Genetic Variation and Inheritance of Diapause Induction in Two Distinct Voltine Ecotypes of *Ostrinia nubilalis* (Lepidoptera: Crambidae)

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ABSTRACT European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), displays a larval diapause in response to short photoperiods and is adapted to a variety of local conditions throughout North America. Hence, the effective photoperiod inducing larval diapause will differ among geographic ecotypes. This study considers the inheritance of photoperiodic larval diapause induction by hybridization and backcrossing two latitudinally distinct ecotypes of the European corn borer collected between 41° N, 96° W and 48° N, 96° W and under a range of photoperiods representative of their respective locations: from 14:10 to16:8 (L:D) h. The ecotype adapted to a bivoltine habitat (southeastern Nebraska) exhibited a shorter critical photoperiod (1480 h) than the ecotype (1533 h), originating from a univoltine habitat (northwestern Minnesota). Reciprocal F_1 crosses exhibited intermediate values with indication of sex-linked inheritance. In addition, the male parent had significantly more influence on diapause incidence of subsequent progeny than the female. The F_2 and backcross progeny further supported the supposition that diapause response is a sex-linked inheritance. The minimum number of genes estimates, and the response from backcross progeny, suggest that diapause response of European corn borer larva may be controlled by only a few loci. The overall results indicated that both ecotypes had adopted unique diapause responses, which ultimately lead to seasonal synchrony in their ecosystems.

KEY WORDS sex-linked, voltinism, diapause induction, genetics, critical photoperiod

Diapause is defined as a state of arrested development enforced by a physiological mechanism rather than by unfavorable environmental conditions (Beck 1962). It is widely distributed among species of insects that encounter great diversity in climatic conditions, obtaining a great photoperiodic response from insect species from different localities. Depending on the species, observed differences among ecotypes may be heritable either due to long-term genetic differentiation or a direct physiological response to environmental conditions (Baldwin and Dingle 1986, Blanckenhorn 1997).

Diapause induction and related traits of European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), have been of interest to scientists almost as early as its introduction to North America. European corn borer is an insect species which enters a well-defined, photoperiodically induced larval diapause as a mature fifth instar between 20 and 29°C (Beck and Apple 1961, Skopic and Bowen 1976, Takeda and Skopik 1985). It is commonly found in most parts of the Northern Hemisphere where it causes significant damage to its main host, corn (*Zea mays* L.) and subsequently, corn-based economies (Krumm et al. 2008). The first positive identification of this species in the United States was made near Boston by Vinal (1917). Since then, European corn borer has spread north, south, and west and has become established in major corn growing areas of the United States. This suggests that longitudinal and latitudinal distribution in North America is closely related to the ability of this species to adapt to several ecological constraints.

Consistent with its large geographic distribution, several differences among ecotypes of the species have been identified, including subtle differences in pheromone blend discrimination (Glover et al. 1987, Sorenson et al. 2005), differences in ability to survive on host plants (Straub et al. 1986, Eckenrode and Webb 1989, Zoerb et al. 2003) and susceptibility to chemical (Kuhr and Davis 1975) and biological (Zoerb et al. 2003) insecticides. In North America, the

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European corn borer is believed to exist as a representation of three ecotypes that are morphologically indistinguishable but have distinct differences in voltinism and sex pheromone communication (Showers et al. 1975). Recently, Coates et al. (2004) documented significant genetic differentiation between samples from Atlantic coast and midwestern U.S. samples and between sympatric uni-and bivoltine ecotypes.

Babcock (1927a,b) expressed a Lamarckian opinion that a borer population under long exposure to a particular seasonal cycle would persist in its diapause and voltinistic characteristics, even if the borers were transferred to different environments. Arbuthnot (1944) suggested that univoltinism functioned as a recessive character. Sparks et al. (1966) examined F_1 crosses of three geographic ecotypes, in field cages and in the laboratory, and concluded that diapause induction was genetically determined, and that the northern population (Minnesota) had more influence on the diapause response of the progeny than did the more southern parents (Iowa and Missouri). They proposed that a multigenetic system was involved in diapause induction. Showers et al. (1972), however, studying European corn borer ecotypes from Alabama, Maryland, and Minnesota, proposed a sex-linked inheritance for diapause induction of the species. Reed et al. (1981) also supported the sex-linked inheritance and lack of dominance. Furthermore, McLeod (1978) concluded that the incidence of diapause was more influenced by the male parent than the female parent. None of these studies, however, used a comprehensive comparison of photoperiod, F_1, F_2, backcrosses, and reciprocal progeny. Reed et al. (1981) suggest that there is an urgent need for genetics research on insects used in intensive research programs and/or insects of economic importance, where there is a potential for genetic mechanisms in population management. Our objective, therefore, was to inspect the genetic components of diapause induction under one temperature by determining the critical photoperiods of two distinct European corn borer ecotypes, their reciprocal hybrids, F_2 crosses, and backcross progeny.

