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EVOLUTION OF DORMANCY AND ITS PHOTOPERIODIC CONTROL IN PITCHER-PLANT MOSQUITOES

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Phenotypic characters which vary over a wide geographic range are not unusual. Good examples relating ecogeographic characters to specific environmental variables are less frequently encountered and usually correlate a physiological function with some aspect of environmental temperature (Brown and Lee, 1969; Snyder and Weathers, 1975; Moore, 1950; Ruibal, 1955; Volpe, 1957). Photoperiodism is also an ecogeographic character and correlates closely with altitude, latitude, and growing season (Danilevskii et al., 1970; Danilevskii, 1965; Masaki, 1972; Bradshaw, 1976). Since natural selection will favor adaptation to a combination of climatic factors impinging upon an organism, ecogeographic characters like photoperiodism which provide a link between the organism and climate in general are more likely to correlate with geography than a character relating to only one aspect of climate.

In our current study, we examine the effects of latitude, altitude, and longitude on photoperiodism and dormancy of pitcher-plant dwelling mosquitoes in eastern North America. The range of the purple pitcher-plant, *Sarracenia purpurea* Lin. extends from the Gulf Coast of the United States north to Labrador and west to Manitoba (McDaniel, 1971). Throughout most of this range, the water-filled leaves serve as the breeding place of mosquitoes, *Wyeomyia haynei* from the Carolinas southwards and *W. smithii* farther north (Dodge, 1947; Carpenter and LaCasse, 1955). The northern limit of *W. smithii* is about 54°N (Haufe, 1952; Burgess and Rempell, 1971; Evans and Brust, 1972). We have studied animals collected from 30°N along the Gulf Coast in Mississippi, Florida, and Alabama to 49°N along the northern Gaspé in Quebec (Fig. 1).

Wyeomyia, like all mosquitoes, develops through four larval inter-molt periods or instars. *W. smithii* undergoes developmental arrest or diapause throughout its range. The onset, maintenance, and termination of this diapause are mediated by day length (Smith and Brust, 1971; Bradshaw and Lounibos, 1972; Evans and Brust, 1972). At day lengths intermediate between long and short, temperature (Lounibos and Bradshaw, 1975) or food level and larval density (Istock et al., 1975) may affect the initiation of diapause. Factors affecting diapause in *W. haynei* have not been previously studied. We shall presently examine the role of photoperiod on the initiation and maintenance of diapause among *Wyeomyia* along latitudinal, altitudinal, and longitudinal gradients.

Dodge (1947) distinguished *W. haynei* from *W. smithii* on the basis of the lateral hairs and papillary structure on the anal segment of the larvae and the presence or absence of silver scales on the scutellum of the adult females. The adult males are indistinguishable (Dodge, 1947; Carpenter and LaCasse, 1955). The lack of more de-

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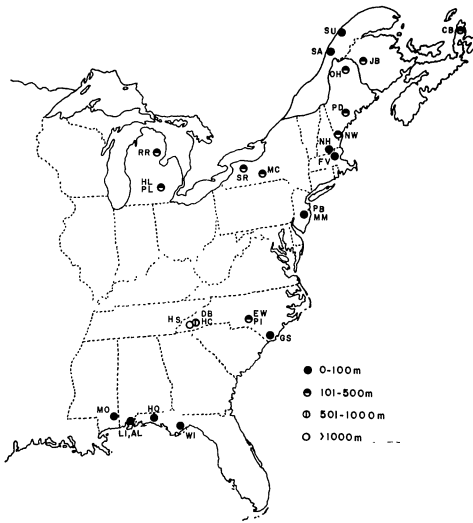
FIG. 1. Map of localities mentioned in the text. When two localities are very close to each other and are at the same elevation, they are labelled as one point. More exact information is provided in Appendix I.

finitive morphological differences, the uniqueness of the pitcher-plant habitat, and their geographic distribution suggested the *W. smithii* and *W. haynei* might be monophyletic or geographic races of a single, polytypic species. Based on a more extensive examination of external morphology, on the physiology of dormancy and its photoperiodic control, and on the inheritance of these physiological and morphological characters, we will argue that there is but one species, *W. smithii*, which has evolved from south to north.

MATERIALS AND METHODS

Collection and Maintenance of Larvae

The locations of collecting sites are provided in Appendix I. Wild-caught larvae were packed in ice after collection and maintained in the laboratory at 5 ± 2C in an ordinary refrigerator without a programmed light regimen until used for experiments. Colonies of adults were placed at 25 ± 1C, 75–95% relative humidity, and a 16:8 light:dark regimen. Adults

and larvae were fed food mixtures described earlier (Bradshaw and Lounibos, 1972).

Critical Photoperiod

Critical photoperiod or critical daylength for diapause termination or initiation is defined as the number of hours of light per day which either promotes 50% development or initiates and maintains 50% diapause. We determined critical photoperiod by exposing larvae to a range of photoperiods at 25 ± 1C for 20 days, plotting percentage development as a function of hours of light per day, and taking the 50% intercept. Rarely did one of the set regimens elicit exactly 50% development; most of our critical photoperiods therefore reflect interpolated values. Critical photoperiods were determined for either the maintenance or initiation of diapause (Appendix I). Preliminary experiments (Bradshaw, unpublished) have indicated that prolonged chilling at 2–7C beyond 4 months reduces the critical photoperiod; however, for up to 3–4 months the critical photoperiod remains constant. Due to factors beyond our control, we could collect larvae at lower altitudes and latitudes only after 4 months of presumed chilling in nature (January–February) and at higher elevations and latitudes only in the early fall (September–October). We were unable to colonize the latter populations in the laboratory; the critical photoperiods discussed in this study therefore reflect a mixture of photoperiods necessary for the maintenance or initiation of diapause. All of the experiments using wild-caught larvae were performed within 45 days of capture. All experiments for the determination of critical photoperiod except those involving certain hybrid populations (Appendix II) spanned daylengths from short to long in 0.5 h increments.

Anal Papillary Phenotypes

The anal papillae of *W. smithii* larvae from all localities ranged from four long,

narrow papillae through two long, ventral and two short dorsal papillae to simply two ventral papillae. Initially, to identify the species in our collections, we fixed larvae in Kahle's solution, then measured the relative length of the anal papillae and examined the lateral hairs of the anal segment in wet mounts under a compound microscope. We found that clearing and mounting or simply preserving the larvae in Kahle's solution markedly altered papilla length and appearance. We adopted a 1–5 scale of papilla phenotypes which could be assessed on living larvae observed through a dissecting microscope. We scored 1 for four long anal papillae of equal length, 2 for two long ventral papillae and somewhat shortened dorsal papillae (dorsal papillae approximately 0.75 the length of the ventral papillae), 3 for two long ventral and medium sized dorsal papillae (dorsal papillae approximately 0.5 the length of the ventral papillae), 4 for two long ventral and small but noticeably present dorsal papillae (dorsal papillae approximately 0.25 or less of the ventral papillae), and 5 for absence of any noticeable dorsal papillae (Fig. 6). We averaged the scores given each of 50–300 third or fourth instar larvae. The mean papillary phenotype for a locality is then the mean of the average score given each instar.

Depth of Diapause

As a measure of the depth or firmness of diapause, we determined T_{50}, the number of long days required to terminate diapause in 50% of a sample population, by long-day to short-day, pulse-chase experiments (Bradshaw and Lounibos, 1972; Bradshaw, 1973). While T_{50} may change after four or more months of prolonged chilling in nature, T_{50} determined with unchilled, laboratory-reared animals is a reliable indicator of the "depth" of diapause or the "firmness" with which it is established. That is, if many long days are required to commit larvae to resumed development, we contend that such larvae are deeper in diapause than those for which

only a few long days are required. To obtain larvae in diapause, we reared them from the day of hatch until the diapausing stage at $23 \pm 1\frac{1}{2}$ C or 25 ± 1 C and short-day photoperiod (as determined for that population by the above critical photoperiod studies). Larvae were then maintained for at least two weeks on short day lengths at the same temperature before experimentation. To determine T_{50}, samples of 20–50 larvae each were first exposed to 0, 1, 2, 3, . . . 20 long days (as determined by prior critical photoperiod studies) at 25 ± 1C, and then to 20, 19, 18, 17, . . . 0 short days, respectively, at the same temperature. After 20 days total experimental time, the larvae were scored for development and the experiment terminated. Percent development was then plotted as a function of number of pulsed long days and the 50% intercept read directly. As in the critical photoperiod studies, exactly 50% development was rarely elicited by our experimental regimens and the T_{50}'s reflect mainly interpolated values.

