at the end of selection. To determine the correlated response of critical photoperiod to selection on development time, larvae within each line were pooled and placed in the short-day room for 30 days to induce diapause. After 30 d they were returned to the long-day room and exposed to an "astronomically" increasing daylength, simulating the approximately linear increase in photoperiod near the vernal equinox in nature. All determinations of astronomic critical photoperiod were made in light-tight cabinets as described in Hard et al. (1992). The photoperiod began at a point 2.5–4.0 hr shorter than the estimated critical photoperiod for that population and subsequently increased 3 min/d. Larvae under this regime, at a density of 20–25 per 150 mm petri dish, were fed and examined for development every 1–3 d (daily near the critical photoperiod) and larval dishes cleaned every 1–2 wk. Sex and photoperiod at pupation were recorded for each pupa. Experiments were run until all larvae had pupated or died, or else for 100 d (5 hr range in photoperiod). Critical photoperiod was calculated as mean $\log_{10}$ photoperiod at pupation.

Correlated response of critical photoperiod under static photoperiod. – To isolate any confounding effect of development time on estimation of critical photoperiod, another sample of 300–550 larvae from throughout the oviposition distribution in each line was divided equally among 6–11 static (constant, unchanging)

photoperiods in 15- or 30-min increments. Choices of photoperiods and intervals were based on experiments of Campbell and Bradshaw (1992) and were clustered around the line-specific critical photoperiod. Fifty larvae, 25 to a petri dish, were exposed to each static photoperiod. The development of the larvae was monitored under these photoperiods for 45 d. Static critical photoperiod was estimated from direct interpolation of 50% development from cohorts with photoperiods near the inflection point of the photoperiodic response curve, where the curve is nearly linear.

Divergence of static critical photoperiod among selected and control lines in each population was determined in reference to the critical photoperiod for the control line (Fig. 2). To maximize discriminating power while minimizing cells with zero entries (0% development or 0% diapause), development at the two treatments above and the two below the critical photoperiod in the control line was analyzed in a three-way contingency table (line × photoperiod × development). The analysis of frequencies involved a loglinear model (Sokal and Rohlf, 1981) that assessed the effects of line (L) and photoperiod (P) on development (D), where D was a dichotomous variable (pupated, not pupated) whose cells were each weighted by the number of observations. The model incorporated all three two-factor interactions (DL, LP, and DP) and the single three-factor interaction (DLP). The significance of the three-factor interaction was tested first; if not significant, the two-factor interaction DL was tested. The other two-factor interactions were not tested. A significant DP interaction is trivial since it indicates only that development depends upon photoperiod, a response already well established for *W. smithii* (Bradshaw and Lounibos, 1977). A significant LP interaction indicates that the slope of the photoperiodic response curve differs among lines. This difference is trivial in the present context as it would be expected if one is sampling different portions of offset sigmoid curves.

For any analysis, 0.5 was added to each cell frequency if more than 20% of cells had low expected frequencies ($\hat{n} < 5$). The simultaneous testing of conditional

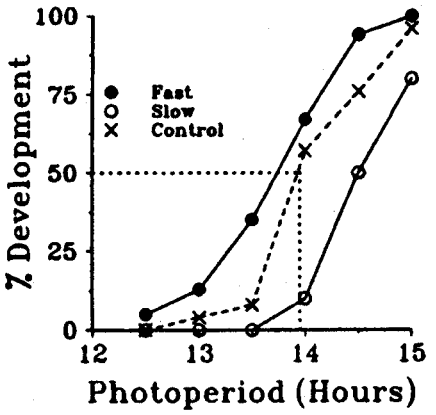

Fig. 2. Testing for correlated responses of static critical photoperiod with a log-linear model. Developmental response was assessed at two photoperiods above and two below the critical photoperiod in the control line.

independence increases the risk of type I error (Sokal and Rohlf, 1981). Therefore, to avoid spurious rejection of null hypotheses of independence, the overall significance level for rejection of independence or goodness-of-fit in individual tests was set to 0.01. Significance of interactions was tested with the likelihood-ratio chi-square, G (Sokal and Rohlf, 1981). Where data near the critical photoperiod were not sufficient to construct a loglinear model, tests of independence of L and D used Fisher's exact test from two-way contingency tables (Sokal and Rohlf, 1981).

