Epistasis and the Genetic Divergence of Photoperiodism Between Populations of the Pitcher-Plant Mosquito, *Wyeomyia smithii*

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ABSTRACT

Parallel crosses between each of two southern (ancestral) and one northern (derived) population of the pitcher-plant mosquito, *Wyeomyia smithii*, were made to determine the genetic components of population divergence in critical photoperiod, a phenological trait that measures adaptation to seasonality along a climatic gradient. Joint scaling tests were used to analyze means and variances of first- and second-generation hybrids in order to determine whether nonadditive genetic variance, especially epistatic variance, contributed to divergence in critical photoperiod. In both crosses, digenic epistatic effects were highly significant, indicating that genetic divergence cannot have resulted solely from differences in additively acting loci. For one cross that could be tested directly for such effects, higher order epistasis and/or linkage did not contribute to the divergence of critical photoperiod between the constituent populations.

CONVENTIONAL evolutionary theory predicts that as a consequence of natural selection, traits that affect fitness should generally have low additive genetic variance and selection would therefore appear to have little opportunity to further increase fitness. However, numerous studies have recently documented that traits affecting fitness often have moderate and even high levels of additive genetic variance and can respond to selection (*e.g.*, HEGMANN and DINGLE 1982; ISTOCK 1983; ROFF and MOUSSEAU 1987; MOUSSEAU and ROFF 1987; ROFF 1990). Such traits may also have high levels of nonadditive genetic variance (FISHER 1958; WRIGHT 1977; FALCONER 1981; GOODNIGHT 1987). GRIFFING (1960) has shown under the infinitesimal model that additive × additive epistatic interactions can make a transient contribution to selection responses, and several investigators have argued that, particularly under the influence of strong genetic drift (*e.g.*, during a founder event), additive × additive epistatic variance can contribute to selection responses by its conversion to additive variance (ROBERTSON 1952; COCKERHAM 1984; BRYANT, MCCOMMAS and COMBS 1986; GOODNIGHT 1988).

The contribution of epistatic variance to the evolution of polygenic traits, especially those affecting fitness, is not well documented (KEARSEY and KOJIMA 1967; HEDRICK, JAIN and HOLDEN 1978; BARKER 1979; COCKERHAM 1984). WRIGHT (1935, 1969) argued that, under stabilizing selection, a trait affecting fitness that is determined solely by additive gene action could produce epistasis with respect to fitness. Epistasis may thus be extremely important in contributing to genetic variation and adaptation. Like dominance, epistasis involving dominance effects may contribute to heterosis (HAYMAN 1960a; HILL 1982), thereby enhancing the rate of population divergence through genetic drift (BRYANT, COMBS and MCCOMMAS 1986). During a founder event, the genetic background may be altered through the reorganization of epistatic variance into additive variance; this "exposed" additive variance, not recognized by natural selection in the ancestral population, may result in a rapid shift of the population to a new peak of fitness on the adaptive landscape (MAYR 1954; CARSON 1968; WRIGHT 1977; TEMPLETON 1980; GOODNIGHT 1987).

The contribution of epistasis to the divergence and adaptation of natural populations is poorly understood. In this paper, we examine the extent to which epistatic components of genetic variance contribute to the differentiation of populations of the pitcher-plant mosquito, *Wyeomyia smithii* (Coq.), in a trait that is critical to long-term fitness and that closely tracks the geographic distance between populations along a climatic gradient.

RATIONALE

Photoperiodism in *W. smithii*: The immature stages of *W. smithii* develop only within the water-filled leaves of the purple pitcher-plant, *Sarracenia purpurea* L. The range of the mosquito in North America follows that of its host from the Gulf of Mexico to north-central Canada (30–54°N Lat.). Present-day distribution, physiology, morphology, and behavior of *W. smithii* all indicate that the evolution of this species in North America has proceeded

from south to north (ISTOCK and WEISBURG 1987; BRADSHAW and HOLZAPFEL 1990). Throughout their range, the mosquitoes overwinter in the leaves in a state of larval diapause initiated, maintained, and terminated by photoperiod (BRADSHAW and LOUNIBOS 1977). During the early spring, 100% of the genotypes are therefore available in the leaves for sampling.