Materials and Methods

Sources of Insect Ecotypes. The laboratory ecotypes of the European corn borer were established from two latitudinally distinct geographic locations in the Corn Belt. The collections consisted primarily of mature fifth instars, ready to diapause under field conditions in September 2000. The European corn borer shows mainly univoltinistic characters in northwestern Minnesota and northeastern North Dakota (Frye 1971, Palmer et al. 1985). Also, the established phenotype above latitude 45° N is known to be univoltine under field conditions (Hudon 1959, Chiang 1961, Showers 1981). Accordingly, the univoltine European corn borer ecotype represented in this study was obtained from a combined collection of three different cornfields in northwestern Minnesota (Polk County, 47.8° N, 96.6° W; Pennington County, 48.1° N, 96.2° W; and Red Lake County, 47.9° N, 96.3° W), and the bivoltine ecotype was represented among combined collections of two areas of southeastern Nebraska (Lancaster County, 40.8° N, 96.6° W; and Saunders County, 41.2° N, 96.5° W). These two ecotypes from northwestern Minnesota and southeastern Nebraska are hereafter be referred to as univoltine (U) and bivoltine (B), respectively. At each site, plants showing signs of larval infestation were dissected and >300 larvae collected. The number of larvae taken from any one plant was limited to four, to reduce the number of siblings and ensure more genetic diversity in the samples.

Population Establishment. Field-collected larvae were disinfected by subjection to phenylmercuric nitrate (0.1 g/liter H_2O; Sigma, St. Louis, MO) as described in Guthrie et al. (1985). In case of insufficient reserves and diapause induction in the field, the collections were introduced to a meridic diet with pupation collection rings (Reed et al. 1972) to simulate an overwintering site and then placed in a growth chamber (model I-35 VLX, Percival, Perry, IA) at 25°C and a photoperiod of 12:12 (L:D) h for 10 d. This procedure ensured appropriate diapause induction with sufficient fat reserves for subsequent diapause development in collections for both ecotypes. The larvae inside pupation rings were placed in plastic round boxes (17.8 by 7.6 cm, diameter × height) containing a stack of moistened paper and chilled under complete darkness at 4°C for 90 d to complete simulated winter development. During this procedure, to maintain humidity, moisture was periodically added to the overwintering larvae by spraying or misting water into the plastic boxes. Upon completion of the chilling procedure, each larva was individually placed in 7 dram vials (U.S. Plastic Corp., Lima, OH) containing moistened cotton balls at the bottom, water source, and a dry cotton at the top served as a plug to prevent larval escape. The appropriately labeled vials were then placed in a growth chamber at 28°C and a photoperiod of 16:8 (L:D) to attain pupation.

Each parental stock was reared for at least two generations on a regular European corn borer diet (Guthrie et al. 1985) to develop synchronization of the ecotypes. The univoltine population was clearly behind in its post diapause development compared with the bivoltine population. Furthermore, there was a substantial amount of variation within each population, which required a synchronization procedure for a fair representation of each population. This was achieved by slowing the development of early pupated individuals at low temperatures between 17 and 22°C, depending on their expected eclosion date. During this early rearing period, and throughout the experiment, the larvae were reared on a full European corn borer diet containing antibiotics to eliminate field-borne diseases and to protect them from any further laboratory-borne infections (Guthrie et al. 1985). In both ecotypes, the moths that emerged from this procedure were mated, and the progeny that resulted from stock population matings were used for the experimental procedures.

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Table 1. Average diapause frequencies (± SE) of European corn borer univoltine (U; from Minnesota) and bivoltine (B; from Nebraska) ecotypes; their F_1, F_2 crosses, and backcross progeny in response to different photoperiods at 27°C