Statistical Analyses

We performed our statistical analyses according to Snedecor (1956). When analysis of variance indicated a significantly greater variation among than within samples, we performed Duncan's multiple range test (Duncan, 1955).

EXPERIMENTAL RESULTS

Larval Morphology

Larvae from the Gulf coast had relatively longer dorsal papillae than described previously for either *W. haynei* or *W. smithii* (Table 1). Larvae from low elevations in North Carolina possessed anal papillae similar to *W. haynei*. Dorsal papillae were lacking altogether among *Wyeomyia* larvae from high elevations in North Carolina or from Massachusetts. On a qualitative basis, we were able to distinguish individuals as belonging to either the Gulf coast populations, the low elevation populations in North Carolina, or the

TABLE 1. *Length of anal papillae, ratio of dorsal to ventral lengths (d:v ratio), and percent of lateral hairs on the anal segment which are bifid (% bifid).*

Collection site	Mean Length Anal Papillae		d:v ratio (n)		% bifid (n)	
	Dorsal	Ventral				
MO, Miss.	0.40 ± .05[a]	0.55 ± .07	0.73 ± .06	(10)	81	(16)
LI, Ala.	0.35 ± .08	0.43 ± .09	0.81 ± .09	(9)	82	(18)
HO, Fla.	0.34 ± .05	0.41 ± .06	0.82 ± .03	(12)	65	(34)
GS, N.C. 15m	0.18 ± .02	0.45 ± .05	0.40 ± .07	(14)	38	(16)
EW, N.C. 150m	0.16 ± .02	0.47 ± .06	0.33 ± .02	(10)	48	(29)
DB, N.C. 890m	—	0.40 ± .02	—	(16)	22	(23)
FV, Mass.	—	0.32 ± .05	—	(13)	60	(20)

[a] ±2 SE.

populations from higher latitudes or altitudes. Thus, all individuals from the Gulf coast possessed four long anal papillae; all individuals from low elevations in North Carolina possessed two long and two short papillae; all larvae from high elevations in North Carolina or from farther north lacked the dorsal papillae altogether. None of the populations we sampled were comprised of larvae with exclusively bifid or trifid lateral hairs on the anal segment. Southern populations did, however, tend to have a higher frequency of bifid hairs although 12 to 20 larvae we examined from Massachusetts also had bifid hairs. The adult females from high elevations in North Carolina (HC) and from Massachusetts (FV) lacked silver scales on the scutellum; those from along the Gulf coast and from low elevations in North Carolina exhibited at least a small patch of silver scales. These results enabled us to distinguish three varieties of *Wyeomyia* from our pitcher-plant collections: *W. smithii* from the north and high elevations in the south, *W. haynei* from low elevations in North Carolina, and a Gulf coast variety, previously undescribed.

Age and Developmental State of Overwintering Larvae

Wyeomyia from all localities overwinter primarily in the third or fourth instar although a small percentage of second stage larvae are also generally present in winter

collections (Fig. 2A). The frequency of fourths is highest in the south or at low elevations. With either increasing altitude or latitude, the percentage of fourths declines and that of thirds increases (Fig. 2A,B).

To see which, if any, of the different instars might be in diapause, we exposed samples of third or fourth instar larvae from the Gulf coast (LI, WI), low elevations in North Carolina (GS, PI, EW), high elevations in North Carolina (DB, HS), and northern localities (MM, HL) to experimental photoperiods ranging from 10 to 15.5 hours of light per day (Fig. 3). Among larvae from Florida and Alabama (WI, LI), photoperiod had no discernible effect upon the duration of the third instar but did have a profound effect on the duration of the fourth. Daylengths below 11.5 hours prevented pupation in most or all of the samples while longer daylengths promoted progressively more rapid development. Among larvae from low elevations in North Carolina (GS, PI, EW), short days appeared to retard but could not prevent third instars from molting to fourths. The effect of photoperiod on the duration of the fourth instar was qualitatively similar to that observed among larvae from Florida and Alabama. At greater elevations in North Carolina (DB, HS) or at higher latitudes (MM, HL), fourth instar larvae comprised less than 1% of the population (Fig. 2). Third instar larvae from these higher altitudes or latitudes were
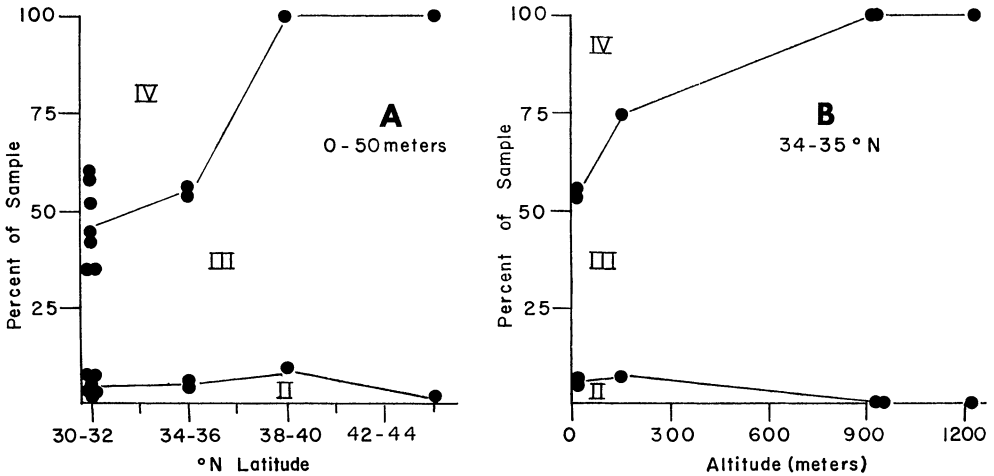
FIG. 2. Age of overwintering larvae. Percent of total sample in the II, III, or IV instar is plotted along (A) a latitudinal gradient and (B) an altitudinal gradient. The percentage of II's is the difference between the bottom curve and the horizontal axis; of III's is the difference between the top and bottom curves; of IV's is the difference between the top curve and 100%. Data above 36°N from Lounibos and Bradshaw (1975). Raw data and collection dates are given in Appendix I.

uniformly prevented from developing by short days (Fig. 3). Among the fourth stage larvae which resulted under intermediate and long-day conditions, there was no apparent effect of photoperiod.

These results indicate that photoperiodically maintained diapause among southern pitcher-plant mosquitoes occurs during the fourth instar. As one moves north, the proportion of *Wyeomia* overwintering as fourth instar larvae declines and, between 150–900 m in North Carolina or between 35 and 40°N latitude, becomes negligible. Concomitantly, over the same range in altitude and latitude, there is a switch from dormancy during the fourth instar to the third larval stage.

Critical Photoperiod

We determined the critical daylengths for the initiation or maintenance of diapause among larvae from 22 localities extending from Alabama to the northern Gaspé, Quebec, and from 10 to 1190 m in elevation (Fig. 1). Critical photoperiod was highly significantly correlated with both altitude and latitude ($r = 0.98$, $F =$

225, $P < 0.001$; t(altitude) $= 8.546$, $P < 0.001$; t(latitude) $= 20.96$, $P < 0.001$). The least squares regression equation relating photoperiod to latitude and altitude is:

$$\text{critical photoperiod} = 6.52 + 0.0013\text{m Alt.} + 0.185 \text{ N Lat.} \quad (1)$$

Consideration of these two variables was sufficient to explain 96% of the variation in critical photoperiods that we observed over this wide geographic range. The quotient of the regression coefficients for latitude and altitude (Eq. 1) gives the conversion factor for correcting latitude for altitude so that

$$\text{altitude-corrected latitude} = \text{°N Lat.} + (\text{m Alt.}/142) \quad (2)$$

Critical photoperiods then correlate closely with altitude-corrected latitude (Fig. 4).