The median (critical) photoperiod for the induction and maintenance of diapause in *W. smithii* increases linearly with latitude and altitude of origin (BRADSHAW 1976: $R^2 = 0.96$). Thus, critical photoperiod reflects seasonal adaptation of *W. smithii* along its evolutionary trajectory in North America. Critical photoperiod is typically considered only as a population-level trait. In the present paper, we modify the method of BRADSHAW and HOLZAPFEL (1990) for scoring the photoperiodic response of individuals, under conditions of increasing daylength. Operationally, diapausing larvae are exposed to short days that increment by 3 min/day. At some point, the increasing photoperiod triggers resumed development and the individual eventually pupates. Herein, we then define the critical photoperiod for an individual as the daylength of the day on which it pupates and for a population as the mean daylength of pupation in a cohort of individuals.

Experimental approach: We sampled *W. smithii* from two southern (30–31°N) and one northern (49°N) population and performed reciprocal crosses between the northern and each of the southern populations to include the F_1, F_2 and first backcross generations. We then determined the mean critical photoperiod and its sampling variance in each cross and generation under increasing photoperiods. Finally, we tested the adequacy of genetic models that incorporate composite additive, dominance, and digenic epistatic effects to explain the differentiation between populations in critical photoperiod.

MATERIALS AND METHODS

Collection and establishment of populations: We collected approximately 2000 overwintering *W. smithii* larvae from each of three localities in eastern North America, two along the Gulf Coast of Florida (WI and CR of BRADSHAW and HOLZAPFEL 1989), and a third from 2500 km further north in western Ontario (DL of BRADSHAW and HOLZAPFEL 1989). Before the onset of experiments, stock colonies were maintained in larval diapause in a controlled-temperature room at 21 ± 0.5° on short-day photoperiod (L:D = 8:16) with 25–30 larvae per 150 × 25 mm plastic Petri dish filled with distilled water. Larvae were fed an emulsion of ground guinea pig chow and freeze-dried brine shrimp (3:1 ratio by volume in dry diet) and their dishes cleaned and water changed every 1–2 weeks. When stocks were allowed to develop to increase population numbers or to initiate experiments, adult cages were maintained and eggs were collected until all adults had died. Stocks were then thinned or experimental animals removed so that a constant proportion was derived from each oviposition date. In this way, as much of the original variability within populations was maintained as possible.

The larvae were then brought out of diapause and reared in the laboratory under quasi-natural conditions modified from BRADSHAW (1986) and briefly summarized below. Experimental larvae brought out of short-day-maintained diapause were transferred to a 2.4 × 4.7 m controlled-environment room with 80 ± 5% relative humidity, an unambiguous long-day photoperiod (L:D = 18:6), and to simulate temperature conditions in nature (BRADSHAW 1980), a daily sine-wave thermoperiod fluctuating from 13–29° (mean = 21°), that lagged the photoperiod by 3 hr.

Developing larvae were fed three times weekly. Adults were allowed to mass swarm in 12-liter acrylic cages. From the day of first adult eclosion until the last adult death, adults were provided with one or more 15–60 ml (volume) freshly cut pitcher-plant leaves for oviposition. Minimum population size in each line was approximately 150 individuals. After 20 generations under this laboratory regimen, approximately 500 larvae were sampled from each of the three populations to initiate population crosses. Assuming the minimum population size of 150 each generation and a 60:40 (male:female) realized sex ratio, the maximum cumulative inbreeding after 20 generations would be 13.0%.

Crosses among populations: To determine the components of genetic variance in critical photoperiod differentiating southern ("ancestral") and northern ("derived") populations of *W. smithii*, crosses were made between each of the two southern populations (WI, CR) and the northern population (DL). Cross derivatives included reciprocal F_1, F_2 and first backcross (B_1 and B_2) hybrids, yielding a total of 4 complete crosses (24 total lines). Unselected fractions of each population were subjected to the same procedures and served as parental controls. To initiate these crosses, diapausing larvae in each of the three parental populations were simultaneously allowed to develop in the controlled-environment room. The resulting pupae were sexed, counted, and assigned proportionately three times weekly to crosses or to parental controls to maximize variability, to maintain sex ratios near unity, and to ensure that sufficient numbers of parents founded each line. From each population, 12–24 adult males and 18–23 adult females were used to found each parental control line; approximately 75 individuals of each sex were used to found each reciprocal F_1 hybrid. For each reciprocal F_2 hybrid, from 47–75 males and 55–75 females were used to found each line; for each of the reciprocal backcrosses, from 18–76 males and 20–75 females were used. Adults were allowed to mass swarm in cylindrical screen cages and oviposit in a shallow 60-mm Petri dish filled with distilled water and approximately 5 cm^2 of freshly cut pitcher-plant leaf fragments. Eggs were collected every 2–3 days and placed on short-day photoperiod to induce and maintain larval diapause until critical photoperiod was determined (approximately 30 days after last oviposition).