Crosses (♀ × ♂)	Photoperiod (daylight/24 h)								
	1400	1433	1450	1466	1500	1533	1566	1583	1600
B	0.95 ± 0.07	0.89 ± 0.06	0.71 ± 0.06	0.57 ± 0.06	0.34 ± 0.06	0.13 ± 0.06	0.03 ± 0.07	0.03 ± 0.07	<0.01 ± 0.08
U	1.00 ± 0.08	1.00 ± 0.07	0.97 ± 0.07	0.95 ± 0.06	0.77 ± 0.06	0.56 ± 0.06	0.22 ± 0.06	0.13 ± 0.07	0.02 ± 0.07
B × U	0.99 ± 0.08	0.96 ± 0.07	0.93 ± 0.06	0.78 ± 0.06	0.54 ± 0.06	0.36 ± 0.06	0.17 ± 0.06	0.10 ± 0.07	<0.01 ± 0.07
U × B	0.97 ± 0.07	0.93 ± 0.06	0.83 ± 0.06	0.66 ± 0.06	0.46 ± 0.06	0.28 ± 0.06	0.07 ± 0.06	0.07 ± 0.07	<0.01 ± 0.07
BU × BU	0.97 ± 0.09	0.94 ± 0.08	0.88 ± 0.08		0.48 ± 0.07	0.29 ± 0.07	0.06 ± 0.08	0.05 ± 0.08	0.01 ± 0.08
UB × UB	0.98 ± 0.09	0.98 ± 0.08	0.92 ± 0.08		0.66 ± 0.07	0.38 ± 0.07	0.11 ± 0.07	0.09 ± 0.07	0.01 ± 0.08
B × BU	0.96 ± 0.09	0.85 ± 0.08	0.79 ± 0.08		0.43 ± 0.07	0.21 ± 0.07	0.05 ± 0.08	0.03 ± 0.08	<0.01 ± 0.08
UB × B	0.96 ± 0.09	0.86 ± 0.08	0.80 ± 0.08		0.37 ± 0.07	0.19 ± 0.07	0.05 ± 0.08	0.04 ± 0.08	<0.01 ± 0.09
U × UB	1.00 ± 0.09	0.98 ± 0.08	0.93 ± 0.08		0.76 ± 0.07	0.56 ± 0.07	0.14 ± 0.07	0.13 ± 0.07	0.02 ± 0.07
BU × U	0.98 ± 0.09	0.97 ± 0.08	0.90 ± 0.08		0.71 ± 0.07	0.38 ± 0.07	0.10 ± 0.07	0.09 ± 0.07	0.02 ± 0.08

General Insect Rearing and Population Maintenance. Each ecotype colony was maintained in the laboratory as a large, outbred population. F_1 adult moths were maintained under a photoperiod of 16:8 (L:D) h at 23°C as the thermophase (corresponding artificial daytime temperature) and 20°C as the scotophase (corresponding artificial nighttime temperature), with a relative moisture content of 70–80% RH inside a cage (58.7 by 58.7 by 62.7 cm, length × width × height) in the growth chamber. Adult moths were provided with abundant water by dental wicks with one side embedded in 10% sucrose solution inside amber colored prescription boxes. These conditions provided a satisfactory egg yield, viability, and adult longevity to provide enough insects for the study and to maintain the colony. The eggs were laid on waxed paper (locally acquired), which had been positioned to cover the inside, top of the cages. The wax paper was collected daily, and eggs were allowed to mature according to needs by manipulating temperature. The cages were monitored at least twice daily to ensure a continuous source of moisture for the adult moths. The eggs were then placed inside 0.95 liter (quart-size) glass jars and provided with moist cotton balls. At the blackhead stage, the eggs were transferred to round rearing boxes (Reed et al. 1972, 254 cm in diameter by 9 cm in depth) containing the meridic diet described by Guthrie et al. (1985), and the hatched larvae were grown under nondiapause conditions of 29°C and a photoperiod of 16:8 (L:D) h for experimental needs.

Population Crosses. Crosses between ecotypes were made by mass-mating females of one population and males of the other under the condition described above. In all instances, the females origin was designated as the first member of the cross (i.e., B♀ × U♂, U♀ × B♂, etc.). Newly emerged (virgin) adults were sex-selected on the day of eclosion and assigned to appropriate cages to produce F_1, subsequent F_2, and backcross progeny. Including two parental stocks, 10 ecotypes/crosses in total were evaluated for diapause induction (Table 1).

Each cage contained an approximately equal number of males and females and ranged from 75 to 200 pairs for each cross. A minimum of 8,000 eggs was obtained, ranging in age from 0 to 10 d, from each cross. To synchronize the egg eclosion time for maximum number of adults from all crosses, all the eggs produced from each cage were collected daily and stored in complete darkness between 16 and 29°C, depending on their relative age. This approach provided synchronization of six to seven sequential egg-collection days to one general eclosion time for each and every cross. The newly hatched 22–25 larvae from each cross and for each day were then introduced into rearing cups (118-ml round polypropylene specimen cups; U.S. Plastic Corp.). In each mating replicate, four to eight rearing cups were randomly assigned as a single replication of a given photoregime for the corresponding cross.