Hybrid Phenotypes

In the following experiments we consider the genetic basis for the stages of diapause, critical photoperiod, and anal papil-
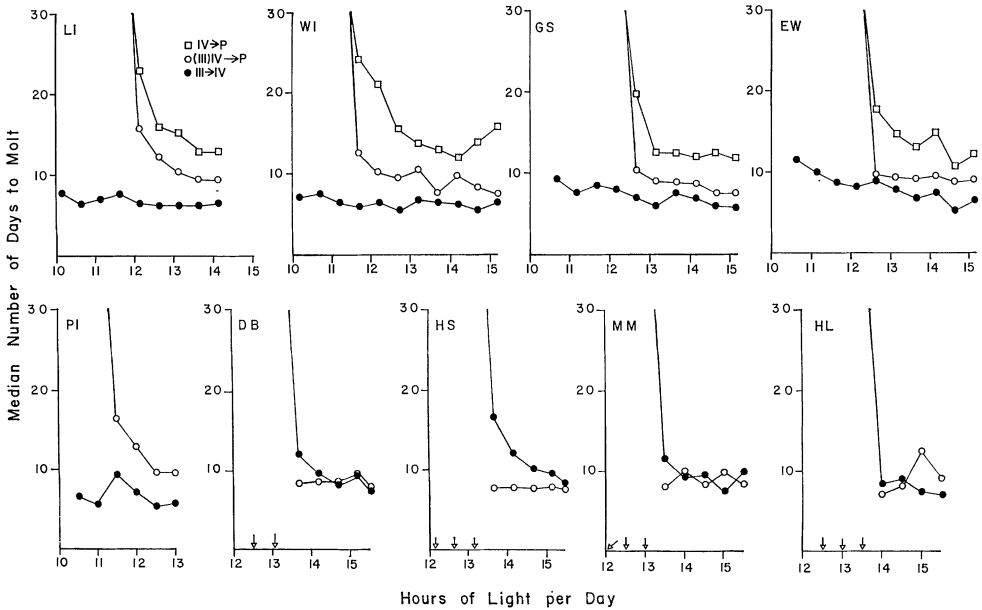
FIG. 3. Developmental state of overwintering larvae. The figures illustrate the time for 50% of the III instar larvae to molt to IV's or of the IV instar larvae to molt to pupae in response to various photoperiods. Solid circles show the median number of days to molt of wild caught III to IV instar larvae. Open circles show the median number of days they subsequently spent as IV's. Squares show the median duration of the IV instar among larvae originally caught as IV in the field. The arrows in DB, HS, MM, and HL indicate the daylengths to which III instar larvae were exposed but for which less than 50% molting to the IV resulted. Sample sizes are given in Appendix I.

lae morphology. Equation 2 enabled us to compute altitude-corrected latitudes for each of our localities. For mass crosses between any populations, we calculated the equivalent mean altitude-corrected latitude as the arithmetic mean of the altitude-corrected latitudes of the parents. We compared the F_1's bred within populations of wild-caught larvae with their F_1 hybrids between populations from Alabama (AL), low elevation North Carolina (EW), high elevation North Carolina (HC), and Massachusetts (FV). In addition we examined F_2's from an AL × FV cross as well as a backcross of AL × (AL × FV). In all cases, the larvae were reared at 24 ± 1.5C and 10 h of light per day, and maintained on this regimen for 30 days as third or fourth instar larvae before scoring for frequency of diapause. We determined critical photoperiods for the main-
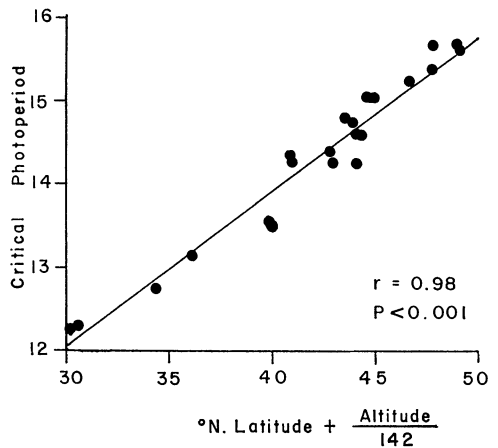

FIG. 4. Critical photoperiod for the initiation and maintenance of III or IV instar diapause from Florida to the northern Gaspé, Quebec. Note that the horizontal axis represents equivalent latitude, that is, latitude corrected for altitude by the conversion factors derived in Eqs. (1–2). Raw data and sample sizes are provided in Appendix I.
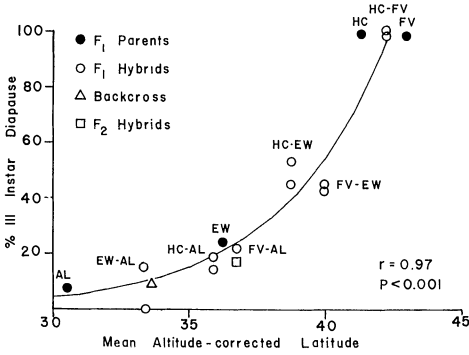
FIG. 5. Proportion of F_1 larvae entering diapause in the III instar. Solid circles, F_1 of inbred parental lines; open circles, F_1 hybrids; triangle, backcross of AL to (FV × AL); square, F_2 of AL × FV. Sample sizes are given in Appendix I.
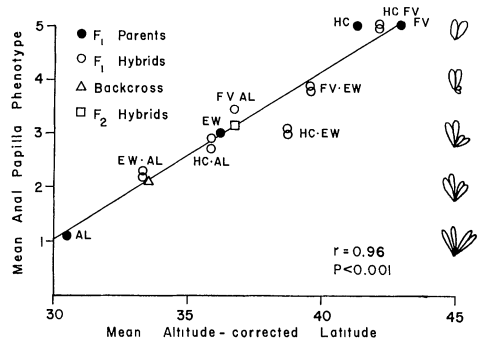

FIG. 6. Mean phenotype of anal papillae among inbred parental and hybrid lines. Sketches of the anal papillae on the right correspond to the numerical score given on the left. The plotted points show the average phenotype of III and IV instar larvae. Confidence limits for individual crosses have been omitted for clarity but are provided in Table 2.

tenance and termination of diapause at this same temperature.

Stage of diapause.—For the initiation of dormancy in the laboratory, we observed a low frequency of third instar diapause among larvae from AL, a somewhat higher frequency among larvae from EW, and an exclusively third instar diapause among larvae from HC and FV (Fig. 5). Hybrid phenotypes were intermediate between those of their parents. Percentage third instar diapause did not vary more than 15% between reciprocal crosses (FV × HC, HC × FV; FV × EW, EW × FV; HC × EW, EW × HC; HC × AL, AL × HC; all crosses are shown as females × males). The increase in the frequency of third instar diapause was best described by an exponential function:

$$\ln(\%\text{ third instar diapause})$$
$$= 0.25 \text{ mean altitude-corrected Lat.}$$
$$- 6.23 \tag{3}$$

Morphology of the anal papillae.—The morphology of the anal papillae of larvae collected in nature ranged from four long, slender papillae along the Gulf coast (LI, AL, WI, HO, MO, and seven other localities in Fla., Ala., and Miss.) to two long and two intermediate, moderately stubby papillae at low elevations in North and South Carolina (EW, PI, GS) to a single

ventral pair of somewhat shortened, stubby papillae at high elevations in North Carolina (HS, HC, DB) or further north (PB, MM, PL, MC, SR, FV, HL, and nine more northern populations in Mich., N.H., Maine, Que., N.B., and N.S.). The number, size, and shapes of anal papillae of F_1 larvae from AL, EW, HC, FV, and their F_1 hybrids was closely correlated with mean altitude-corrected latitude (see Table 2 and Fig. 6):

$$\text{Mean papillary phenotype}$$
$$= 0.31 \text{ mean altitude-corrected Lat.}$$
$$- 8.30 \tag{4}$$

Two way analyses of variance among hybrid papillary mean phenotypes (Table 2) indicate significant differences between crosses ($F = 59.37$; $P < 0.001$) and between instars ($F = 21.05$; $P < 0.001$) where the anal papillae of larvae diapausing in the third instar were north biased with respect to those of larvae diapausing in the fourth instar. Of three reciprocal crosses, only HC × AL and AL × HC had significantly different mean anal papillary phenotypes (difference between means = 0.20; difference required for significance at the 1% level of confidence by Duncan's Multiple Range Test = 0.18), and showed

TABLE 2. *Papillary morphology of F_1 hybrids, given in numbers of papillae. See also Fig. 6.*