Analysis of cross means: For each of the line-cross derivatives resulting from a pair of the parental populations, critical photoperiod and its standard error were determined from approximately 125 individuals. To simulate the almost linear increase in photoperiod near the vernal equinox in nature, diapausing larvae in each line were exposed to photoperiods that increased 3 min/day from a photoperiod 1.0–1.5 hr shorter than the estimated critical photoperiod for that population. This technique was feasible because critical photoperiod in *W. smithii* is the same for the onset and termination of diapause in unchilled larvae (BRADSHAW and LOUNIBOS 1972). All photoperiod determinations were made in light-tight 57 × 37 × 38cm cabinets; light within

the cabinets was provided by a 4-W cool-white fluorescent bulb isolated in a light-baffled, clear acrylic tube. To minimize potential temperature gradients within cabinets caused by the fluorescent bulbs, ambient air (3 m^3/min) was blown from the outside of the cabinets, through the acrylic tube, and back to the outside. Change in daylength was programmed by Chrontrol CD-4 electronic timers; each timer controlled four separate cabinets independently. Larvae, at a density of 20–25 per 150-mm dish, were fed and checked for development every 1–3 days and larval dishes cleaned every 1–2 weeks. Sex and photoperiod at development were recorded for each pupa.

The size and number of available cabinets prevented concurrent measurement of critical photoperiods in all lines; consequently, the critical photoperiods of the F_1 derivatives and their parental controls were determined in one generation, and the critical photoperiods of the F_2 and backcross derivatives and another set of parental controls were determined in the next. This procedure produced two estimates of critical photoperiod for each parental control line. In order to minimize the effect of environmental differences between successive generations, the means of all lines, including parental controls, were transformed to deviations from midparent (unweighted mean of the means of each replicated parental control).

Distributions of individual critical photoperiod, while approximately normally distributed, were often positively skewed. Moreover, protandry in *W. smithii* resulted in a slight sexual dimorphism in critical photoperiod. MATHER's scaling tests A, B, and C (see MATHER and JINKS 1982, pp. 71–76) were all significantly negative, suggesting that a transformation that foreshortens the distribution was appropriate for these data. Therefore, in this analysis critical photoperiod in each line was transformed by $\log_{10}$, a procedure that reduced skewness and stabilized coefficients of variation. Log transformation also eliminated significant differences in critical photoperiod due to sex and therefore allowed pooling of data from males and females within a line.

For each original pair of parental populations, the critical photoperiods of the six resulting derivatives and their standard errors were analyzed with a technique originally developed by CAVALLI (1952) and HAYMAN (1958, 1960a,b) for the genetic analysis of crosses between inbred lines. The joint scaling test (MATHER and JINKS 1982) is applicable to lines or populations that are not homozygous as long as close relatives are not mated. The critical photoperiods of the six lines resulting from crosses between any two parental populations were used to derive estimates of composite additive, dominance, and digenic epistatic effects for this trait. The expected means for the six lines in the presence of digenic epistasis, using the F_2 as the reference line, were estimated with the formulae given in HAYMAN (1958) and HILL (1982).

The weighted least-squares model that incorporates composite additive, dominance, and digenic epistatic effects is (HAYMAN 1958):

$$\hat{\mathbf{x}} = (\mathbf{C}^T\mathbf{E}^{-1}\mathbf{C})^{-1}\ \mathbf{C}^T\mathbf{E}^{-1}\mathbf{y} \quad (1)$$

$$\mathrm{Var}(\hat{\mathbf{x}}) = (\mathbf{C}^T\mathbf{E}^{-1}\mathbf{C})^{-1} \quad (2)$$

$$\hat{\mathbf{y}} = \mathbf{C}\mathbf{x} = \text{the fitted mean values} \quad (3)$$

where $\hat{\mathbf{x}}$ is the vector of mean, additive, dominance, additive × additive, additive × dominance, and dominance × dominance epistasis parameters $\hat{m}$, $\hat{d}$, $\hat{h}$, $\hat{j}$ and $\hat{l}$, respectively, $\mathbf{C}$ is the matrix of coefficients for these parameters from the equations for predicted line means ($\mathbf{C}^T$ its transpose), $\mathbf{E}$ is a diagonal matrix of error variances (squared standard errors) of the line means, $\mathbf{y}$ is the vector of observed line means, and $\hat{\mathbf{y}}$ is the vector of predicted line means. The observed line means were first tested for fit to a model incorporating only the composite mean, additive, and dominance parameters $\hat{m}$, $\hat{d}$ and $\hat{h}$, and the corresponding matrix of coefficients. Under the assumption of normality, goodness-of-fit of the log-transformed line means to the additive-dominance model was tested with the chi-square statistic derived by HAYMAN (1958). The level of significance used was $P < 0.05$.