Correlated response of depth of diapause. – The depth of diapause (T_{50} of Bradshaw and Lounibos, 1977) is defined as the number of long days required to stimulate development in 50% of a diapausing population (Saunders, 1982). To determine depth of diapause, a sample of 150–500 larvae from throughout the oviposition distribution in each line was divided into 3–10 equal cohorts of 50 larvae, which were then exposed to 0 (control) or from 1 to 10 long days (L : D = 17 : 7) followed by short days (L : D = 8 : 16). The number of long days in an exposure was determined from experiments of Campbell and Bradshaw (1992) and was line-specific. Subsequent development was monitored for 45 d. Depth of diapause and its divergence among experimental lines was estimated with the same technique as static critical photoperiod (Fig. 2), but with number of long days experienced as the independent variable.

Results

Development time and critical photoperiod in the base populations

Mean development time (Fig. 3A) in the base population (F_2 lab generation) was not significantly correlated with latitude of origin ($r^2 = 0.47$, $P > 0.05$). Mean development time of the four more northern populations fell within one standard

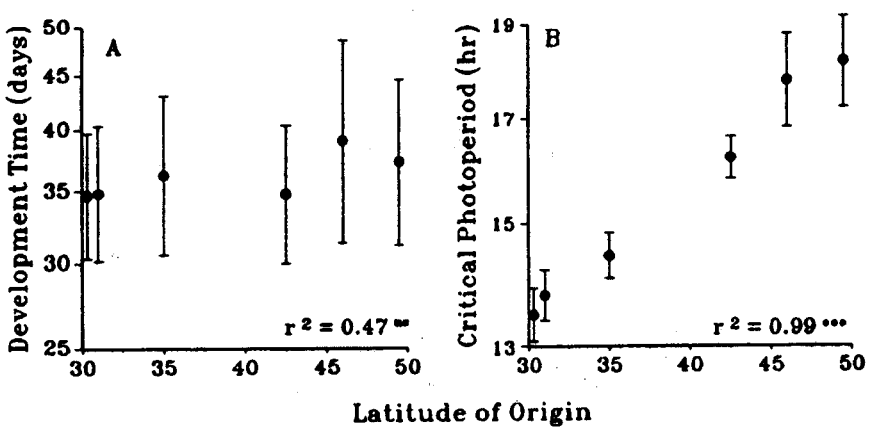

Fig. 3. Mean development time (A) and mean critical photoperiod (B) in the base population (F_2 lab generation). The error bars show ± one standard deviation.

deviation of the mean of either of the Florida populations. By contrast, mean critical photoperiod (Fig. 3B) in the base population (F_2 lab generation) was tightly correlated with latitude ($r^2 = 0.99$, $P < 0.001$). The mean of the most northern population exceeded the mean of either Florida population by more than nine standard deviations of the mean of the Florida populations. Our present populations are therefore consistent with earlier studies involving 12 or more populations that also showed significant correlation between critical photoperiod and latitude (Bradshaw and Lounibos, 1977; Bradshaw and Holzapfel, 1990) but not between development time and latitude (Bradshaw and Holzapfel, 1990).