Cross[c]	III instar	(n)	IV instar	(n)	III/IV dif.[a]	Recip.[b]
EW × FV	3.93 ± .16**	(30)	3.72 ± .14	(43)	$P < 0.01$	n.s.
FV × EW	3.91 ± .29	(22)	3.71 ± .34	(21)	$P < 0.01$	
EW × HC	3.10 ± .07	(81)	3.00 ± .00	(24)	n.s.	n.s.
HC × EW	3.00 ± .00	(24)	3.00 ± .00	(28)	n.s.	
FV × AL	3.52 ± .36	(21)	3.35 ± .18	(71)	$P < 0.05$	
F_2*	—		3.13 ± .33	(61)	—	
bc × AL*	2.32 ± .51	(19)	1.90 ± .14	(181)	$P < 0.01$	$P < 0.01$
AL × HC	2.94 ± .09	(32)	2.46 ± .10	(194)	$P < 0.01$	
HC × AL	3.00 ± .00	(9)	2.81 ± .11	(53)	$P < 0.05$	
AL × EW	—		2.20 ± .20	(35)	—	
EW × AL	2.50 ± .27	(15)	2.11 ± .07	(83)	$P < 0.01$	

[a] Significance of difference between the morphology of the III and IV instars according to Duncan's Multiple Range Test ($F = 21.05$; $P < 0.01$).
[b] Significance of difference between reciprocal crosses according to Duncan's Multiple Range Test ($F = 59.4$; $P < 0.01$).
[c] Females × males.
* F_2 of AL × FV and backcross, bc, of AL × (AL × FV).
** Mean ± 2 SE.

a paternal bias. We were also able to mimic the papillary phenotype of low elevations in North Carolina (EW) by hybridizing the Alabama (AL) population with either a Massachusetts (FV) or high elevation North Carolina (HC) population. This same mean papillary phenotype was retained in the F_2 hybrid of FV × AL (Fig. 6). We obtained virtually identical phenotypes by crossing AL × EW and by backcrossing FV × AL with AL. Figure 7 shows that the phenotypic variance of anal papillae was correlated with map distance between the localities where we collected their parents ($r = 0.79$; $P < 0.001$). The anal papillae of larvae at HC are identical to those from FV; yet, F_1 larvae resulting from crossing FV times EW show greater phenotypic variance than the F_1 larvae of HC times EW (Fig. 7). Similarly, greater phenotypic variance is observed among the F_1 progeny of AL times FV than AL times HC. The variance observed in the F_2 of FV × AL was significantly greater than the mean F_1 ($F = 2.66$; $P < 0.01$) or mean backcross ($F = 1.64$; $P < 0.001$) variance (Fig. 7). Mean backcross variance was also significantly greater than the mean variance observed among the F_1 ($F = 1.63$; $P < 0.05$).

Critical photoperiod—The critical photoperiod of hybrid and parental F_1's was closely correlated with mean altitude-corrected latitude (Fig. 8):

$$\text{Critical photoperiod} = 5.66 + 0.21 \text{ mean altitude-corrected Lat.} \quad (5)$$

In particular, we could create the photoperiodic phenotype of low elevation North
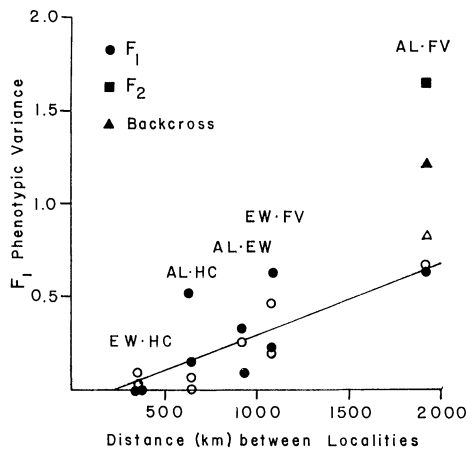

FIG. 7. Phenotypic variance of papillary morphology as a function of approximate distance between localities of the parents. Open figures represent variance estimates for III and solid figures the variance estimates for IV instar larvae. Variances for both III and IV instar larvae entered into the regression but those for the F_2 and backcross did not.
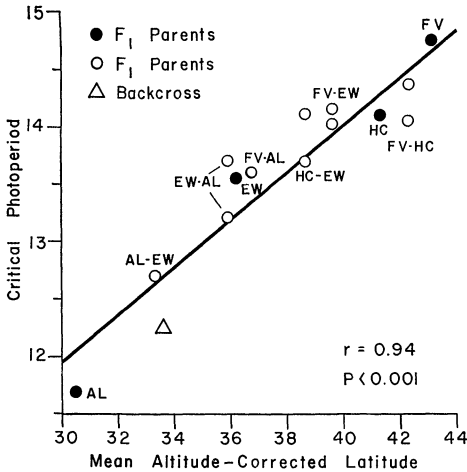
FIG. 8. Critical photoperiod for the initiation of diapause among F_1 larvae. Symbols and the sequence of parentals and hybrids are as in Figs. 5–6. Increments of photoperiods and sample sizes are provided in Appendix II.

FIG. 9. Depth of diapause as measured by T_{50}, the number of pulsed long days required to terminate diapause in 50% of the sample. T_{50}'s for the termination of III instar diapause were used to calculate the altitude:latitude conversion factor, 49m:°N (Eqs. 6–7), which was then used to correct for altitude of IV instar diapausing populations. Actual T_{50}'s and sample sizes are given in Appendix I.

Carolina (EW) either by crossing an Alabama population times a Massachusetts population (AL × FV) or by crossing an Alabama population times a high altitude North Carolina population (HC). In addition, the backcross AL × (AL × FV) resulted in a critical photoperiod similar to that of the F_1 hybrids obtained from the AL × EW cross (Fig. 8).

Depth of Diapause

To estimate the depth of diapause, we determined T_{50}, the number of pulsed long days required to terminate diapause in half the sample population. Among larvae diapausing in the third instar (CB7, CB5, FV, HL, PL, MM, HS, and DB; one determination each except HS and DB, two determinations), T_{50} was significantly correlated with both altitude and latitude ($r = 0.945$, $F = 29.35$, $P < 0.001$; t(altitude) = 3.66, $P < 0.01$; t(latitude) = 7.07, $P < 0.001$):

$$T_{50} = -4.8 + 0.16°N \text{ Lat.}$$
$$+ 0.0033m \text{ Alt.} \qquad (6)$$

Consideration of these two variables was therefore sufficient to explain 89% of the variation in T_{50} that we observed among
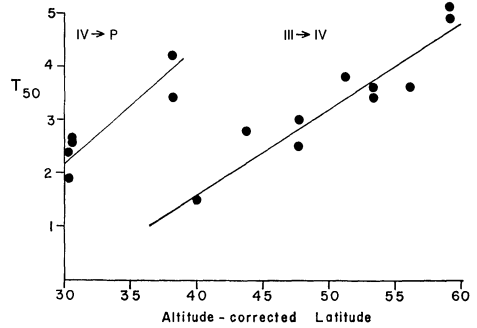
larvae from elevations in excess of 900 m or from latitudes greater than 39°N. The quotients of the regression coefficients for latitude and altitude (Eq. 6) gives a conversion factor for correcting latitude for altitude so that

Altitude-corrected latitude
   $= °N \text{ Lat.} + (m \text{ Alt.}/49).$ (7)

A rise of 49 m altitude thus has the equivalent effect on T_{50} among third instar diapausing larvae as an increase in latitude of 1°N.

We did not have colonies of *Wyeomyia* which diapaused in the fourth instar from sufficient variations in altitude and latitude to warrant an independent analysis of these factors. Rather, we used Eq. 7 to convert altitude to equivalent latitude for T_{50} among populations diapausing in either the third or the fourth instar (Fig. 9). T_{50} for the termination of fourth instar diapause (two determinations each from LI, WI, and EW) was correlated with altitude-corrected latitude ($r = 0.91$; $P < 0.001$) and

T_{50}(III to IV)
   $= 0.16$ altitude-corrected Lat. $- 4.8$ (8)

T_{50}(IV to Pupa)
  = 0.18 altitude-corrected Lat. − 5.2. (9)

Analysis of covariance indicates that the slopes of these two lines do not differ significantly from each other ($F = 0.117$; $P > 0.05$).

Initiation of Diapause

To examine the effect of photoperiod on early development, we reared larvae from nine localities (WI, LI, GS, EW, DB, MM, FV, HL, and CB) on a variety of photoperiods ranging from 12–17 hours of light per day at 25 ± 1C. We observed larvae daily from the day of hatch until they pupated or had spent 20 days as a third or 30 days as a fourth instar larva, i.e., had entered diapause.