Rejection of the additive-dominance model indicates that epistasis and/or linkage are contributing to the genetic divergence of the populations. Otherwise additive and dominance effects alone are sufficient to explain the genetic divergence. In the presence of substantial epistasis, estimates of composite additive and dominance effects are unreliable (HAYMAN 1958, 1960a).

Estimates of the composite effects $\hat{m}$ to $\hat{l}$ were obtained from the vector $\hat{\mathbf{x}}$ after the appropriate genetic model was fitted. For the full six-parameter model, whose adequacy could not often be tested because the number of significant parameters usually equalled the number of observed lines, $\hat{\mathbf{x}}$ provided estimates of the digenic epistatic effects $\hat{i}$, $\hat{j}$ and $\hat{l}$ (HAYMAN 1958). The composite effects are sums of weighted line means; therefore, their sampling errors were calculated as the square root of the sum of the sampling variances of the contributing line means, weighted by the corresponding squared coefficients in the equations for the line means (HAYMAN 1958). These sampling errors are provided by the diagonal elements of the matrix $\mathbf{C}^T\mathbf{E}^{-1}\mathbf{C})^{-1}$ (M. LYNCH and B. WALSH, unpublished manuscript).

Analysis of cross variances: The joint scaling test was also used to interpret the variances of critical photoperiod in the line crosses. Assuming that gene action is additive and that the environmental variance is independent of the genetic background (*i.e.*, no significant genotype × environment interaction or correlation), the expected phenotypic variances for the six lines can be estimated by the methods of HAYMAN (1960b) and LANDE (1981). A maximum likelihood method for estimating the variance components involves the model (HAYMAN 1960b; COCKERHAM 1986):

$$\hat{\mathbf{c}} = (\mathbf{M}^T\mathbf{V}^{-1}\mathbf{M})^{-1}\mathbf{M}^T\mathbf{V}^{-1}\mathbf{v} \quad (4)$$

where $\hat{\mathbf{c}}$ is the vector of least-squares estimates of the variance components, $\mathbf{M}$ is the matrix of coefficients in the equations for the predicted line variances under an additive model (HAYMAN 1960b; LANDE 1981), $\mathbf{M}^T$ is the transpose of $\mathbf{M}$, $\mathbf{V}$ is the sampling variance-covariance matrix for the line variances and $\mathbf{v}$ is the vector of observed phenotypic variances. If individuals in the different lines are unrelated, then under the assumption of normality the diagonal elements of $\mathbf{V}$ are equal to $2v_i^2/n_i$ (the sampling variance of the variances), where v_i is the observed variance of the ith line and n_i is the sample size.

The equations for the predicted line variances for line i include terms for the additive genetic variance, $\sigma^2(A_i)$, and the environmental variance, σ_E^2. The coefficient for σ_E^2 in any line is equal to the sum of the coefficients for the additive genetic variances in the two parental populations, $\sigma^2(A_1)$ and $\sigma^2(A_2)$; therefore, if both parental populations are not completely homozygous ($\sigma^2(A)_1$) and $\sigma^2(A_2) > 0$), $\mathbf{M}$ is singular. To eliminate this problem, the column of coefficients for σ_E^2 were removed from $\mathbf{M}$ and the variance component vector $\hat{\mathbf{c}}^T$) reduced to $[\sigma^2(P_1), \sigma^2(P_2), \sigma_s^2]$, where $\sigma^2(P_1)$, and $\sigma^2(P_2)$ are the parental phenotypic variances and σ_s^2 is the segregational variance (M. LYNCH and B. WALSH, unpublished manuscript). Following HAYMAN's (1960b) maximum

likelihood procedure, the elements of $\hat{\mathbf{c}}$ were then computed a second time by use of Equation 4 after substituting the elements of $\hat{\mathbf{v}} = \mathbf{M}\hat{\mathbf{c}}$, the vector of expected phenotypic variances, into **V**. This procedure was iterated until the estimates of $\hat{\mathbf{c}}$ stabilized. The chi-square statistic used to test goodness of fit to the additive model was (M. LYNCH and B. WALSH, unpublished manuscript):

$$\chi^2_{k-3} = \sum_{i=1}^{k} \frac{(v_i - v_i)^2}{2v_i^2/n_i} \quad (5)$$

where k is the number of lines.

