Genetic Coordination of Diapause in the Pitcherplant Mosquito, *Wyeomyia smithii* (Diptera: Culicidae)

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ABSTRACT Six populations of *Wyeomyia smithii* from 30–49°N latitude maintain significant heritability for and consistently positive genetic correlations among three components of photoperiodically mediated larval diapause: (1) critical photoperiod, (2) required day number, and (3) depth of diapause. These traits then form genetically coordinated syndrome of traits that reinforce each other in a continuum from diapause-prone to diapause-resistant genotypes. We propose that this syndrome maintains genetic flexibility in a habitat that imposes fluctuating stabilizing selection on diapause.

KEY WORDS Insecta, *Wyeomyia smithii*, genetic correlation, photoperiodism

DIAPAUSE IS A PERVASIVE developmental condition that enables many arthropods to escape unfavorable conditions. At temperate latitudes, the onset of diapause is mediated by photoperiod in a wide variety of insects (Beck 1980, Danks 1987, Tauber et al. 1986) as now documented in hundreds of species. Historically, diapause has been considered from an ecological and physiological viewpoint; evolutionary adaptive significance has been inferred largely from phenotypic correlations within and between populations (Andrewartha 1952, Lees 1955, Danilevskii 1965, Saunders 1982). Evolutionary inference must, however, be based on heritable and not just phenotypic variation and covariation of traits (Reznick 1985, Partridge & Harvey 1985, Bell & Koufopanou 1986). Genetic studies "have generally emphasized only one or two traits, that is, the geographically variable critical photoperiod for diapause induction and the duration of diapause" (Tauber et al. 1986, 208–209). The opportunities for and immediate constraints to adaptation are not necessarily reflected by interpopulation variation but must be resolved at the level of genetic variation and covariation within populations. Diapause is a complex developmental and physiological state with diverse components. In temperate milkweed bugs, for example, diapause is integrated into a genetically cohesive but flexible diapause–migration flight syndrome (Dingle 1978, Hegmann & Dingle 1982). In *Aedes triseriatus* (Say) direct selection for shorter critical photoperiod for embryonic diapause elicits a correlated decline in embryonic diapause at higher temperatures, an increase in the critical photoperiod mediating larval diapause, and an increase in the sensitivity of

larval diapause to embryonic photoperiod (Sims 1985). In this article, genetic variation and covariation of some components of larval diapause in another mosquito, *Wyeomyia smithii* (Coquillett) are examined.

Wyeomyia smithii oviposits into and completes its preadult development only within the water-filled leaves of the purple pitcher plant, *Sarracenia purpurea* L. The range of *W. smithii* follows that of its host plant from the Gulf of Mexico to north-central Canada from Labrador to Saskatchewan (30–54° N). *W. smithii* overwinters in a larval diapause that is induced and maintained by short days and averted or terminated by long days; diapause is photoreversible by long days at any time up to and including the diapause stage itself (Smith & Brust 1971, Bradshaw & Lounibos 1972, Evans & Brust 1972, Lounibos & Bradshaw 1975). Southern populations at low elevations below 36° N latitude enter diapause in the fourth instar; northern populations at higher latitudes, altitudes, or both, enter diapause in the third instar. The critical photoperiod for the onset and maintenance of diapause increases linearly with latitude or altitude, regardless of the diapause instar (Bradshaw 1976). The depth of diapause, however, increases with latitude in a saw-tooth pattern with a rapid decrease in depth of diapause at the latitudinal transition from fourth to third instar diapause (Bradshaw & Lounibos 1977). Herein we consider genetic variation and covariation of critical photoperiod (CPP) (number of hours of light per day experienced from day of hatch that results in 50% development [and 50% diapause]), depth of diapause (DOD) (number of long days experienced by diapausing larvae that results in 50% resumed development when subsequently followed by short days), and the required day number (not previously determined

in *W. smithii*) (RDN) (number of long days experienced from day of hatch that results in 50% development when subsequently followed by short days).