There was no apparent effect of photoperiod on the duration of the first instar within any population. Among populations originating from Florida and Alabama or low elevation North Carolina, there was no distinct influence of photoperiod on the duration of the second instar (Fig. 10: WI, LI, GS, EW). At higher elevations in North Carolina and further north, photoperiod had a striking effect on the median duration of the second instar (Fig. 9: DB, MM, FV, HL, CB). In these latter populations, long days hastened and short days retarded the completion of the second instar. The maximum duration of the second instar usually occurred at about the critical photoperiod or at slightly shorter daylengths. The difference between the shortest and longest median duration of the second instar was less than 2.5 days among larvae from the Gulf coast (WI, LI) or low elevation in North Carolina (EW, GS). This difference ranged from 5.5–10 days among larvae from high elevations in North Carolina (DB) and from New Jersey, Massachusetts, and Michigan (MM, FV, HL, respectively). The difference between the shortest and longest median duration of the second instar declined to 3.5 days for a population obtained from Nova Scotia (CB), although

photophases as long as 17 h failed to hasten the completion of this instar to shorter than 7 days.

The duration of the third instar was strongly affected by photoperiod among larvae from all localities. Along the Gulf coast and at low elevations in North Carolina (LI, WI, GS, EW), long days promote the rapid completion of the third instar and short days retard it. The maximum duration of the third instar, like that of the second, occurs slightly short of the critical photoperiod. At high altitudes in North Carolina and further north, short days initiate and long days avert diapause in the third instar. The only population which exhibits a substantial proportion of both third and fourth instar diapause originates from EW (Fig. 11). The F_1 larvae from EW enter fourth instar diapause at very short daylengths. With increasing daylength, the proportion of larvae diapausing as thirds increases, peaking sharply at the critical photoperiod, 13.15 h of light per day.

These results show that photoperiod, apart from its qualitative role in determining diapause, plays an important role in prediapause development. When diapause occurs in the fourth instar, photoperiod does not affect the duration of the second but has a strong influence on the third instar. Similarly, when diapause occurs in the third instar, photoperiod does not affect the duration of the first but exerts a major influence on the length of the second instar.

DISCUSSION

Systematics of the Pitcher-plant Mosquito

The absence of silver scutellar scales on adult females and the absence of dorsal anal papillae on the larvae might be used to distinguish *W. smithii* from *haynei* and Gulf coast, but the lateral hairs on the abdominal segment are at best a questionable quantitative character. Similarly, Gulf coast might be distinguished from *W.*
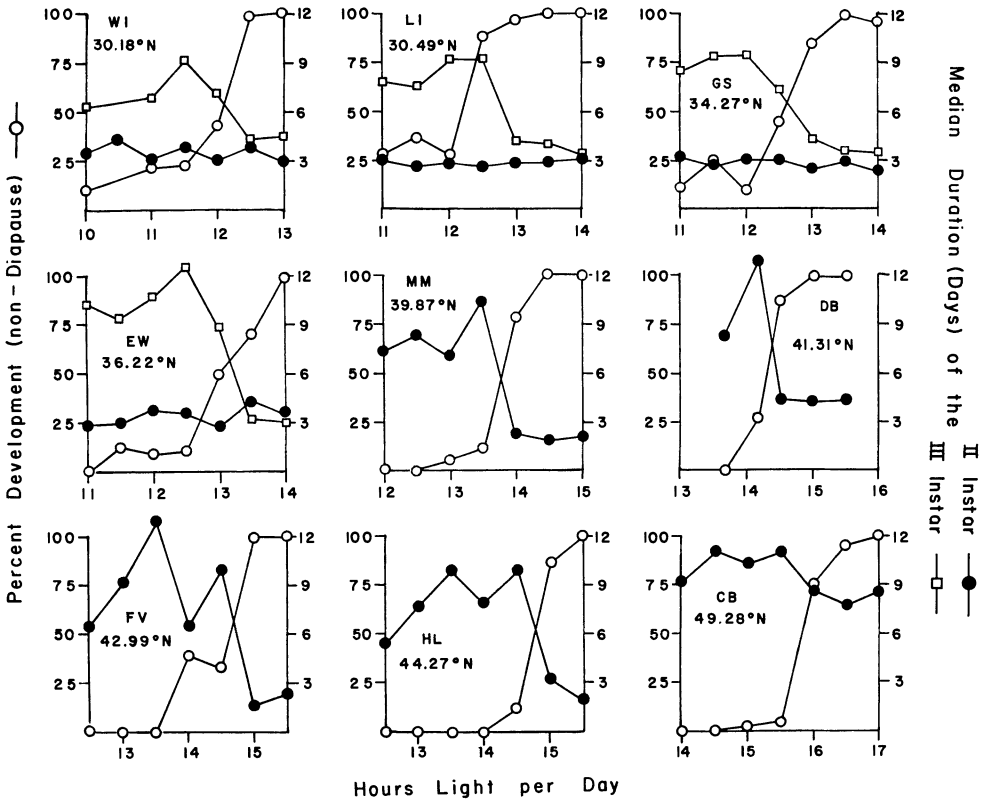
FIG. 10. Initiation of diapause and duration of prediapause development in the II and III instars. The open circles plot percent development (nondiapause) as a function of photoperiod to give reference points for the duration of previous stages. The latitudes given in the figures are altitude-corrected latitudes; hence, DB appears between MM and FV. From WI to EW, the duration of the II instar is little affected by photoperiod; thereafter, photoperiod has a substantial effect on its duration until CB (here represented as a composite of all three Cape Breton localities) where the second instar is long, regardless of photoperiod. From WI to EW, photoperiod has a quantitative effect on the duration of the third instar; north of EW, daylength imposes a qualitative diapause/no-diapause effect on the III instar. Sample sizes are given in Appendix I.

haynei on the basis of longer relative length of the dorsal papillae on the larvae but is indistinguishable as an adult. None of the three varieties of *Wyeomyia*, *smithii*, *haynei*, or Gulf coast, appears to be a distinct species. We therefore lump them all into one species, *W. smithii*, and distinguish three geographical races.

Apart from the morphological reasons above, there are two major bases for our assertion of a single species of *Wyeomyia* living in the pitcher-plant, *Sarracenia purpurea*. First, populations from as far away as Alabama and Massachusetts readily form fertile hybrids in the laboratory. Second, the phenotype of *W. haynei* larvae (EW) may be created at will in the laboratory by hybridizing the Gulf coast phenotype (AL) with populations from either high elevations in North Carolina (HC) or Massachusetts (FV), both "*W. smithii*." The latter observation holds true not only for anal papillary morphology (Fig. 6) but also for the stage of dormancy (Fig. 5) and its photoperiodic control (Fig. 8). We therefore conclude that these
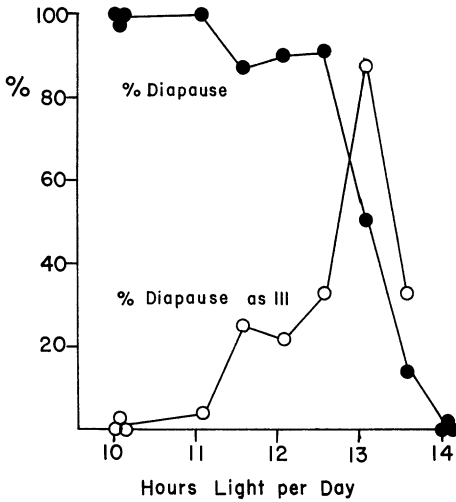
FIG. 11. Initiation of diapause at Eastwood (EW) in the North Carolina Piedmount. EW is the northernmost of the southern IV instar diapausing populations. Solid circles show the total percent of the population entering diapause; the open circles show the percent of those which enter diapause which do so in the III rather than the IV instar.

characters, morphological and physiological, distinguish geographic races whose phenotypes are the consequence of living at different altitudes and latitudes. *W. smithii* should exhibit a geographic cline in these characters whose phenotypes are predicted by the intercepts in Figures 5, 6, and 8. We have tried on several occasions to find pitcher-plant localities between 400–800 m in North Carolina and between 36–40°N in southeastern Virginia. So far, we have been unsuccessful, not because *S. purpurea* does not occur at these latitudes and altitudes (McDaniel, 1971), but, we believe, due to the destruction of many habitats. We found, for instance, specific localities described by Fernald and Long on herbaria specimens in the University of North Carolina and Duke Herbaria, which are now either paved or have gone through ecological succession to habitats where *S. purpurea* do not live.