Finally, for each cross the minimum number of effective factors contributing to the divergence among populations, n_E (WRIGHT 1968; LANDE 1981), was estimated. The procedure for estimating n_E and its sampling variance incorporated the modifications of COCKERHAM (1986), which correct for sampling variances in the estimates from the parental populations and incorporate information from all cross derivatives. The approximate variance of n_E was calculated from the estimated line and segregational variances by the formulae derived in LANDE (1981).

RESULTS

Line means: The relationships between the mean critical photoperiods of the derivative lines, for reciprocal and pooled crosses, are shown in Figure 1. The contributions of maternal or cytoplasmic effects to the differences between population means in critical photoperiod were assessed by comparing means of reciprocal crosses and F_2 derivatives; where no significant effects were detected, the potential effects of sex-linked genes were similarly tested in males in reciprocal F_1 derivatives (CARSON and LANDE 1984). Only two of the comparable means differed significantly, backcrosses of WI × DL and DL × WI females to WI males ($t = 2.71$, $P < 0.01$), and backcrosses of CR × DL and DL × CR females to DL males ($t = 2.82$, $P < 0.01$) (Table 1). However, these two pairs of means differed by only about 2 SE; therefore, reciprocal crosses were pooled for analysis by joint scaling tests.

For both crosses, the line means show evidence of nonadditivity. An additive model (4 d.f.) of divergence in the WI-DL cross is strongly rejected (pooled reciprocals: $\chi^2 = 877.8$; $P < 0.001$). The additive model is also strongly rejected in the CR-DL cross (pooled reciprocals: $\chi^2 = 758.6$; $P < 0.001$). The low hybrid means in both WI-DL and CR-DL crosses (Figure 1, E and F) suggests an excess of dominance effects from the populations with shorter critical photoperiods (WI, CR). However, the significantly lower mean in the F_2 (relative to the F_1) in both crosses suggests that epistatic interactions and/or linkage are important. Indeed, rejection of an additive-dominance model (3 d.f.) in both the WI-DL cross (pooled reciprocals: $\chi^2 = 865.4$; $P < 0.001$) and the CR-DL cross (pooled reciprocals: $\chi^2 = 687.3$; $P < 0.001$) confirms the significant and substantial contribution of epistasis and/or linkage to divergence.

Epistatic variation must contribute to the divergence of critical photoperiod in these populations, regardless of the extent of linkage. In the CR-DL cross, the significant deviation of the F_1 hybrid from the midparent (Figure 2; $F_1 - 0.5[P_1 + P_2] \pm 2$ SE $= \hat{h} - \hat{i}$) is unbiased by linkage and is highly significant (CR-DL: -0.0115 ± 0.0012***). Since the estimates of composite dominance ($\hat{h}$) and additive × additive epistasis ($\hat{i}$) are both positive but only the latter is significantly different from zero (Table 2), then their significant difference ($\hat{h} - \hat{i}$) must be due to the epistatic term. Consequently, at least additive × additive epistasis contributes to the divergence of these populations, regardless of the degree of linkage present. In the WI-DL cross, to test for the presence of higher order genic interaction and/or linkage, the nonsignificant $\hat{i}$ term was dropped from the model, reducing the 6 × 6 **C** matrix in Equations 1–3 to 6 × 5, producing one degree of freedom and providing a means to determine whether higher order epistasis and linkage are important (Table 2; see MATHER and JINKS 1982, Ch. 5). Estimating the five components $\hat{m}$, $\hat{d}$, $\hat{h}$, $\hat{j}$ and $\hat{l}$ proceeded exactly as for the compos-