Photoperiodic Response Curves and Critical Photoperiod. The photoperiodic response curves of both parent ecotypes, F_1, F_2, and reciprocal backcrosses were obtained by rearing each cross under different photoregimes between 12 and 17 h light per day at 27°C. The duration of light was increased from 12 h to 17 h at increments of 0.5 h in each subsequent treatment. However, after initial run, all of the 13.5 h or lower photoperiods resulted in 95–100% diapause response for all crosses, as opposed to the ≥16 h photoregimes, which produced little or no diapause. Therefore, the experiment was concentrated on the narrow range of 14–16-h photoperiods.

Due to large numbers of growth chamber needed for experimentation, all necessary light:dark cycles were attained by hand-transferring the groups of larvae (4–10 cups per photoperiod/cross treatment) between continually dark and continually illuminated growth chambers at an appropriate time for each treatment. To avoid erratic light–dark signals to experimental insects, the growth chambers were held in an artificially lighted room, and all the transfers between the growth chambers were made with the help of a flashlight while the room lights were off.

Classification of Diapause and Nondiapause. After the control larvae (photoperiod of 16:8 [L:D] h and 27°C) completed their development and pupated, experimental insects were checked for their developmental stage. Individuals that remained as larvae were assumed to be in diapause (Beck 1989). The diapause character in European corn borer is not morphologically distinguishable (Beck 1980); therefore, this trait

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is characterized by comparison of control groups grown under conditions that would not produce diapause. The pupated individuals were taken out of the box and the remaining larvae continued to experience their respective photo-regime until all data were collected. At 27°C, it took ≈25–26 d to complete larval development for control groups of all parents and crosses. However, because the data collection period covered ≈1 wk for all experimental replicates, the initial readings were checked again on the last day of data collection, and the newly pupated individuals also were counted for final analysis. The number of individuals pupating within this time period was not >5% of any given group of crosses within any replicates.

Analyses. The experimental design was completely randomized. Each treatment combination (cross × photoperiod) was represented with four to 10 rearing cups, holding 22–25 larvae in each. The cups were randomly positioned inside the growth chambers. Each mating was replicated three times throughout the calendar year. In the last mating replication, the parental ecotypes were in the seventh generation of laboratory breeding.

The photoperiod/diapause response data were analyzed with PROC PROBIT to estimate the critical photoperiods for each group of insects (SAS Institute 2001). Any two critical photoperiod (50% response) estimates were considered significantly different if their 95% fiducial limits (FL) did not overlap. Similarly, lines representing photoperiod/diapause percentage responses of parents, F_1, F_2, and backcross progeny were plotted using the estimated responses from probit analysis (SAS Institute 2001). The sensitivity to photoperiodic changes was estimated based the differences of between 25 and 75% response times from the regression line. The method of Lande (1981) was followed for estimating the minimum effective number of freely segregating genes involved in the induction of diapause.

Results

In general, all of the matings successfully produced more than enough egg masses to carry out the necessary experiments. The average responses of the two parents, F_1, F_2, and backcross progeny to varying photoperiods are presented in Table 1. As expected, the diapause incidence was inversely proportional to increasing daylength between 14 and 16 h in all the crosses. In general, there were significant differences among the ecotypes at most of the photoperiods tested. However, these differences diminished when the tested photoperiod approached 14–16 h, mostly producing diapausing or pupated individuals in all crosses, respectively. Therefore, the triggering mechanism for continuous development (i.e., pupation) became operational at photoperiods between 14 and 16 h. Although, 16-h daylength promoted continuous development, there were always a few individuals failing to pupate in all ecotypes within 30 d at 27°C. However, these individuals did not constitute >5% of any given cross. Similarly, some individuals pupated even under <13.5-h photoregime. The percentages of individuals avoiding diapause under short-day conditions were not statistically different, but were slightly higher in the bivoltine population than in the univoltine population (Table 1). These individuals may have either escaped the given photoregime, or they may have rare genetic alleles yielding their unique phenotypes under those conditions. In fact, a discontinued selection effort with the bivoltine population showed signs of an increase in the percentage of individuals avoiding diapause under short-day conditions (data not shown). Furthermore, there are examples in the literature where a pure nondiapause strain of European corn borer was bred (under diapause-promoting conditions) to use as part of a pest management method (Showers et al. 1990). The unique responses from those individuals probably have a genetic basis in this study. However, their relative importance for our findings is probably very limited, because they do not constitute >5% of any cross.