Altitude and Latitude

There are numerous studies on the ef-

fect of latitude on photoperiodism in insects and mites (Beck, 1968; Lees, 1955; Danilevskii, 1965; Danilevskii et al., 1970; Tauber and Tauber, 1972), but only a few studies have taken altitude into account. Accommodation to latitude and altitude can take place with or without modification of the critical photoperiod. Certain lepidoptera (Danilevskii et al., 1970) have adapted to high elevations by shifting the stage which is sensitive to photoperiod in response to lower temperatures. Croft (1971) examined photoperiodic response in the mite, *Typhlodromus occidentalis*, from four localities in California and the Pacific Northwest. Using his data on critical photoperiod, altitude, and latitude, we have calculated an altitude:latitude conversion factor of 363 m:°N. This extremely high value suggests that *T. occidentalis* employs a mixed temperature: photoperiod strategy over its range of habitats. The possible divergence of adaptation to latitude and altitude is illustrated by *Pieris napi meridonalis* (Danilevskii et al., 1970). In eastern Europe at low elevations, the critical photoperiod of this butterfly increases from 13–18 h of light per day over 17°N latitude. Yet, in the southern Caucasus, the critical photoperiod remains constant from sea level to 1600 m. *W. smithii* provides an example of an alternative strategy, namely, changing the critical photoperiod continuously over a wide range of altitudes and latitudes. The factor for converting units of altitude to those of latitude, 142 m:°N (Eq. 2), may be used to predict not only the hybrid critical photoperiods (Fig. 8) but hybrid anal papillary phenotypes as well (Fig. 6). We interpret this similar relationship to mean that similar environmental variables, presumably climatic, are acting upon the two systems. We can correlate critical photoperiod or mean anal gill phenotype closely with a number of climatic variables: mean annual temperature, mean number of freeze-free days, Julian date of first freeze, or mean number of days when the temperature is

90F (31.2C) or higher. But, since both critical photoperiod and mean anal papillary phenotype themselves correlate closely with altitude and latitude, as do the climatic variables, identifying strict causality is not possible. Both growing season and maximum summer temperatures clearly increase at lower latitudes and altitudes. Photoperiodic organisms will adapt to later and milder winters in the southern low altitudes by shorter critical photoperiods. Concomitant with the longer growing season, however, is an increase in mean summer temperature (Baldwin, 1968). Pitcher-plant dwelling *Wyeomyia* do not have to surface to respire. Ordinarily, mosquito larvae use their anal papillae for salt and water balance but the use of anal papillae to augment oxygen transpiration is not without precedent (Clements, 1963, p. 54–56). If *W. smithii* employs its anal papillae as a respiratory surface, the reduction in number and size of anal papillae may correspond to an increase in the amount of dissolved oxygen available in the leaves of *S. purpurea* on cooler mountains or at more northern latitudes.

There are several investigations which provide a basis for estimating altitude: latitude equivalencies for ecogeographic characters. Hopkins (1938), proposed his "bioclimatic law" which states that the date of a phenological event should advance 4 days for an increase of 1° in latitude, 5° in longitude, or 400 ft in altitude (122 m: °N). Using the data of Johnston (1954), we calculated a conversion factor of 142 m: °N ($r = 0.94$; $P < 0.01$; $n = 6$) for the onset of breeding of song sparrows in the Pacific Northwest. We also calculated equivalences of 138 m: °N ($r = 0.59$; $P < 0.001$; $n = 35$) for timberline in continental Europe (Dansereau, 1957, p. 108), 50 m: °N ($r = 0.71$; $P < 0.001$; $n = 25$) for critical thermal maximum and 75 m: °N ($r = 0.94$; $P < 0.001$; $n = 32$) for the lower lethal limit in frogs and toads (Brattstrom, 1968). Reader, Radford, and Lieth (1974) found a conversion factor of 317 m: °N ($r = 0.94$; $P < 0.001$; $n = 133$) for

the average dogwood-redbud flowering dates in eastern North America. They are quick to point out that the effect of geography on phenological events is confounded by microclimatic variations due to soil, slope, aspect, etc. The correlation of litter size and latitude in North American mammals varies according to whether one examines pedators or prey and whether or not the prey hibernate (Lord, 1960). Reader et al. (1974) describe the difficulty in correlating phenological events with specific climatic variables—including dates predicted by Hopkins (1938) law. Hardwick and Lefkovitch (1971) found that geography (altitude, latitude, and longitude) explained only 27% of the variation in the distribution of the moths in the genus *Euxoa* in western North America. Sample size and an index of habitat accounted for 68% of the variation and when geography was added as a supplemental variable, only an additional 6% of the variation was explained. Hardwick and Lefkovitch point out that "the major habitats in western North America may be defined largely on a geographic basis, and climatic temperature and rainfall gradients are to a large extent a function of latitude and altitude."

The clear expression of the photoperiodic phenotype in *W. smithii* probably relates to two observations. First, the primary adaptive significance of photoperiodism is to enable organisms to time their physiology and development in appropriate concert with the changing seasons. Second, the pitcher-plant may itself experience a wide variety of micro-habitats: wet pine savannahs along the Gulf coast and low elevations in North Carolina; alder thickets at high elevations in North Carolina; cedar swamps in New Jersey; cattail marshes, quaking bogs, and marl pits farther north. However, the larvae of *W. smithii* experience only one micro-habitat over their entire range: the water-filled leaves of a single species of plant. Critical photoperiod in *W. smithii* thus represents an ecogeographic character closely related

to climate and uniquely free of environmental vagaries which obscure the significance of the altitude:latitude equivalencies discussed above.

The difference in the conversion factor of 142 m:°N in *W. smithii* and Hopkins' law (122 m:°N) may be due to the differences in daylength for a given calendar date on a southern mountain and the same date at low elevation further north (Bradshaw, 1976). For a given calendar date between the vernal and autumnal equinoxes, daylengths are longer in the north than in the south. Consequently, *W. smithii* on a southern mountain would use a shorter daylength to enter diapause on the same calendar date as a population further north. To find a population on a southern mountain which used the same critical photoperiod as the population further north, one would have to go still higher on the mountain. "Hopkins' bioclimatic law in temperate and north temperate latitudes may thus be expected to underestimate the effect of altitude on photoperiodism" (Bradshaw, 1976).

Anal Papillae and the Evolution of Wyeomyia

The 90 or so members of the genus *Wyeomyia* are mainly Neo- and subtropical; only the pitcher-plant dwellers range into the temperate zone north of Florida (Carpenter and LaCasse, 1955; Ross, 1964; Stone, Knight, and Starke, 1959). This distribution suggests that the origins of the pitcher-plant mosquito were tropical (Ross, 1964). In addition, the number of anal papillae is a highly conservative character. Whereas there are more than a few species of mosquitoes whose larvae have one pair of anal papillae reduced in size, we know of only one other North American mosquito, *Culex bahamensis* (Carpenter and LaCasse, 1955), which has but a single pair. The two other North American species of *Wyeomyia*, *W. mitchelli* and *W. vanduzeei*, are restricted to bromeliads in southern Florida and both have four anal papillae. We therefore conclude that four

long anal gills in *Wyeomyia* is primitive and that *W. smithii* has evolved in a northwardly direction in temperate North America, probably following the post-glacial invasion of *Sarracenia purpurea* into the north temperate zone.

When a northern, two papillae phenotype is crossed with the Gulf coast, four long papillae phenotype, the mean papillary phenotype of the F$_1$ hybrid reflects the relative equivalent latitudes of the parents (Fig. 6). For example, AL × HC yields F$_1$ progeny whose dorsal papillae are longer than those of AL × FV, even though larvae from both HC and FV possess only two anal papillae. The implication is that HC, from a lower equivalent latitude, has apparently retained more genes which code for longer anal papillae. We noted above that F$_1$ hybrids which diapaused in the third instar tended to have more northern mean papillary phenotypes than the F$_1$ of the same cross which diapaused in the fourth instar. While this observation is strongly suggestive of pleiotropy, we are unable to say whether the tendency of HC × AL hybrids to have more southern mean papillary phenotypes than FV × AL results from positive selection for pleiotropic properties, from gene flow from lower elevations, or both.