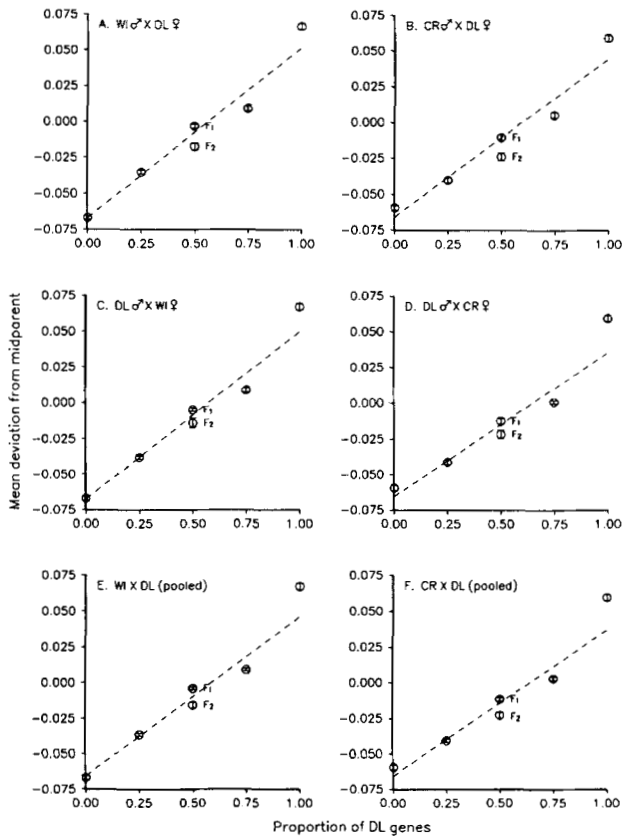

FIGURE 1.—Mean critical photoperiod (log hr) in derivative lines of two crosses of populations of *W. smithii* (WI-DL and CR-DL) as a function of the proportion of genes from the DL (common) population. Critical photoperiods (± 2 SE) are expressed as mean deviations from midparent. The dashed lines connect the maximum likelihood expectations of an additive model The results of the reciprocal and pooled crosses are shown. In each comparison, additive and additive-dominance models are strongly rejected (see text).

TABLE 1

Tests for maternal/cytoplasmic effects (reciprocal B_1 and B_2 hybrids and reciprocal F_2 hybrids) and sex-linked effects (reciprocal F_1 males) on divergence in critical photoperiod between two southern populations (WI, CR) and one northern population (DL) of *W. smithii*

Test	Cross			
	WI ♂ × DL ♀	DL ♂ × WI ♀	CR ♂ × DL ♀	DL ♂ × CR ♀
B_1 (WI/CR)	1.1537 ± 0.0020 (143)	1.1501 ± 0.0017 (127)	1.1509 ± 0.0018 (145)	1.1496 ± 0.0020 (131)
B_2 (DL)	1.1957 ± 0.0022 (130)	1.1944 ± 0.0014 (133)	1.1993 ± 0.0025 (120)	1.1951 ± 0.0017 (133)
F_2	1.1686 ± 0.0024 (133)	1.1719 ± 0.0033 (143)	1.1700 ± 0.0027 (143)	1.1721 ± 0.0028 (119)
F_1 males	1.1802 ± 0.0020 (73)	1.1794 ± 0.0016 (85)	1.1794 ± 0.0025 (57)	1.1796 ± 0.0020 (66)

Reciprocal B_1 and B_2 hybrids are from F_1 females backcrossed to the same parental line of males. Values are mean critical photoperiods (log hr) ± 2 SE. Sample sizes are in parentheses.

TABLE 2

Estimates (± 2 SE) of composite effects contributing to the divergence in critical photoperiod between two southern populations (WI, CR) and one northern population (DL) of *W. smithii*

Term	Pooled cross		
	WI-DL (full model)[a]	WI-DL (1 d.f.)[b]	CR-DL (full model)[a] B_1
$\hat{m}$	−0.0157 ± 0.0020***	−0.0141 ± 0.006***	−0.0228 ± 0.0020***
$\hat{d}$	−0.0455 ± 0.0014***	−0.0455 ± 0.0014***	−0.0435 ± 0.0017***
$\hat{h}$	0.0033 ± 0.0043[NS]	0.0041 ± 0.0008***	0.0039 ± 0.0088[NS]
$\hat{i}$	0.0074 ± 0.0042[NS]		0.0154 ± 0.0087***
$\hat{j}$	0.0215 ± 0.0018***	0.0201 ± 0.0018***	0.0158 ± 0.0022***
$\hat{l}$	0.0398 ± 0.0130***	0.0462 ± 0.0041***	0.0374 ± 0.0112***

[a] Estimated from the full 6-parameter model incorporating the composite effects $\hat{m}$ to $\hat{l}$.
[b] Significant components estimated from a 5-parameter model after dropping $\hat{i}$ from the model.
[NS] not significant ($P > 0.05$); ***, $P < 0.001$.

ite additive-dominance model. The results of this test ($\chi^2 = 3.04$, 1 d.f., $P > 0.05$) indicate that higher order epistasis and/or linkage do not make a significant contribution to the divergence of critical photoperiod between these populations. Consequently, composite additive and dominance effects and digenic epistatic effects involving dominance are necessary and sufficient to explain genetic divergence between WI and DL.