We address the central question of whether the genetic covariation of these traits within populations produces a reinforcing or compensating diapause syndrome. If the traits are all positively genetically correlated, then they are reinforcing. Diapause-prone individuals would enter diapause at longer photoperiods, require more long days to avert diapause, and enter a deeper diapause than diapause resistant individuals. By contrast, if the traits are both positively and negatively correlated, then they are compensating. For example, an increased tendency to enter diapause acquired by genes promoting entry into diapause at longer photoperiods would be offset by genes requiring fewer long days to avert diapause, sustaining a shallower diapause, or both. To determine whether patterns of reinforcement, compensation, or both, persist throughout the range of *W. smithii*, we consider three southern (30–35° N latitude) and three northern (42–49°N latitude) populations.

Approach and Rationale

If one applies direct selection on one trait and obtains a correlated response in a second trait, then the heritabilities of both traits as well as the genetic correlation between them must be nonzero (Falconer 1981, 286). Our basic approach was to impose divergent selection for increased or decreased CPP and RDN, and, from the direct and correlated response of CPP, RDN, and DOD, to identify significant heritabilities of and genetic correlations among these traits. Initially, we determined the CPP, RDN, and DOD in the base population. We imposed selection for an increase or decrease in CPP or RDN and assessed response to selection by exposing the selected lines and the unselected controls to CPP, RDN, and DOD of the base population. As illustrated in Fig. 1, a significantly higher percentage of diapause in a selected than in a control line indicated an increase in the trait being assessed. A significantly lower percentage indicated a decrease in the trait being assessed. We interpreted a decrease in CPP, RDN, or DOD to reflect a decreased tendency to diapause, and, conversely, for an increase in these traits. If direct selection for a decrease in one trait elicited a correlated decrease in the other traits, this response was interpreted as reinforcing; if direct selection for a decrease in one trait elicited an increase in one or both of the other traits, this response was interpreted as compensating. Because of differences in the intensity of selection within and among populations, we were unable to make quantitative estimates of the heritabilities and genetic correlations. We interpreted any
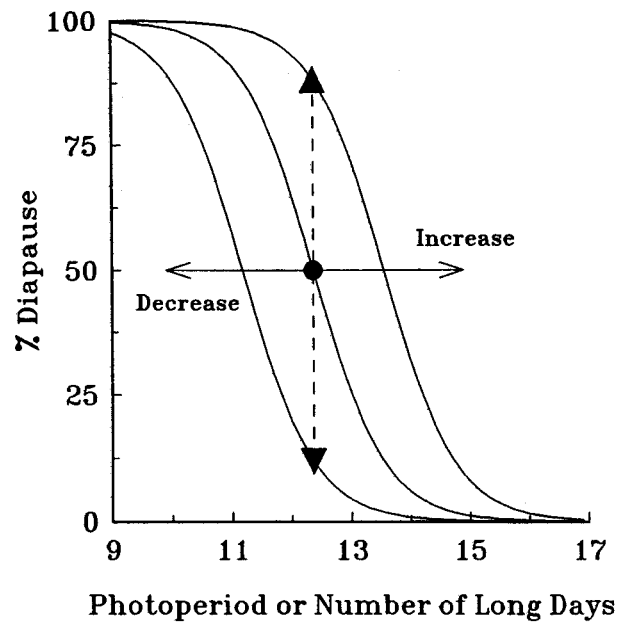

Fig. 1. Response to selection and its interpretation. Diapause in the base population is designated by a solid circle and in the selected lines by a solid triangle for a decrease (▼) or increase (▲) in the trait being assessed. If selected and control lines are exposed to conditions eliciting a 50% diapause in the base population, then a decrease in the trait will result in a lower incidence of diapause and an increase in the trait will result in a higher incidence of diapause in comparison to the control.

significant differences in direct response between selected lines or between selected and control lines to indicate a significant, nonzero heritability. Likewise, we interpreted any significant correlated response to indicate a significant, nonzero heritability of both the selected and correlated traits and a significant, nonzero genetic correlation between them with the sign of the genetic correlation determined from the sign of the correlated response.