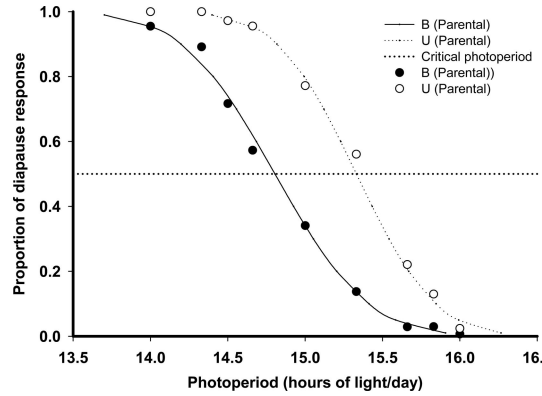


Fig. 1. Photoperiodic response curves for two geographic ecotypes of the European corn borer reared at 27°C. B denotes the bivoltine ecotype originating from southeastern Nebraska, and U denotes the univoltine ecotype from northwestern Minnesota. Each sigmoid line represents the expected responses from corresponding crosses. Each point indicates the average of three replications at a given photoperiod.

Parental Ecotypes. Photoperiodic response curves for parental ecotypes are presented in Fig. 1. Diapause response of the bivoltine parent was noticeably distinct from that of the univoltine parent. Between 14.5- and 15.5-h photoregimes, diapause percentage was significantly higher in the univoltine parent than in the bivoltine parent (Table 1). Although 14.80 h was considered as long days by 50% of individuals in the bivoltine ecotype, this photoperiod was seen as short days by the majority of the univoltine ecotype (95% diapause). When the univoltine ecotype reached 50% pupation at 15.33 h >90% of bivoltine ecotype attained pupation (Table 2; Fig. 1). Consequently, there seemed to be a 0.53-h difference between the critical photoperiod of both parental ecotypes (Table 2).

F_1 Progeny. The progeny of crosses between univoltine and bivoltine parents showed diapause induction at a somewhat intermediate rate compared with

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Table 2. Critical photoperiod (CP) and sensitivity of European corn borer univoltine (U; from Minnesota) and bivoltine (B; from Nebraska) ecotypes; their F_1, F_2 crosses; and backcross progeny based on their response to various photoperiods at 27°C

Crosses (♀ × ♂)	CP[a] (FL)	25%[b] (FL)	75%[b] (FL)	Sensitivity[c]
B	14.80 (14.74–14.86)	15.12 (15.06–15.20)	14.48 (14.41–14.55)	0.64
U	15.33 (15.27–15.39)	15.60 (15.53–15.69)	15.06 (14.99–15.12)	0.54
B × U	15.10 (15.04–15.17)	15.43 (15.35–15.52)	14.77 (14.69–14.84)	0.66
U × B	14.97 (14.90–15.04)	15.31 (15.22–15.41)	14.63 (14.54–14.71)	0.68
BU × BU	15.02 (14.91–15.12)	15.34 (15.24–15.46)	14.70 (14.55–14.82)	0.64
UB × UB	15.17 (15.09–15.26)	15.47 (15.38–15.57)	14.88 (14.75–14.97)	0.59
B × BU	14.90 (14.85–14.96)	15.24 (15.18–15.30)	14.57 (14.49–14.64)	0.67
UB × B	14.87 (14.78–14.95)	15.19 (15.11–15.28)	14.55 (14.43–14.64)	0.65
U × UB	15.30 (15.18–15.41)	15.58 (15.47–15.74)	15.01 (14.85–15.14)	0.57
BU × U	15.19 (15.10–15.27)	15.49 (15.41–15.59)	14.88 (14.77–14.97)	0.61

[a] Critical photoperiod (CP) based on estimated 50% diapause response from probit line.
[b] Estimated daylight (hours) that would induce a 25% and 75% diapause response from probit line.
[c] Sensitivities based on changes in estimated times (hours) that would induce 25% and 75% diapause response in each crosses.

those of parental ecotypes (Fig. 2). This suggests that diapause response is not a completely dominant gene. Furthermore, the responses of reciprocal F_1 crosses to varying photoperiods were significantly different from each other at most of the photoperiods tested (Fig. 2). Based on 95% confidence intervals, critical photoperiods for the two F_1 crosses showed no overlap, and the U × B F_1 cross had a lower critical photoperiod (14.97 h) than that of B × U F_1 cross (15.10 h) (Table 2). Both critical photoperiods were statistically different than those of parental ecotypes (Table 2), indicating a sex linkage in the inheritance of diapause induction, with the male parent having more influence on the following F_1 progeny.

F_2 Progeny. The diapause responses of F_2 progeny are presented in Table 1 and graphically depicted in Fig. 3. In the reciprocal F_2 hybrids, diapause incidence was significantly higher in the UB × UB cross than in the BU × BU crosses at most of the photoperiods (Table 1). The critical photoperiod was estimated to be 15.17 h for UB × UB cross and 15.02 h for BU × BU cross (Table 2). Based on fiducial confidence limits, despite the lesser number of BU × BU F_2 progeny, the difference between B[times] U F_1 and BU × BU F_2 progeny was not statistically different for critical photoperiods. However, the UB × UB F_2 cross showed a significantly higher critical photoperiod than its progenitor: the U × B F_1 cross. Overall, the diapause responses of F_2 progeny were opposite of the results from F_1 crosses (Figs. 2 and 3).