Direct response of development time to selection

Development time showed a detectable response to six generations of divergent selection in five of the six populations (Fig. 4A). In all populations except FL2, lines selected for fast development developed significantly faster than lines selected for slow development. Similarly, realized heritabilities (h^2_{DT}) were significantly different from zero ($P < 0.05$) in all populations except for FL2 (Tab. 2). The

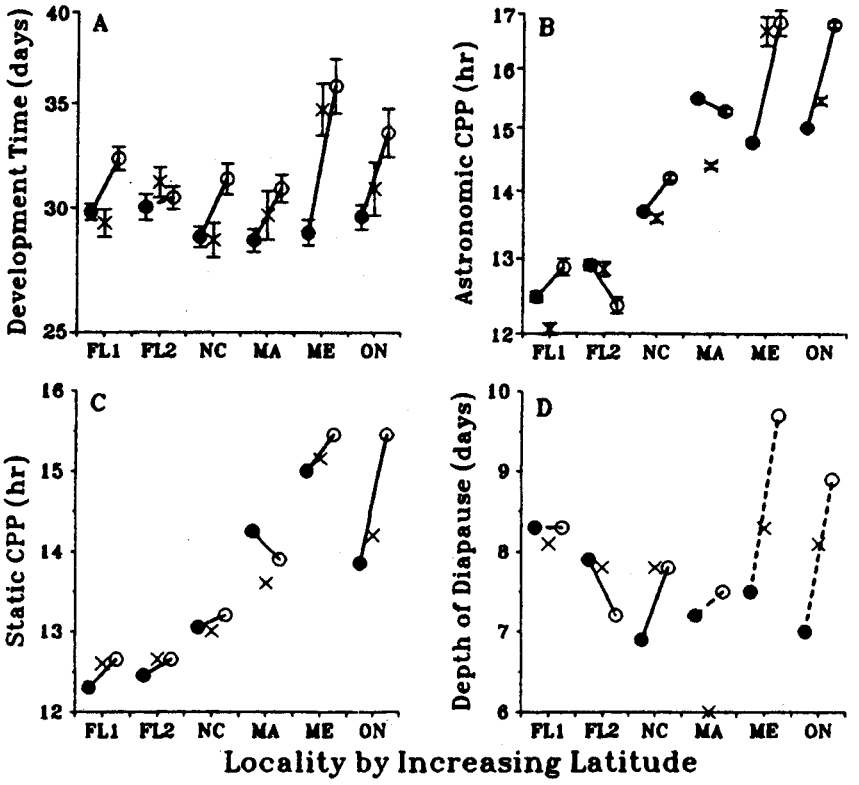

Fig. 4. Direct (A) and correlated responses (B–D) to selection on development time. Line means (±2SE in A and B) identified by ● Fast, × Control, ○ Slow. Difference between Fast and Slow lines shown by — significant (A–B, $P < 0.05$; C–D, $P < 0.01$) or - - - non-significant.

Table 2. Realized heritability of development time (h^2_{DT} estimated from the ratio of total response to total selection differential on development time, S_{DT}) and total correlated response of astronomical critical photoperiod (CR_{ACPP}), static critical photoperiod (CR_{SCPP}), and depth of diapause (CR_{DOD}) to selection on development time.

Pop	S_{DT}[a]	$h^2_{DT} \pm 2$S.E.	CR_{ACPP}[b]	CR_{SCPP}[d]	CR_{DOD}[c]
FL1	0.776	0.044 ± 0.019	0.014	0.35	0.0
FL2	0.767	0.009 ± 0.013	−0.019	0.20	−0.7
NC	0.869	0.043 ± 0.019	0.016	0.15	0.9
MA	0.875	0.038 ± 0.018	−0.007	−0.35	0.3
ME	1.039	0.090 ± 0.026	0.057	0.45	2.2
ON	0.723	0.074 ± 0.024	0.048	1.60	1.9

[a] log(days)
[b] log(hours)
[c] hours
[d] days

heritability of development time was not significantly correlated with latitude ($r^2 = 0.112$, $P > 0.05$).

Correlated response of phenological traits to selection

Astronomic critical photoperiod. – Direct selection on development time elicited significant differences in astronomic critical photoperiod between fast- and slow-developing lines in all six populations (Fig. 4B). Fast lines had significantly shorter critical photoperiods in two southern (FL1, NC) and two northern (ME, ON) populations. By contrast, slow lines had significantly shorter critical photoperiods in one southern (FL2) and one northern (MA) population.