Materials and Methods

Collection and Maintenance. Stock populations of *W. smithii* were collected as overwintering larvae in March and June 1988, along a latitudinal gradient from 30 to 49° N latitude (Table 1). Larvae were maintained in the laboratory for at least three generations before the start of the experiments. All experiments were run at in walk-in controlled temperature rooms at 21±0.5°C. Short photoperiods (8:16 [L:D] h) were used to initiate and maintain diapause and long photoperiods (17:7 [L:D]) to avert or terminate diapause. Intermediate photoperiods were provided in light-sealed cabinets within the controlled temperature rooms. All transfers between cabinets, rooms, or both, were made during the common photophase. Larvae were raised according to standard techniques we have used during

Table 1. Localities of origin (designated as in Bradshaw & Holzapfel [1989]) of *W. smithii* used in this study and starting population sizes for each of the selected lines

Population	State or province	County	°N Latitude	°W Longitude[a]	Starting population	
					CPP and RDN	Control
WI	Fla.	Liberty	30	85	1,000	200
CR[2]	Fla.	Okaloosa	31	87	595	100
GS	N.C.	Brunswick	34	78	350	100
FV	Mass.	Middlesex	43	72	1,000	200
KC	Maine	Aroostook	46	68	1,000	200
DL	Ontario	Kenora	49	94	820	150

[a] All localities lie below 400 m elevation.
[b] CR is 190 km WNW of WI.

the past 20 y to provide low density and high food without overfeeding. Twenty to 25 larvae were reared per plastic petri dish (150 by 25 mm), half filled (80 ml) with distilled water and fed an emulsion of ground, sifted, guinea pig chow, and freeze-dried brine shrimp (3:1 by vol) three times weekly and cleaned as needed. Pupae were collected three times weekly and transferred to plastic dessert dishes one-quarter filled (40 ml) with distilled water and placed in either cylindrical cages (200 by 150 mm [height by diameter]) or rectangular cages (500 [high] by 250 [wide] by 400 mm [long]) and raised to adults in a controlled environment room imposing a daily sine-wave thermoperiod from 12 to 28°C (mean, 21°C), 80% RH, and a long-day photoperiod of 17:7 [L:D]. Adults were allowed access to pesticide-free raisins and to a 70-ml glass dish half filled with distilled water and either a whole leaf or leaf fragments from their pitcherplant host. The bottom of the cages were lined with filter paper and kept moist with distilled water.

Determination of Traits in the Base Population. CPP for the initiation of diapause was determined (as in Bradshaw & Lounibos 1972) by exposing larvae (starting at day of hatch) to a range of photoperiods in 0.5-h increments. Percent diapause as a function of hours of light per day experienced by larval cohorts was plotted after 60 d. CPP was estimated by taking the 50% intercept, usually interpolated, directly from this plot.

Required day number was determined by exposing adults, eggs, and larvae to a varying number of long days (17:7) then switching larvae to short days (8:16). Larvae were observed for 60 d from day of hatch. Percent diapause was plotted as a function of the number of long days (since day of hatch) experienced by larval cohorts. RDN was estimated by taking the 50% intercept, usually interpolated, directly from this plot.

Depth of diapause was determined (as T_{50} in Bradshaw & Lounibos 1977) by placing and maintaining larvae on short days from day of hatch until they reached their diapausing instar, at which time they were switched to long days and received a predetermined number of long days before being returned to short days. Larvae

were observed for 40 d after initial switch to long day and percent diapause was plotted as a function of the number of long days experienced by larval cohorts. DOD was estimated by taking the 50% intercept, usually interpolated, directly from this plot.

Divergent Selection. We sought to impose as strong a selection differential as possible and still maintain a population of 50 or more adults after selection. We started with 350–1,000 individuals in each selected line (Table 1) and, from the determination of parameters in the base population, imposed a selection differential that was calculated to produce 50–100 selected adults each generation. If the number of selected adults fell below 50, we relaxed (but never reversed) selection by 15 min (CPP) or 1 d (DOD, RDN) in the next generation. If the number of selected adults fell between 50 and 100, selection was maintained at the same level in the next generation. If the number of selected adults exceeded 100, selection was increased by 15 min (CPP) or 1 d (DOD, RDN) in the next generation.