The deviation of the line means from additivity is not the result of a scaling effect. Neither the $\log_{10}$ transformation nor a "variance stabilizing" transformation based on regression of standard deviation on mean among population derivatives suggested by KLECZKOWSKI (1949) and independently by WRIGHT (1968, Ch. 10) result in an adequate fit of the means to the additive model. The means and variances of the hybrids relative to the parental populations suggest that a more extreme transformation is necessary, but in fact none may be adequate. The interacting factors, whether they be due to epistasis or linkage, may be too strong; the low F_1 variance relative to that of either parent (Figure 2) indicates that a transformation that removes interaction effects will be difficult to find. As a result, the failure to produce additivity of means is more likely due to the failure of the genetic assumptions rather than to scaling effects.

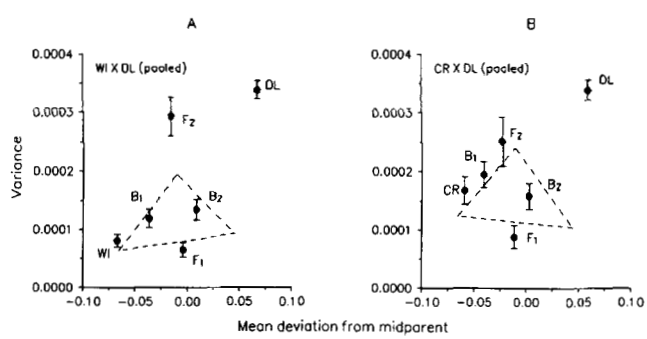

FIGURE 2.—Variances of critical photoperiod ((log hr)^2) in derivative lines of two crosses of populations of *W. smithii* (WI-DL and CR-DL) as a function of their means. The results of the pooled crosses are shown. Values are observed variances and means ± 2 SE (the dispersions about the means all lie within the solid symbols); lines forming a triangle connect the maximum likelihood predictions of an additive model, with F_1 and backcross hybrids at the midpoints of the edges connecting the vertices representing the parental lines and F_2 hybrids (LANDE 1981). The additive model for each comparison is strongly rejected (see text).

Line variances: The relationships between the variances of critical photoperiod and their means, for pooled reciprocal crosses, are shown in Figure 2. An additive model (4 d.f.) for the variances is strongly rejected in both the WI-DL cross (pooled reciprocals: $\chi^2 = 1070.4$; $P < 0.001$) and the CR-DL cross (pooled reciprocals: $\chi^2 = 751.4$; $P < 0.001$). In the WI-DL cross, the high F_2 variance relative to the backcrosses, but especially the high variance in DL, contribute to rejection of additivity, and indicate that strong epistatic effects (or linkage) are in part responsible for the divergence between the populations. For the CR-DL crosses, the high variance in DL is primarily responsible for rejection of additivity. In both crosses, but especially in CR-DL, the low variance in the F_1 suggests an excess of dominance effects from the populations with shorter critical photoperiods may exist in addition to epistasis. The variance-stabilizing transformation has no detectable effect on the results of these tests.

From the analysis of line variances, the estimated minimum number of effective factors, n_E, contributing to the divergence in critical photoperiod between WI and DL (± 2 SE) is 19.4 ± 4.0; that contributing to the divergence between CR and DL is 14.0 ± 3.6. However, these estimates are unreliable in the presence of such strong nonadditive variation (see DISCUSSION).

DISCUSSION

The results of these crosses indicate that epistatic interactions among loci affecting critical photoperiod make a substantial contribution to the genetic divergence between the southern (ancestral) and northern (derived) populations of *W. smithii*. The pattern of means in the crosses (Figure 1) suggests that directional dominance, toward southern populations, in genes affecting critical photoperiod may contribute to latitudinal divergence, but the joint scaling tests on these means, as well as the patterns of the variances (Figure 2), indicate that simple dominance is totally inadequate to explain the differentiation of these populations. The F_1 mean includes the effects of dominance × dominance epistasis, which in both crosses is apparently large (Table 2). In one cross (CR-DL), additive × additive and possibly all three types of digenic epistasis are important; in the other (WI-DL), both additive × dominance and dominance × dominance epistatic effects appear to be important. In the latter comparison, we found no evidence for effects of higher order epistasis or linkage on divergence after dropping the nonsignificant additive × additive epistatic term from the model. These results indicate that trigenic or higher order epistasis and/or linkage do not make a significant contribution to the divergence of critical photoperiod between the WI and DL populations. The patterns of means and variances are remarkably similar for both crosses, implying that genes determining critical photoperiod in the northern and those in the two distinct southern populations interact consistently.