As we had expected, due to the sex linkage apparent from F_1 crosses, a slight shift toward the bivoltine parent in BU × BU diapause response was observed in the critical photoperiod. Lepidopteran females are known to have only one sex chromosome (WZ), whereas males have two copies of the sex chromosome (ZZ). Therefore, the F_2 female progeny in the BU × BU cross may have either univoltine or bivoltine type sex chromosome because their father B × U F_1 cross has both bivoltine and univoltine type sex chromo-

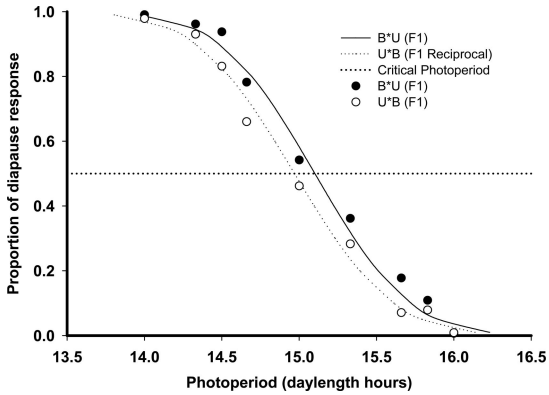


Fig. 2. Photoperiodic response curves for reciprocal F_1 crosses of the two parental ecotypes reared at 27°C. B denotes the bivoltine ecotype originating from southeastern Nebraska, and U denotes the univoltine ecotype from northwestern Minnesota. Female parent in each cross is represented first. Each sigmoid line represents the expected responses from corresponding crosses. Each point indicates the average of three replications at a given photoperiod.

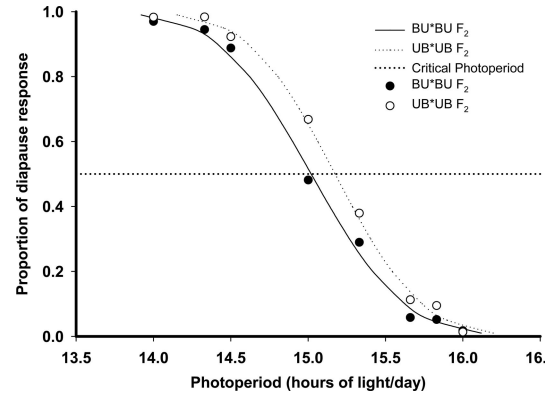


Fig. 3. Photoperiodic response curves for reciprocal F_2 crosses of the two parental ecotypes reared at 27°C. B denotes the bivoltine ecotype originating from southeastern Nebraska, and U denotes the univoltine ecotype from northwestern Minnesota. Female parent in each cross is represented first. Each sigmoid line represents the expected responses from corresponding cross. Each point indicates the average of three replications at a given photoperiod.

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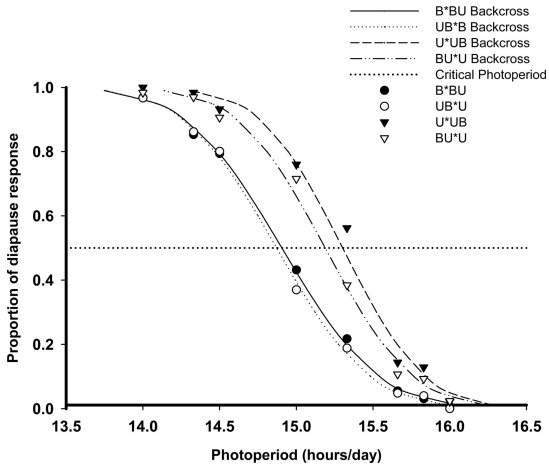


Fig. 4. Photoperiodic response curves for maternal and paternal backcrosses of the two parental ecotypes and F_1 hybrids reared at 27°C. B denotes the bivoltine ecotype originating from southeastern Nebraska, and U denotes the univoltine ecotype from northwestern Minnesota. Female parent in each cross is represented first. Each sigmoid line represents the expected responses from corresponding crosses. Each point indicates the average of three replications at a given photoperiod.

some originating from both parental ecotypes. However, the F_1 female progeny (B × U) may have only univoltine type sex chromosome from their fathers, leading their response toward the univoltine parent. Similarly, compared with the U × B F1 cross, a shift in diapause response toward univoltine parent was observed from UB × UB cross, due again to sex linkage. However, the extent of the shift in diapause response seemed a little bit more drastic for UB × UB cross, as it was lower than the BU × BU cross in many photoperiods tested (Table 1).