Selection for decreased values of traits resulted in direct development and a rapid turnover of generations. Selection for increased values resulted in diapause. Because we waited for 40–60 d of nondevelopment to score an individual as diapausing, selection for increased values required twice the generation time as selection for increased values. Consequently, we imposed decreasing selection for four generations and increasing selection for two generations.

For each population, we maintained a control line of 100–200 individuals that developed continuously on long days without diapause for four generations.

To select for a shorter CPP, larvae were exposed to a photoperiod that would induce ≈70% diapause (as determined above). Pupae were collected and raised to adults; any larvae remaining after 60 d were discarded. To select for a longer CPP, larvae were exposed to a photoperiodic condition that would induce ≈30% diapause. Collected pupae were discarded. Larvae remaining after 60 d were placed on long days (17:7) to induce development.

To select for low RDN, larvae were exposed to a number of long-day cycles (17:7) that would induce ≈70% diapause. Pupae were collected and raised to adults; any larvae remaining after 60 d were discarded. To select for high RDN, larvae were given (from day of hatch) a number of long-day cycles that would induce ≈30% diapause. Pupae were discarded. Larvae remaining after 60 d were placed on long days to induce pupation.

Response to Selection. The determination of any genetic correlation between the three photoperiodic traits studied was carried out using the selected lines from the above experiments. These experiments yielded five lines from each population: a control (unselected) line, lines selected for increased and decreased CPP, and lines selected for increased and decreased RDN. To determine response to selection, subsamples of each of these lines were exposed to the conditions that maintained 50% diapause in the base population for the CPP, RDN, and DOD. A significant difference between the selected lines or between a selected and the control line would then reflect a significant heritability for CPP or RDN or a significant genetic correlation between CPP or RDN and RDN, CPP, or DOD (Fig. 1). Significance was assessed using a nonparametric G test for independence of development (number pupated, not pupated) between lines (increase, decrease, and control) (Sokal & Rohlf 1969).

Through the process of trying to maximize selection each generation, several lines were lost or fell to low numbers. The results are based on those lines that survived in sufficient numbers to provide a minimum sample size of 50. Sample sizes in Fig. 2 ranged from 50 to 286.

Voucher specimens were not retained at the end of the experiments.

Results

The direct and correlated responses to selection on CPP (Fig. 2 A–C) are straightforward. All direct responses (Fig. 2A), significant and nonsignificant, are in the direction of selection. In the one population where selected lines declined to low numbers (GS), there was still a significant correlated response in DOD (Fig. 2C) so that the heritability of CPP is significantly nonzero in all populations.

There was a significant correlated response in RDN to directional selection on CPP in all five populations with adequate numbers (Fig. 2B). Selection for decreasing (increasing) CPP resulted consistently in a decreasing (increasing) RDN, including both significant and nonsignificant values. This pattern of correlated response indicates a significant heritability of RDN and a nonzero, positive genetic correlation between CPP and RDN in five of the six populations.

There was a significant correlated response in DOD in three of the six populations but not in the other three (Fig. 2C). Similarly to RDN, the pattern of correlated response in DOD indicates a significant heritability of DOD and also a nonzero, positive genetic correlation between CPP and DOD.

The direct responses to selection on RDN are not as straightforward (Fig. 2E). The three significant responses for a decreasing RDN are all in the direction expected. The two significant responses for an increasing RDN are both opposite to the direction expected. In one of the latter cases (WI), the direct response to decreasing RDN differs significantly and in the direction expected from the response to increasing RDN. In the other case (CR), the contrasting selected line did not survive but the significant correlated response of RDN to direct selection on CPP in Fig. 2B indicates a significant heritability of RDN in this population. In both cases, the percentage diapause in the control population was much higher (87% in WI, 73% in CR) than the 50% expected from RDN in the base population. Consequently, the negative heritability implied by direct response to increasing selection on RDN at WI and CR may be artifacts of the low (but also unexplained) incidence of development in the control line. Finally, for one population (GS), there is no significant direct (Fig. 2E) or correlated (Fig. 2 B, D, and F) response involving RDN. These results indicate that there is significant heritability of RDN in five of the six populations but do not reveal whether the heritability at GS is simply too low to be detected by our experimental protocol or is effectively zero.