The increase in phenotypic variance observed in the F$_2$ and backcross generations (Fig. 7) indicates that anal papillary morphology is under polygenic control. The correlation of phenotypic variance with distance rather than phenotypic divergence suggests that different genes are involved in the formation of visibly identical anal papillae among the FV and HC populations. Apparently the same adaptive norm, the absence of dorsal papillae and reduction of ventral papillae, has been arrived at through distinct evolutionary pathways.

Depth of Diapause

The depth of dormancy, or diapause intensity, is generally positively correlated with latitude (Danilevskii, 1965; Tauber

and Tauber, 1972). In some cases, southern forms may experience a more intense diapause which maintains their dormant state despite transient periods of developmentally favorable temperatures (Danilevskii, 1965; Danilevskii et al., 1970). In *W. smithii*, the depth of dormancy as indicated by T_{50} appears to be a discontinuous or saw-toothed function of equivalent latitude (Fig. 9), although the similarity in slopes for third and fourth instar diapause suggests that related adaptive strategies are involved. In addition, the altitude:latitude conversion factor for T_{50} is 49 m:°N, approximately ⅓ the value for critical photoperiod or anal papillae (Figs. 4, 6).

In the north, third instar larvae enter a firm diapause (Fig. 10). Farther south, diapause in the third instar becomes shallower until T_{50} approaches one long day. When less than one long day is required to terminate diapause in the third instar, the larvae do not diapause in that stage but enter a firm fourth stage diapause. Going still farther south, the firmness of diapause in the fourth instar continues to decrease until, on the Gulf coast, the larvae undergo a somewhat shallow dormancy. The important point is that when viewed as remoteness from pupation, the pattern observed in Fig. 9 represents a continuum. In the mild climate along the Gulf coast, *Wyeomia* enter dormancy in the last larval instar and require little input from the environment to resume development. Proceeding northward, not only do winters become more severe, but also vernal climates become more unpredictable. Polymorphisms which vary the timing of development are not unusual among other insects (Bradshaw, 1973; Lounibos and Bradshaw, 1975) and the increase in the depth of dormancy with equivalent latitude may represent an analogous adaptation in *Wyeomyia*. Among southern populations of *Wyeomyia*, a shallow fourth instar diapause becomes somewhat more conservative by requiring a greater stimulation (higher T_{50}) for the resumption of devel-

opment. Nonetheless, fourth instar diapausing larvae are still developmentally close to the sensitive pupal stage and to committing themselves to a more unpredictable aerial environment. The next steps in the northward progression are to enter first a shallow and then progressively deeper dormancies at an earlier age, i.e., the third instar. Climatic factors are probably important in the evolution of specific phenotypes but the fitness which these phenotypes realize during a given spring will be strongly affected by the weather during that spring. Both the water in the leaf of a pitcher-plant and the surrounding bog may act as a buffer against freezing (Patterson, 1971). *Wyeomyia* are more protected from capricious weather if they remain in the aquatic confines of these leaves.

Increasing the depth of dormancy (i.e., lengthening T_{50}) may retard development in the spring, thereby protracting the generation time. Moving diapause to an earlier stage has no such effect. At a given locality, it requires the same generation time to go from a diapausing third stage larva in the spring to a diapausing third stage larva in the fall as it does to go from a fourth to a fourth.

We wish to emphasize that we are using T_{50} as an indicator of the depth or firmness with which diapause is established. The actual termination of diapause in nature will be affected not only by the immediate temperature and photoperiod but also by chilling received during the winter and spring. Such chilling can alter the responsiveness of diapausing larvae to short and intermediate as well as long days (Bradshaw, 1974). Correlation of T_{50} with altitude and latitude serves as a warning for the interpretation of ecogeographic characters. Had we examined T_{50} among only the northern *W. smithii*, we would have arrived at a very different altitude: latitude conversion factor than we did for critical photoperiod. T_{50} is confounded with the stage of diapause and the more appropriate ecogeographic character to be

considered might be the time from the onset of diapause terminating stimuli to pupation or adult emergence. This character is a composite of at least four parameters: T_{50}, the effects of chilling, the stage of dormancy, and adaptive and acclimative responses to temperature. The difficulty in constructing this composite character further underscores the elegance of the photoperiodic phenotype in *W. smithii*.

Evolution of Dormancy and its Photoperiodic Control

Both the present-day distribution of the genus *Wyeomia* and the anal papillary morphology argue that evolution of pitcher-plant mosquitoes in North America has proceeded in a south to north direction. We propose that dormancy has likewise evolved from south to north so that diapause in the fourth instar is primitive while that in the third is derived. Our principal argument is that while a second, fourth instar diapause may be induced among northern populations (Lounibos and Bradshaw, 1975), we have been consistently unable to evoke a third instar diapause among any of the populations from the Gulf coast (Fig. 10, LI, WI). To be sure, photoperiod has a quantitative effect on the duration of the third instar at these latter localities but short days did not induce diapause among any of them. Moreover, we found no discernible effect of photoperiod on development of larvae overwintering as third instars along the Gulf Coast (Fig. 3, LI, WI).

The effect of photoperiod on the relative durations of the second, third, and fourth instars reveals the manner in which third instar diapause may have evolved (Fig. 10). Along the Gulf coast (WI, LI) and the North Carolina coastal plain (GS), the second instar is little affected by photoperiod but the third instar is definitely prolonged under the influence of short days, particularly those daylengths proximal to the critical photoperiod. On the piedmount of North Carolina (EW), photoperiod has an even more pronounced effect on the du-

ration of the third instar and, proceeding higher in either latitude (MM) or altitude (DB), short days prolong the third instar indefinitely, i.e., induce diapause.

We can propose at least two reasons why the prediapause instar might be prolonged under short-day conditions. (1) The larvae may require additional time to store nutritional reserves prior to diapause. *W. smithii* in Massachusetts may initiate diapause as early as August (Lounibos and Bradshaw, 1975) when daylengths are declining but temperatures are still quite warm. Although they are capable of feeding and growing while in diapause (Bradshaw and Lounibos, 1972), their growth rate during dormancy is relatively slow. In addition, when temperatures approach freezing, larvae may void their guts (Patterson, 1971), presumably lessening the probability of ice-crystal formation. Prolongation of the second instar prior to diapause may thus afford the larvae an additional period of active feeding while temperatures are still warm. Extra prolongation of the prediapause instar at or near the critical photoperiod (Fig. 10, MM, DB, HL, EW, LI, WI) would be desirable since late in the summer these daylengths would constitute the warmest "short days" encountered under natural conditions. (2) Photoperiods at or near the critical photoperiod are neither long nor short and may represent ambiguous cues. Some insects may be able to discern the direction of change in photoperiod as well as its absolute duration. For example, in the lacewing, *Chrysopa carnea* (Tauber and Tauber, 1970), 12 h of light per day may be interpreted as long, short, or intermediate, depending upon the photoperiods previously experienced. For the termination of diapause, *W. smithii* appears to rely solely upon the absolute length of day (or night) (Bradshaw, 1971). While we do not know whether prior photoperiodic experience is important for the initiation of diapause in *Wyeomyia*, extended prolongation of the second instar at or near the critical photoperiod would permit a longer period

of time to assess absolute daylength or the direction in which it was changing. In extreme northward populations, the length of the second instar is less variable in response to different daylengths (Fig. 10, CB). In such circumstances where *W. smithii* is likely univoltine and third instar diapause occurs in every generation, selective pressure for a differential photoperiodic response would decline.

Evolution of third instar dormancy has taken place via the increased effect of photoperiod on the prediapause instar. Initially, short days retard development during the stage prior to diapause. Through evolutionary time, this effect may be progressively enhanced. Eventually, short days promote an indefinite prolongation of development or diapause in the former prediapause instar. Entering into a third instar diapause on the North Carolina piedmount (Fig. 11) under the influence of intermediate photoperiods may represent a conservative response to ambiguous cues, leaving more developmental options open to an individual. Farther north or at high elevations, diapause becomes routinely conservative, i.e., is entered exclusively in the third instar. It is, however, interesting to note that the ancestral, fourth instar, form of diapause can be invoked in the spring when ambiguous conditions may again occur (Lounibos and Bradshaw, 1975). The spruce budworm in eastern Canada normally enters diapause in the second larval instar but about 5% of the population is capable of entering a secondary diapause in the fourth instar (Harvey, 1961). Nymphs of the dragonfly, *Anax imperator*, in England may diapause in either an early or late nymphal stage or both (Corbet, 1956). *A. imperator* can thus become either uni- or semi-voltine depending upon environmental conditions. Levins (1969) and Cohen (1970) have presented models for the optimal timing of diapause. These approaches have emphasized diapause as a physiological trigger or discontinuous rate process. However, at least some insects such as the spruce budworm, emperor

dragonfly, and pitcher-plant mosquito appear capable of maintaining finer gradations between dormancy and continuous development.