Backcross Progeny. The diapause responses of backcrosses were mostly intermediate between the respective parental and F_1 crosses from which they were derived (Fig. 4). The critical photoperiod for the B × BU cross (14.90 h) was not statistically different from that of the bivoltine parent (Table 2). Similarly, the UB × B cross had a critical photoperiod value of 14.87 h.

Backcrossing F_1 progeny to the univoltine parent also resulted in higher diapausing rates in most of the photoperiods. The critical photoperiod was 15.30 h for the U × UB and 15.18 h for BU × U backcross, which was not significantly different from that of the univoltine parent (Table 2). In general, the results from backcrosses were consistent with the sex linkage schemes observed from F_1 and F_2 progeny. However, the influence of paternal effects from F_1 males was not as clear as it was in those of the parental males on the backcross progeny.

Number of Estimated Genes. Based on intercept values and SEs, the method of Lande (1981) produced an estimate of the minimum number of genes (*ne*) for the ecotypes tested (Table 3). The four methods applicable to this study showed varying estimates from 0.92 to 6.4, with the majority being within 0.92–1.62. The highest estimate (6.4), based on method 3, was at least 4 times more than the next highest estimate. The higher estimate was primarily a function of one of the four backcrosses (U × UB) having a much higher variance component than any other crosses. Given the fact that estimates in Lande's approach are based largely on sample variances, which are sensitive to equal gene effect and linkage, they tend to substantially underestimate the actual variance (Zeng et al. 1990). The information from these estimates may be limited or misleading in the current study. Therefore, the number of genes involved in the diapause response of the European corn borer may be higher than estimated here.

Table 3. Probit regression statistics and minimum number of genes estimates (Lande 1981) based on intercept values and SEs of European corn borer univoltine (U; from Minnesota) and bivoltine (B; from Nebraska) ecotypes and their crosses

Crosses (♀ × ♂)	Intercept ± SE[a]		
B	31.22 (1.87)	ne_1	1.53
U	38.18 (2.47)	ne_2	1.62
B × U	31.12 (2.07)	ne_3	6.40
U × B	29.86 (2.11)	ne_4	0.92
BU × BU	31.79 (2.92)		
UB × UB	34.55 (2.85)		
B × BU	30.01 (1.47)		
UB × B	31.12 (2.22)		
U × UB	36.24 (4.22)		
BU × U	33.68 (2.55)		

ne Estimates of minimum number of genes operating in the base population tested. The subscript numbers (1, 2, 3, 4) are Lande's first, second, third, and fourth methods, respectively.

[a] Intercept of the probit line followed by the SE.

Discussion

The ecotypes and their crosses we studied showed sigmoidal photoperiod–diapause response curves: a typical long day response of insects that are active in summer (Saunders 1982). The two parental ecotypes reflected their location origins in their preadaptive diapause characteristics. The more southern population had a comparably shorter critical daylength as noted for many other insect species (Beck 1980, Saunders 1982). This agrees with findings in many other European corn borer studies (Beck and Apple 1961, Sparks et al. 1966, Showers et al. 1972, Skopic and Bowen 1976, Takeda and Skopik 1985). This study has shown that the diapause response to changing photoperiods has a genetic basis in this species, as the parental responses were stable even after generations of laboratory breeding.

The intermediate responses from F1 crosses indicate that diapause inheritance is not controlled by a dominant gene. The intermediate response of F_1 hybrids is generally viewed as a characteristic of polygenic inheritance (Danilevsky 1961, Beck 1980, Tauber et al. 1986). However, the immediate backcrosses to parental ecotypes in our study very closely

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resembled parental responses. This rapid recovery to parental type response suggests the number of genes involved may be from only a few loci. Furthermore, estimation of the minimum number of loci (Lande 1981) suggested that diapause response in European corn borer to critical diapause inducing conditions was due to only a few loci. However, given the fact that the methods followed here have been shown in previous studies to be prone to underestimating the factors involved (Zeng et al. 1990), there may be more genes operating in the ecotypes tested.

Due to the uncertainty about the number of genes involved, it is difficult to determine the nature of dominance relationships of the alleles studied (i.e., nondiapause versus diapause). Furthermore, the results from the reciprocal F_1 crosses clearly indicated that a gene or genes on the sex chromosome is mainly, although not exclusively, responsible for the differences in the incidence of diapause between the two ecotypes tested in this study. This further complicates inheritance of the diapause response. However, the data from F_1 crosses suggest that the sex-linked gene(s) is probably dominant in nature. Given that the U $\times$ B cross response was similar to the bivoltine parent rather than intermediate, and that the response from the B $\times$ U cross was intermediate between the bivoltine and univoltine parents (Fig. 2), it seems to suggest that the sex-linked part of the diapause character in the bivoltine parent may be dominant over that of the univoltine parent.