The significant correlated responses of CPP (Fig. 2D) and of DOD (Fig. 2F) to direct selection on RDN are consistent. Selection for decreasing (increasing) RDN elicits a decreasing (increasing) CPP or DOD in three of the six populations. These results indicate that in one southern (WI) and two northern (KC, DL) populations, there is a positive genetic correlation between RDN and CPP; likewise, in one southern (WI) and two northern (FV, KC) populations, there is a positive genetic correlation between RDN and DOD.

The significant correlated responses by DOD to direct selection on CPP (Fig. 2C), RDN (Fig. 2F), or both, indicate a significant heritability of this trait in all but one southern (CR) and one northern (DL) population. As in the RDN, these results do not reveal whether the heritability of DOD in the CR and DL populations is simply too low to be detected by the experimental protocol or is effectively zero.

Discussion

The frequent, significant direct and correlated responses shown in Fig. 2 and obtained after
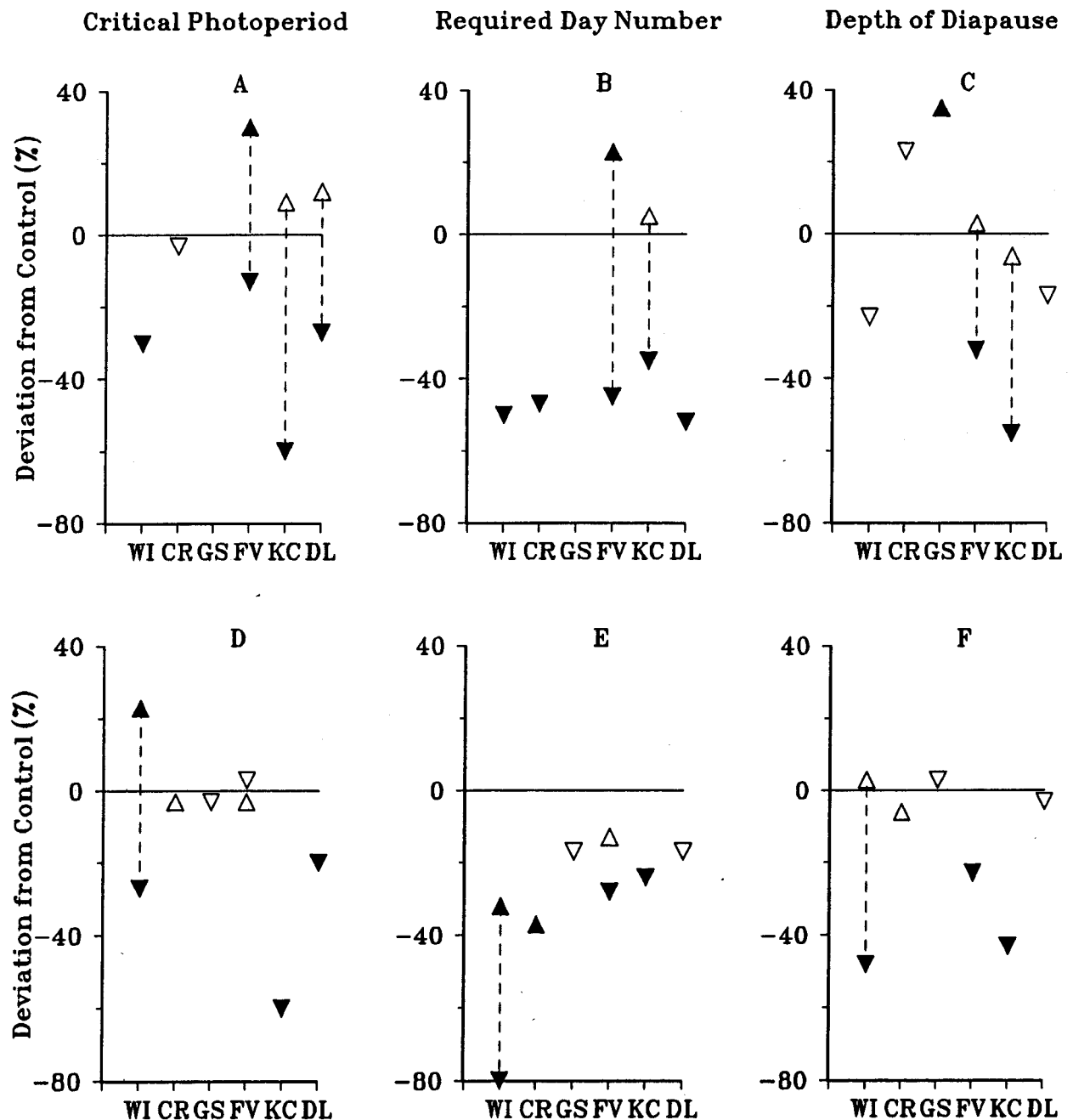
Fig. 2. Direct and correlated response to direct selection on CPP (A–C) and RDN (D–F). Vertical axis plots deviation of selected lines from the control line (percentage of diapause selected minus control) after four generations for decreasing (▼) or two generations for increasing (▲) selection. Significance of deviation from controls is determined by *G* tests for enumeration data and is indicated by open triangles ($P > 0.05$) or solid triangles ($P < 0.05$). A significant difference ($P < 0.05$) between selected lines is indicated by a dashed line joining them; otherwise, the difference is not significant ($P > 0.05$). Localities on the horizontal axis are ranked from south to north (Table 1).