The low sampling variances that were observed in these lines, if underestimated, might affect variance estimates for divergence in critical photoperiod. The true variance of a line mean is the sum of the variance of its family means, divided by the number of families, and the variance within families, divided by the product of family size and the number of families. Our estimates of line variances assume all variance is within-family variance and would underestimate the true variances unless the between-family variance was zero. The magnitude of this bias is unknown. However, the procedures used in these experiments to mass swarm adults and to sample their offspring from throughout the oviposition distribution in establishing derivative lines should have minimized the contribution of between-family variance to the estimates of line variances. From Figure 2, R. LANDE (personal communication) has noted that the observed segregational variance differs from the segregational variance predicted from the additive model by a factor of approximately two or less. Inflating our sampling variances by a factor of two had no effect on the rejection of the additive and additive-dominance models. Indeed, the sampling variances would have to be increased nearly a hundred fold to alter our conclusion that epistasis contributes significantly to the genetic divergence of these populations.

Our method of estimating critical photoperiod would also lead to underestimates of variances if the starting photoperiod exceeded that which might trigger development in some individuals. However, based on the photoperiodic responses of these populations observed two generations earlier, we set our starting photoperiods at levels to avoid this specific problem.

We estimated that the minimum number of effective factors, n_E, contributing to the divergence between populations was on the order of 14–20. We do not place much reliance on these estimates. Linkage, unequal gene effects, and nonadditive gene action all tend to bias n_E downward (LANDE 1981; ZENG, HOULE and COCKERHAM 1990; but see MATHER and JINKS 1982). The large differences in variances between the F_1 and F_2 of both crosses (Figure 2) do not clearly support the hypothesis that critical photoperiod is under polygenic control with the many constituent loci each contributing minor effects, as suggested by the estimates of n_E. The strong nonadditive (epistatic) effects (Table 2) probably contribute to biased estimates of n_E. These estimates are also sensitive to the haploid chromosome number and recombination index (DARLINGTON 1937) and to line variances (ZENG,

HOULE and COCKERHAM 1990). The haploid number of chromosomes in *W. smithii* is three (RAI 1963; MOEUR and ISTOCK 1982), and this low number should result in a correspondingly low estimate of n_E (ZENG, HOULE and COCKERHAM 1990). The recombination index for *W. smithii* is unknown, but based on that estimated for another mosquito in the same tribe (Sabethini), *Sabethes cyaneus* (MUNSTERMANN and MARCHI 1986), it is on the order of 6–8. Unlike *Drosophila*, recombination usually occurs in male mosquitoes (IQBAL *et al.* 1973), so our estimates are probably not affected by sexual differences in recombination. It would therefore appear that the major possible sources of error in our estimates of n_E are (1) tendencies to underestimate due to low haploid chromosome number, linkage, and strong epistatic effects, and (2) a tendency to overestimate due to downward-biased line variances (or to additive × dominance epistasis, MATHER and JINKS 1982). For our study, we purposefully chose for comparison the most distant populations available to us. We do not therefore know whether epistatic effects become important immediately upon the isolation of populations or accumulate with progressive population differentiation. From patterns of isozyme variation, ISTOCK and WEISBURG (1987) concluded that in *W. smithii*, gene flow was continuous within a bog of pitcher-plants but was interrupted at even the local between-bog level. Crosses similar to the ones we have performed here between southern, ancestral populations and progressively more northern, derived populations should reveal the evolutionary pattern of epistasis in this species. Most models dealing with the potential for and constraints to adaptive evolution consider the maintenance of strictly additive genetic variation and covariation within populations (*e.g.*, LANDE 1980a,b, 1982; CHEVERUD 1984; ROSE 1985; VIA and LANDE 1985; GILLESPIE and TURELLI 1989; CHARLESWORTH 1990). Our results show that the genetic differentiation of populations within a single species can involve substantial and perhaps overwhelming nonadditive, multiple epistatic effects. If similar results are obtained from other studies, then models assuming strictly additive genetic effects, even if appropriate at the within-population level, may tell us little about the actual genetic divergence of populations.

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