Comparison to Literature. This view is contrasted by the report of Sparks et al. (1966) who concluded that diapause response of the northern parent (MN) seemed dominant over the more southern parents (IA and MO). A direct comparison of the data are difficult between the two studies, due to limited crosses in the Sparks et al. (1966) study (only parents and F_1) and limited photoperiod tested. Apart from limited photoperiods and crosses, the Sparks et al. (1966) study further suffers from lack of mating replications. The study's methodology clearly states four replications of photoperiods (14.25 and 15.00 h), each of which consists of 30 larvae from a single egg mass source. This effectively illustrates that, at best, four pairs of moths were used, for a total of four replications. In comparison, our study used 75–200 pairs of moths for each photoperiod in each mating replication. Assigning a single egg mass source to each photoperiod, would drastically limit replication to single pair mating. Our pool of at least 8,000 eggs from 75 to 200 pairs of moths was synchronized for eclosion and randomly assigned for each photoperiod. This approach effectively replicates photoperiods, temperature, growth chambers used, and most importantly, matings that occurred under laboratory conditions, throughout the calendar year.

Although diapause response is an "all or none" individual trait, for genetic studies, it is measured based on the response of the population (Danilevsky 1961, McLeod 1978, Beck 1980) because only a single response could be measured from an individual in its lifetime. Our study, and many others (McLeod 1978, Reed et al. 1981), clearly suggest that there is also an appreciable amount of genetic variability in a given European corn borer population for diapause determination, suggesting that the population consists of individuals with a variety of genotypes and diapause responses.

Our results for sex-linked inheritance of diapause response are in accordance with several other studies (Arbuthnot 1944, Showers et al. 1972, McLeod 1978, Reed et al. 1981). Despite not being aware of photoperiod involvement in diapause determination, Arbuthnot (1944) concluded that the nondiapause trait was dominant over the diapause trait, with a strong influence of male parents in the subsequent progeny. The study by Showers et al. (1972) conducted under field conditions, suggested that sex-linked inheritance might be responsible for the diapause response. Despite limited crosses and photoperiods, the data of McLeod (1978) matched ours very closely.

In conclusion, the results of our study add to the mounting evidence that variation in diapause response of the European corn borer larvae is a seasonal adaptation cued mostly by the photoperiod experienced by developing larvae (Beck 1980, Skopic and Bowen 1976, Takeda and Skopik 1985). A difference as little as 0.54 h in daylight caused a change from a mostly diapausing state (25%) to pupation (75%) in the univoltine parent (Table 2). The same change was obtained at 0.64 h for the bivoltine population. This result suggests that the univoltine ecotype is more sensitive to changes in photoperiod than the bivoltine ecotype. Also, this sudden change in univoltine ecotype could be interpreted to suggest a greater reliance on photoperiod for diapause determination. This was consistent with the results from Beck and Apple (1961) and Showers et al. (1975) who reported higher sensitivity to photoperiod in northern ecotypes of the European corn borer.

In addition, sensitivity, in this context, may refer to variability of diapause response gene(s) in the base ecotypes. Thus, the univoltine ecotype probably has limited genetic variability compared with the bivoltine ecotype for a diapause induction mechanism, because the univoltine ecotype is forced to change its response from diapause to pupation in a relatively short time. Because environmental conditions act to select the genotypes that are adapted to localities, it is reasonable to expect less genetic variability in the univoltine ecotype originating in northern latitudes, where environmental conditions exert higher pressures on the European corn borer larvae to avoid late summer pupation.

Arbuthbot (1944) reported an apparent homozygous univoltine strain of the European corn borer, but he was unable to do the same for a multivoltine strain in his research. This is consistent with our finding that the univoltine ecotype had limited genetic variability, which would make it easier to isolate a pure or mostly univoltine strain under the laboratory conditions. In the absence of any photoperiodic information in our study, however, it is impossible to make any direct

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comparison or draw any further conclusions regarding this supposition.

Limitations. We examined crosses at only one temperature for diapause induction, and given that temperature is the second most important ecological factor for diapause induction, it is conceivable that genetic variation not detected in this study may be expressed, or the ones detected may be suppressed, at another temperature regime. A pattern of inheritance may appear differently in another environment. In general, genetic analysis of diapause determination is challenging and time-consuming due to the complexity of extrinsic and intrinsic characters involved. Therefore, development of pure lines whose individuals would respond more or less identically to given conditions (i.e., photoperiod and temperature) is essential to further investigate and understand this life-history trait. This approach may help reduce within population variation and clarify the potentiality of alleles involved on developed lines.

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