only four (decreasing) or two (increasing) generations of selection suggest significant heritabilities of and genetic correlations among CPP, RDN, and DOD. Substantial heritabilities of diapause-related traits are consistent with earlier studies in *W. smithii* (Bradshaw & Holzapfel 1990) as well as in other insects (Dingle et al. 1977, Sims 1985, Sauer et al. 1986). Where indicated by significant correlated responses, all the genetic correlations are positive. Consequently,

these traits reinforce rather than compensate for one another. This reinforcement means that there is a genetically coordinated diapause syndrome in *W. smithii*: Diapause-prone individuals enter diapause at longer photoperiods, after experiencing more long days, and enter a deeper diapause than do diapause-resistant individuals.

There were no clear geographic trends in response to selection as shown in Fig. 2. As related above, the significant direct and correlated re-

sponses provided evidence for a significant heritability of CPP in all six populations, of RDN in five of the six populations, and DOD in four of the six populations. All significant correlated responses included at least one northern and one southern population and there were no significantly negative correlated responses. We therefore conclude that the individual traits either make a non-significant contribution to the diapause syndrome in *W. smithii* or reinforce each other throughout the range of populations we considered (30–49° N).

We emphasize that this diapause syndrome relates to genetic variation and covariation within rather than between populations. At any one locality, variation in the end of the favorable season may impose a fluctuating stabilizing selection that maintains genetic variability in phenologically relevant traits (Dingle et al. 1977, Istock 1981, Sauer et al. 1986). Mediation of diapause by photoperiod maintains greater genetic flexibility if the components of diapause reinforce rather than compensate for one another. The end of the favorable season, as marked by the date of the first hard frost, decreases continuously with latitude and altitude (Visher 1954); variation in the date of the first hard frost does not. Taylor & Spalding (1986) report that variation in "the occurrence of the first hard frosts over many years along a latitudinal gradient . . . from South Carolina to Maine demonstrates no consistent latitudinal trend" (p. 79). Thus, selection for genetic flexibility in diapause should be maintained relatively uniformly over this range. We propose that the persistence of significant heritabilities and positive genetic correlations in diapause traits throughout the range of *W. smithii* (Fig. 2) represents the consequences of this sustained selection.

Among populations of *W. smithii*, critical photoperiod is tightly correlated with latitude and altitude (Bradshaw 1976). Depth of diapause increases generally with latitude and altitude but shows a reversal in this trend where the diapausing stage changes from the fourth to the third instar (Bradshaw & Lounibos 1977). Thus, although the diapausing traits considered here are positively genetically correlated within populations and this correlation structure persists over the range of *W. smithii*, correlations do not prevent local departure of individual traits from the diapause-prone to diapause-resistant continuum. Genetic coordination of diapause traits does not preclude their evolutionary flexibility.