Static critical photoperiod. – The relationship of static critical photoperiods between lines among populations (Fig. 4C) showed the same general geographic pattern as the astronomic critical photoperiods (Fig. 4B) with the notable exception of the FL2 population. In the FL2 population, the line selected for fast development had a significantly shorter static critical photoperiod than the line selected for slow development, a reversal of the relationship between the same lines exposed to an astronomic photoperiod. As in the response to astronomic photoperiods, the relationship of static critical photoperiods (Fig. 4C) between the lines selected for fast and slow development and the control line was erratic, especially for the MA population.

Depth of diapause. – Direct selection on development time elicited a significant correlated response in depth of diapause in only two southern populations (FL2 and NC in Fig. 4D). Relative to the line selected for slow development, the line selected for fast development entered a deeper diapause in the FL2 population and a

shallower diapause in the NC population. For the northern populations, the number of long days required to induce 50% development in a cohort exceeded the maximum number of long days to which we exposed the cohort. The depths of diapause shown in Fig. 4D for northern populations are based on extrapolations. However, the absolute differences in depths of diapause between lines in these northern populations are often larger than those in the southern populations where line differences are significant. Consequently, the lack of a significant difference in depth of diapause between lines selected for fast and slow development in these populations may be due to a lack of discriminating power in our statistical test than to a lack of response.

Discussion

Phenotypic evolution of demography and phenology

Development time in *W. smithii* exhibits low but detectable genetic variability in populations from throughout the species's range. The range of heritabilities of development time observed in the present study (Tab. 2) contrasts with the heritability estimate of 0.22 in a New York (42.5° N) population from parent-offspring regression when offspring cohort size was restricted to 20–25 (Istock et al., 1976). Istock's heritability was based on unweighted cohort means, not individuals, and on cohorts reared in glass jars with a highly regimented fish-food diet. Thus, Istock was able to minimize the effects of individual variation and environmental variance (V_e) in his experiments relative to our estimates under near-natural conditions. We know of no ecological difference between localities that would account for this discrepancy. Kennedy Bog (Istock et al., 1976) is a typical boreal bog at the same latitude as our Massachusetts locality and is ecologically similar to our other two localities. Istock's and our 3 northern localities are ecologically distinct from the wet pine savannahs of our 3 southern localities; yet we obtained similar heritabilities in populations from southern and northern regions (Tab. 2). Regardless, the important point is not the discrepancy between the magnitude of Istock's and our estimates of the heritability of development time in *W. smithii*; rather, both studies show that there is a sufficient genetic variability underlying development time to permit considerable response to natural selection.

Development time, along with other demographic traits, varies among populations but not in concert with the geographical gradient of climate and density-dependent development (Fig. 3A; Bradshaw and Holzapfel, 1989, 1990). Mean development time of derived (northern) populations has diverged less than one standard deviation of ancestral (Florida) populations, indicating weak directional selection over this gradient.

By contrast with development time, critical photoperiod in *W. smithii* exhibits high genetic variability both among (Bradshaw and Lounibos, 1977; Bradshaw and Holzapfel, 1990) and within (Bradshaw and Holzapfel, 1990; Scheiner and Istock, 1991; Campbell and Bradshaw, 1992; Hard et al., 1993) populations. The heritability of this trait ranges from about 0.2 to 0.8, and additive genetic variance increases

with latitude (Hard et al., 1993). At the same time, critical photoperiod itself is tightly correlated with latitude and, hence, climate (Fig. 3). Mean critical photoperiod of the most derived popualtion (ON) has diverged more than nine standard deviations from ancestral (FL) populations (Fig. 4B). These results indicate that there has been both strong directional selection on this trait along the climatic gradient of North America and optimizing selection within each population.

The differences in geographic variation of development time and critical photoperiod lead us to conclude that these traits have evolved independently over *W. smithii*'s evolutionary trajectory (its pattern of dispersal, colonization, and differentiation through space and time). The question remaining is whether this independent evolution has taken place because of or despite an underlying genetic correlation between development time and critical photoperiod.