SUMMARY

Pitcher-plant dwelling mosquitoes in North America range from the Gulf Coast to Labrador and northern Manitoba. From the Gulf Coast to the Northern Gaspé, Quebec, the larvae overwinter in a state of developmental arrest (diapause) in the leaves of *Sarracenia purpurea*. Along the Gulf Coast, larvae enter a shallow diapause in the fourth instar. The depth of diapause increases northwards to the Carolina piedmount. At higher elevations in North Carolina or farther north, larvae diapause in the third larval instar. As in the case of fourth instar diapause, the depth of dormancy increases with latitude or altitude. Despite diapause in different stages, the critical photoperiod mediating its onset and maintenance varies continuously, 1 h for each increase of 5.4°N latitude or 769 m altitude. The ratio of these parameters, 142 m : °N, permits calculation of equivalent latitude for any locality. Both the stage of diapause as well as the critical photoperiod among F_1 hybrids from various localities were closely correlated with the average equivalent latitude of the parents. The southern race, formerly *Wyeomyia haynei*, possesses four long anal papillae along the Gulf Coast and two long and two short anal papillae at low elevations in North Carolina. *W. smithii* from high elevations in North Carolina or from farther north have but two ventral papillae. *W. haynei* from southern Alabama or the North Carolina piedmount form fertile hybrids with *W. smithii* from high elevations in North Carolina or low elevations in Massachusetts. The anal papillary morphology of the F_1 hybrids is closely correlated with the equivalent latitude, calculated from photoperiodic response, of the parents. F_1 hybrids between the Gulf Coast variety and animals from high elevations in North Carolina or from Massa-

chusetts (both *smithii*) resemble *haynei* from low elevations in North Carolina with respect to critical photoperiod, morphology of the anal papillae, and stage of dormancy. *W. smithii* is therefore probably polytypic and includes *W. haynei* as a geographic subspecies. Both present-day distribution of the genus and the anal papillary morphology indicate that the southern form is primitive. Although the northern *W. smithii* can be induced to enter a second, fourth instar diapause (Lounibos and Bradshaw, 1975), short days can only retard but not halt development in the third instar among the Gulf Coast variety. Going north from the Gulf Coast, short days increasingly prolong the third instar until diapause occurs in that earlier stage. We therefore conclude that the direction in evolution of dormancy has proceeded from south to north and has taken place via the progressive influence of photoperiod on the prediapause instar.

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APPENDIX I. *Alphabetical listing of localities. Below are given the state, county, longitude (Long), latitude (Lat), and altitude (Alt) of each locality mentioned in the text and/or used in the figures and/or tables. Under Crit. PPD are provided the critical photoperiods shown in Fig. 4 for the initiation (i) or termination (t) of third (III) or fourth (IV) instar diapause with the mean number of individuals used at each photoperiod (n). Under T$_{50}$ are provided the actual T$_{50}$'s and sample sizes (n) for populations shown in Fig. 9. Under Init. are given the average sample sizes for each photoperiod for each population shown in Fig. 10. Under Termin are given the mean sample sizes for each photoperiod shown in Fig. 3. Under % collection are given the percentages of the total collection in the second (II), third (III), or fourth (IV) instar along with the collection size (n) for collections made between 20-1-73 and 28-1-73.*

Loc	State	County	Long	Lat	Alt	Fig. 4 Crit. PPD	Fig. 9 T$_{50}$ (n)	Fig. 10 Init. (n)	Fig. 3 Termin III	Fig. 3 Termin IV	Fig. 2 % collection II	Fig. 2 % collection III	Fig. 2 % collection IV	(n)
AL	Alabama	Baldwin	88	31	20						5	40	55	(962)
BG	Alabama	Mobile	88	31	10						3	32	65	(582)
CB2	Nova Scotia		61	47	440									
CB5	Nova Scotia		60	46	220		3.8 (14)	19						
CB7	Nova Scotia		61	47	410		3.6 (21)							
CI	Alabama	Washington	88	31	50						4	31	65	(409)
DB	N. Carolina	Jackson	83	35	900	14.35tIII (25)	3.6 (23) 3.4 (14)	25	38	—	0	100	0	(843)
EW	N. Carolina	Moore	80	35	150	13.15tIII (21)	3.4 (15) 4.2 (18)	21	40	32	9	65	26	(1703)
FV	Massachusetts	Middlesex	72	43	60	14.25tIII (10)	2.8 (14)	15						
GS	N. Carolina	Brunswick	78	34	20	12.75tIV (25)		25	39	23	4	52	44	(1635)
HC	N. Carolina	Macon	83	35	890	14.35tIII (24)					0	100	0	(399)
HL	Michigan	Livingston	84	43	260	14.25tIII (23)	3.0 (24)	12	24	—				
HO	Florida	Santa Rosa	87	31	40						6	38	55	(524)
HS	N. Carolina	Macon	83	35	1190	14.40tIII (30)	5.1 (25) 4.9 (15)		38	—	+	100	0	(263)
JB	New Brunswick		68	47	270	15.65tIII (22)								
LI	Alabama	Baldwin	88	30	10	12.30tIV (27)	2.6 (17) 2.6 (17)	27	34	37	8	44	48	(1796)
MC	New York	Tompkins	76	43	260	14.60tIII (45)								
MM	New Jersey	Burlington	75	40	10	13.80tIII (22)	1.5 (22)	22	28	—				
MO	Mississippi	George	89	31	80						7	46	47	(828)

APPENDIX I.

Loc	State	County	Long	Lat	Alt	Fig. 4 Crit. PPD	Fig. 9 T$_{50}$ (n)	Fig. 10 Init. (n)	Fig. 3 Termin III	Fig. 3 Termin IV	Fig. 2 % collection II	Fig. 2 % collection III	Fig. 2 % collection IV	(n)
NH	New Hampshire	Rockingham	71	43	80	14.80tIII (18)								
NW	Maine	Cumberland	70	44	110	15.05tIII (14)								
OH	Maine	Aroostook	68	46	250	15.40tIII (18)								
PB	New Jersey	Ocean	74	40	10	13.75tIII (17)								
PD	Maine	Penobscot	69	45	250	15.25tIII (21)								
PI	N. Carolina	Moore	80	35	150				25	—				
PL	Michigan	Washtenaw	84	42	260	14.35tIII (22)	2.5 (44)							
RE	N. Carolina	Brunswick	79	34	10						4	49	47	(478)
RR	Michigan	Ogemaw	84	45	200	15.05tIII (21)								
SA	Quebec		70	48	30	15.70tIII (21)								
SR	New York	Monroe	78	43	150	14.75tIII (45)								
SU	Quebec		68	49	30	15.70tIII (21)								
TE	Florida	Liberty	85	30	10						2	46	52	(149)
WI	Florida	Liberty	85	30	10	12.25iIV (35)	1.9 (19) 2.4 (24)	35	31	30	8	41	51	(1522)

APPENDIX II. *Hybridization studies listed by equivalent latitude (Eq Lat). The crosses are given as females × males. Under initiation are provided the sample sizes from which percentages of third instar diapause were calculated. Under Term are provided the increment, 0.5 or 1.0 h, of successive photoperiods and the average sample size at each photoperiod ( ). The F_2 cross is $AL \times FV$ and the backcross is $AL \times (AL \times FV)$.*

Cross	Eq Lat	Fig. 5 Init	Fig. 8 Term
FV × FV	42.99	354	0.5(23)
FV × HC	42.15	525	0.5(32)
HC × FV	42.15	237	0.5(17)
HC × HC	41.30	300	0.5(25)
FV × EW	39.61	54	1.0(15)
EW × FV	39.61	79	1.0(14)
HC × EW	38.76	53	1.0(14)
EW × HC	38.76	152	0.5(7)
FV × AL	36.74	96	0.5(15)
F_2	36.74	74	1.0(13)
EW × EW	36.22	160	0.5(11)
HC × AL	35.89	126	1.0(9)
AL × HC	35.89	247	0.5(15)
backcross	33.61	200	0.5(15)
EW × AL	33.35	99	0.5(12)
AL × EW	33.35	35	———
AL × AL	30.48	337	0.5(20)