Acknowledgment

References Cited

Andrewartha, H. G. 1952. Diapause in the relation to the ecology of insects. Biol. Rev. 27: 50–107.

Beck, S. D. 1980. Insect photoperiodism. Academic, New York.

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

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

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

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, London.

Bradshaw, W. E. & L. P. Lounibos. 1972. Photoperiodic control of development in the pitcher-plant mosquito, *Wyeomyia smithii*. Can. J. Zool. 50: 713–719.

1977. Evolution of dormancy and its photoperiodic control in pitcher-plant mosquitos. Evolution 31: 546–567.

Danilevskii, A. S. 1965. Photoperiodism and seasonal development of insects. Oliver & Boyd, London (English translation).

Danks, H. V. 1987. Insect dormancy: An ecological perspective. Biological Survey of Canada (Terrestrial Arthropods), Ottawa.

Dingle, H. 1978. Migration and diapause in tropical, temperate, and island milkweed bugs. *In* H. Dingle [ed.], Evolution of insect migration and diapause. Springer, New York.

Dingle, H., C. K. Brown & J. P. Hegmann. 1977. The nature of genetic variance influencing photoperiodic diapause in a migrant insect, *Oncopeltus fasciatus*. Am. Nat. 111: 1047–1059.

Evans, K. W. & R. A. Brust. 1972. Induction and termination of diapause in *Wyeomyia smithii* (Diptera;Culicidae), and larval survival studies at low and subzero temperatures. Can. Entomol. 104: 1937–1950.

Falconer, D. S. 1981. Introduction to quantitative genetics. Longman, New York.

Hegmann, J. P. & H. Dingle. 1982. Phenotypic and genetic covariance structure in milkweed bug life history traits, pp. 177–85. *In* H. Dingle & J.P. Hegmann [eds.], Evolution and genetics of life histories. Springer, New York.

Istock, C. A. 1981. Natural selection and life history variation: Theory plus lessons from a mosquito, pp. 113–127. *In* R. F. Denno & H. Dingle [eds.], Insect life history patterns. Springer, New York.

Lees, A. D. 1955. Physiology of diapause in arthropods. Cambridge University Press, Cambridge, United Kingdom.

Lounibos, L. P. & W. E. Bradshaw. 1975. A second diapause in *Wyeomyia smithii*: seasonal incidence and maintenance by photoperiod. Can. J. Zool. 53: 215–221.

Partridge, L. & P. H. Harvey. 1985. Costs of reproduction. Nature (Lond.) 316: 20.

Reznick, D. 1985. Costs of reproduction: an evaluation of the empirical evidence. Oikos 44: 257–267.

Sauer, K. P., H. Spieth & C. Grüner. 1986. Adaptive significance of genetic variability of photoperiodism in Mecoptera and Lepidoptera, pp. 153–172. *In* F. Taylor & R. Karban [eds.], The evolution of insect life cycles. Springer, New York.

Saunders, D. S. 1982. Insect clocks. Pergamon, Oxford.

Sims, S. R. 1985. Genetic control of embryonic and larval diapause in *Aedes triseriatus*, pp. 359–369. *In* L. P. Lounibos, J. R. Rey & J. H. Frank [eds.], Ecology of mosquitoes: proceedings of a workshop. Florida Medical Entomology Laboratory, Vero Beach.

Smith, S. M. & R. A. Brust. 1971. Photoperiodic control of the maintenance and termination of larval diapause in *Wyeomyia smithii* (Coq.) with notes on oogenesis in the adult female. Can. J. Zool. 49: 1065–1073.

Sokal, R. R. & F. J. Rohlf. 1969. Biometry. Freeman, San Francisco.

Tauber, M. J., C. A. Tauber & S. Masaki. 1986. Seasonal adaptations of insects. Oxford University Press, New York.

Taylor, F. & J. Spalding. 1986. Geographic patterns in the photoperiodic induction of hibernal diapause, pp. 66–85. *In* F. Taylor & R. Karban [eds.], The evolution of insect life cycles. Springer, New York.

Visher, S. S. 1954. Climatic atlas of the United States. Harvard University Press, Cambridge, Mass.