Evolutionary coordination of demography and phenology

The correlated responses of astronomic critical photoperiod to direct selection on development time (Fig. 4B) show, in each population, significant differences between lines selected for fast and slow development. Variation in responses to the astronomic photoperiods includes variation in the photoperiodic switch point but also variation in both the depth of diapause and the duration of post-diapause development leading to pupation. Variation in response to static photoperiods (Fig. 4C) reflects primarily variation in the photoperiodic switch point only, since the experimental period allowed sufficient time (45 d) for mosquitoes deep in diapause to terminate diapause and complete post-diapuase development. The relative response of fast and slow developing lines to astronomic photoperiods coincides with the response to static photoperiods in five of six populations. In the sixth population, FL2, the line selected for fast relative to slow development has a longer apparent critical photoperiod in response to the astronomic regime (Fig. 4B) but a shorter critical photoperiod in response to the static regime (Fig. 4C). At the same time, the line selected for slow development enters a significantly shallower diapause than the line selected for fast development, in contrast to all the significant and non-significant trends in the other five populations (Fig. 4D). Thus, for the FL2 population, the lower astronomic critical photoperiod in the slow than in the fast line results from a shallower diapause in the slow than fast line; the true genetic correlation between development time and photoperiodic switch point in this population is positive, reflecting a lower critical photoperiod in the fast than slow line as indicated in Fig. 4C.

From these observations, we make two conclusions. (1) A genetic correlation exists between development time and critical photoperiod in all populations. (2) In five of the six populations, as predicted by Istock et al. (1976) and implied by Scheiner and Istock's (1991) results, fast developing larvae have shorter critical photoperiods than slow developing larvae. These observations are consistent with a positive genetic correlation between these traits. Because, for a given photoperiodic switchpoint, fast-developing genotypes are better able to replace themselves and

enter diapause before the end of season arrives, natural selection should favor such a correlation in seasonal insects (Istock et al., 1976; Taylor, 1980, 1986a; Istock, 1983). Thus, a positive genetic correlation between these traits is indicative of genetic coordination of demography and phenology.

We believe that the similar correlated responses for most populations in static critical photoperiod and depth of diapause between lines selected for fast and slow development constitutes definitive evidence that, in general, demography and phenology are genetically correlated in ancestral as well as derived populations of *W. smithii*. In this case, the independent evolution of critical photoperiod and development time (Fig. 3) has occurred despite and not because of their underlying genetic correlation. From theoretical arguments, Via and Lande (1985) and Zeng (1988) predicted that a genetic correlation between traits may affect their short-term covariation and adaptation but should not prevent their independent evolution in the long term. Both previous theory and our empirical results with *W. smithii* support the conclusion that the evolution of correlated traits can be affected more by the nature of their joint relationship to fitness than by the direction of their genetic correlation. Genetic coordination of life-history traits does not then preclude their evolutionary flexibility.

Evolutionary genetics of life history

Herein we propose that the independent evolution of genetic correlations among life-history traits in *W. smithii* may reflect the reorganization of genetic relationships following repeated founder events. Several observations are important to our proposition. First, none of the genetic correlations is equal to unity, providing at least the potential for the independent evolution of correlated traits (Via and Lande, 1985; Zeng, 1988). Second, gene flow in *W. smithii* may be substantial within bogs but is disrupted even between nearby bogs (Istock and Weisburg, 1987). Third, the relationship between the control and selected lines in Fig. 4 varies among populations but this variation shows no clear correlation with latitude. Fourth, differentiation of critical photoperiod in *W. smithii* along the south-north climatic gradient in North America has involved potentially interacting loci, particularly additive × additive and dominance × dominance epistasis (Hard et al., 1992, 1993). Different gene–gene interactions are apparent in crosses among southern as well as between southern and northern populations. Epistatic effects change in both magnitude and sign but no component of epistasis is significantly correlated with latitude. We have argued (Hard et al., 1993) that this pattern of restricted gene flow and changing genetic interactions may be due to repeated founder events during the northward evolution of *W. smithii*.

Because of *W. smithii*'s tight host specificity, pitcher plants must have spread to new localities before the establishment of mosquito populations. At the same time, *W. smithii* is a weak flyer "extremely prone to death by desiccation" (Istock and Weisburg, 1987). Natural populations were therefore probably founded by a few individuals colonizing a resource-rich habitat. Breakup of dominance and epistatic

interactions during a founder event potentially can alter the genetic background of a population, including the release of formerly covert additive genetic variation (Goodnight, 1987, 1988). Even if genetic effects appear additive on a phenotypic scale, stabilizing selection can create extreme epistasis on a fitness scale so that the genetic background can "have a major influence on the adaptive value of a particular allele which may be advantageous in some gene combinations and deleterious in others" (Lande, 1980). Thus, following a founder event, selection for dominance and/or epistatic modifiers of pleiotropic effects can lead to the attainment of a new Wrightian (fitness) peak in the local adaptive landscape (Dobzhansky, 1955; Mayr, 1963; Carson and Templeton, 1984). Such reorganizations need not lead to "genetic revolutions" as these authors suggested, but may lead instead to local "genetic uprisings" that produce novel gene interactions within populations and, hence, intraspecific variation among populations without generating incipient speciation.

Given the relationship between *W. smithii* and its host plant, we believe that genetic uprisings have been recurrent events in this mosquito's northward migration during at least the present interglacial period. Such uprisings during successive founder-flush episodes can result in independently altered genetic architectures, encompassing not only additive effects, but also pleiotropic, dominance, and epistatic relationships. During these episodes, the optimum phenotype and underlying genetic correlations may change even if the ecological setting in a new habitat is identical to that in the ancestral habitat. In this way, genetic uprisings may create adaptive opportunities that might not otherwise be possible, including the transient disruption and subsequent coordination of phenological and demographic traits.

Acknowledgments

We thank C. Kleckner and R. King for expert assistance in maintaining stock populations of mosquitoes. L. Heisler, M. Lynch, R. Lande, and two anonymous reviewers provided thoughtful consideration of the manuscript. M. Lynch and B. Walsh provided us with an unpublished manuscript that aided much of our analysis of realized heritability. We greatly appreciate intellectual feedback from C. Istock, P. Service, F. Taylor, and M. Lynch during the formative period of this research. This study was supported by National Science Foundation Grant BSR-8717151 to W. Bradshaw and by National Institutes of Health Genetics Training Grant 5 T32 GM 07413-15 to J. Hard.

References

Beck, S. D. 1980. Insect Photoperiodism. Academic Press, N.Y.

Bell, G. and V. Koufopanou. 1986. The cost of reproduction. Oxf. Surv. Evol. Biol. 3: 83–131.

Bradshaw, W. E. 1976. Geography of photoperiodic response in a diapausing mosquito. Nature (London) 262: 384–386.

Bradshaw, W. E. 1980. Thermoperiodism and the thermal environment of the pitcher-plant mosquito, *Wyeomyia smithii*. Oecologia (Berlin) 46: 13–17.

Bradshaw, W. E. 1983. Interaction between the mosquito *Wyeomyia smithii* and the midge, *Metriocnemus knabi*, and their carnivorous host *Sarracenia purpurea*, pp. 161–189. *In* J. H. Frank and L. P.

Lounibos (eds), Phytotelmata: Terrestrial Plants as Hosts for Aquatic Insect Communities. Plexus Publishing, Medford, New Jersey, USA.

Bradshaw, W. E. 1986a. Variable iteroparity as a life-history tactic in the pitcher-plant mosquito *Wyeomyia smithii*. Evolution 40: 471–478.

Bradshaw, W. E. 1986b. Pervasive themes in insect life cycles, pp. 261–275. *In* F. Taylor and R. Karban (eds), The Evolution of Insect Life Cycles. Springer-Verlag, London, U.K.

Bradshaw, W. E. and C. M. Holzapfel. 1983. Life cycle strategies in *Wyeomyia smithii*: seasonal and geographic adaptations, pp. 169–187. *In* V. K. Brown and I. Hodek (eds), Diapause and Life Cycle Strategies in Insects. Dr. W. Junk, The Hague.

Bradshaw, W. E. and C. M. Holzapfel. 1986. Geography of density-dependent selection in pitcher-plant mosquitoes, pp. 48–65. *In* F. Taylor and R. Karban (eds), The Evolution of Insect Life Cycles. Springer-Verlag, N.Y., USA.

Bradshaw, W. E. and C. M. Holzapfel. 1989. Life-historical consequences of density-dependent selection in the pitcher-plant mosquito, *Wyeomyia smithii*. Am. Nat. 133: 869–887.

Bradshaw, W. E. and C. M. Holzapfel. 1990. Evolution of phenology and demography in the pitcher-plant mosquito, *Wyeomyia smithii*, pp. 47–67. *In* F. Gilbert (ed.), Insect Life Cycles: Genetics, Evolution and Co-ordination. Springer-Verlag, London, U.K.

Bradshaw, W. E. and L. P. Lounibos. 1972. Photoperiod control of development in the pitcher-plant mosquito, *Wyeomyia smithii*. Can. J. Zool. 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.

Bradshaw, W. E. and D. L. Phillips, 1980. Photoperiodism and the photic environment of the pitcher-plant mosquito, *Wyeomyia smithii*. Oecologia (Berlin) 44: 311–316.

Campbell, M. D. and W. E. Bradshaw. 1992. Genetic coordination of diapause in the pitcherplant mosquito, *Wyeomyia smithii*. Ann. Entomol. Soc. Am. 85: 445–451.

Carson, H. L. and A. R. Templeton. 1984. Genetic revolutions in relation to speciation phenomena: the founding of new populations. Ann. Rev. Ecol. Sys. 15: 97–131.

Clark, A. G. 1987. Senescence and the genetic-correlation hang-up. Am. Nat. 129: 932–940.

Cole, L. C. 1954. The population consequences of life history phenomena. Quart. Rev. Biol. 29: 103–137.

Danilevskii, A. S. 1965. Photoperiodism and Seasonal Development of Insects. Oliver and Boyd, Edinburgh, U.K.

Danks, H. V. 1987. Insect Dormancy: An Ecological Perspective. Biological Survey of Canada (Terrestrial Arthropods), Ottawa, Canada.

Dingle, H. 1986. The evolution of insect life cycle syndromes, pp. 187–203. *In* F. Taylor and R. Karban (eds), The Evolution of Insect Life Cycles. Springer-Verlag, N.Y. USA.

Dobzhansky, Th. 1955. A review of some fundamental concepts and problems of population genetics. Cold Spring Harbor Symp. Quant. Biol. 20: 1–15.

Goodnight, C. 1987. On the effect of founder events on the additive genetic variance. Evolution 42: 441–454.

Goodnight, C. 1988. Epistasis and the effect of founder events on the additive genetic variance. Evolution 42: 441–454.

Gromko, M. H., A. Briot, S. C. Jensen and H. H. Fukui. 1991. Selection on copulation duration in *Drosophila melanogaster*: predictability of direct response versus unpredictability of correlated response. Evolution 45: 69–81.

Gupta, A. P. and R. C. Lewontin. 1982. A study of reaction norms in natural populations of *Drosophila pseudoobscura*. Evolution 36: 934–948.

Hard, J. J., W. E. Bradshaw and C. M. Holzapfel. 1992. Epistasis and the genetic divergence of photoperiodism between populations of the pitcher-plant mosquito, *Wyeomyia smithii*. Genetics 131: 389–396.

Hard, J. J., W. E. Bradshaw and C. M. Holzapfel. 1993. The genetic basis of photoperiodism and its evolutionary divergence among populations of the pitcher-plant mosquito, *Wyeomyia smithii*. Am. Nat. 142: (in press).

Hill, W. G. 1972. Estimation of realized heritabilities from selection experiments I. Divergent selection. Biometrics 28: 747–765.
Istock, C. A. 1983. The extent and consequences of heritable variation in fitness characters, pp. 61–96. *In* C. R. King and P. S. Dawson (eds), Population Biology: Retrospect and Prospect. Columbia University Press, N.Y., USA.
Istock, C. A. and W. G. Weisburg. 1987. Strong habitat selection and the development of population structure in a mosquito. Evol. Ecology 1: 348–362.
Istock, C. A., J. Zisfein and K. J. Vavra. 1976. Ecology and evolution of the pitcher-plant mosquito. 2. The substructure of fitness. Evolution 30: 535–547.
Lande, R. 1980. Genetic variation and phenotypic evolution during allopatric speciation. Am. Nat. 116: 463–479.
Lees, A. D. 1955. The physiology of diapause in arthropods. Cambridge Monogr. Exp. Biol. 4: 1–151.
Lewontin, R. C. 1965. Selection for colonizing ability, pp. 77–94. *In* H. G. Baker and G. L. Stebbins (eds), The Genetics of Colonizing Species. Academic Press, N.Y., USA.
Livdahl, T. P. 1979. Environmental uncertainty and selection for life-cycle delays in opportunistic species. Am. Nat. 113: 835–842.
Lynch, M. and B. Walsh, Principles of Evolutionary Quantitative Genetics. Unpubl. MS.
Mayr, E. 1963. Animal Species and Evolution. The Belknap Press of Harvard University Press, Cambridge, MA, USA.
Murphy, G. I. 1968. Patterns in life history and the environment. Am. Nat. 102: 391–403.
Saunders, D. S. 1982. Insect Clocks. 2nd ed. Pergamon Press, Oxford, U.K.
Scheiner, S. M. and C. A. Istock. 1991. Correlational selection of life history traits in the pitcher-plant mosquito. Genetica 84: 123–128.
Service, P. M. and M. R. Rose. 1985. Genetic covariation among life-history components: the effect of novel environments. Evolution 39: 943–945.
Slobodkin, L. B. 1961. Growth and Regulation of Animal Populations. Holt, Rinehart and Winston, N.Y.
Sokal, R. R. and F. J. Rohlf. 1981. Biometry: The Principles and Practice of Statistics in Biological Research. 2nd ed. W. H. Freeman and Co., N.Y., USA.
Stearns, S. C. 1976. Life-history tactics: a review of the ideas. Quart. Rev. Biol. 51: 3–47.
Tauber, C. A. and M. J. Tauber. 1981. Insect seasonal cycles: genetics and evolution. Ann. Rev. Ecol. Syst. 12: 281–308.
Tauber, M. J., C. A. Tauber and S. Masaki. 1986. Seasonal Adaptations of Insects. Oxford University Press, N.Y., USA.
Taylor, F. 1980. Optimal switching to diapause in relation to the onset of winter. Theor. Pop. Biol. 18: 125–133.
Taylor, F. 1985. Estimating the ends of the sensitive period for diapause induction in arthropods. J. Theor. Biol. 117: 319–336.
Taylor, F. 1986a. Toward a theory for the evolution of the timing of hibernal diapause, pp. 236–257. *In* F. Taylor and R. Karban (eds), The Evolution of Insect Life Cycles. Springer-Verlag, N.Y., USA.
Taylor, F. 1986b. The fitness function associated with diapause induction in arthropods. I. The effects of age structure. Theor. Pop. Biol. 30: 76–92.
Taylor, F. 1986c. The fitness function associated with diapause induction in arthropods. II. The effects of fecundity and survivorship on the optimum. Theor. Pop. Biol. 30: 93–110.
Via, S. and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39: 505–522.
Zeng, Z.-B. 1988. Long-term correlated response, interpopulation covariation, and interspecific allometry. Evolution 42: 363–374.

Received 16 February 1993;
accepted 9 June 1993.
Corresponding Editor: W. Gabriel