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Transient effects of photoperiod on reproduction in the Mexican bean beetle

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ABSTRACT. Mexican bean beetles (Coccinellidae: *Epilachna varivestis* (Mulsant)) have an adult diapause and show a graded response to photoperiods intermediate between those that lead to the fastest gonadal maturation and those that lead to diapause. The rate of maturation is reduced by a few to several days at day lengths corresponding to dates after the summer solstice, and such delays in reproduction may be of ecological and evolutionary importance. Multinomial probit analysis is demonstrated as a method for studying more than two classes of response to a photoperiodic regime.

Key words. Diapause induction, Mexican bean beetle, *Epilachna varivestis*, Coccinellidae, probit analysis.

Introduction

Studies of the induction of adult diapause almost always categorize individuals as either diapause or non-diapause; they do not recognize explicitly that under intermediate environmental conditions individuals may show intermediate responses, which may be ecologically and evolutionarily important, and, therefore, deserve explicit consideration. They may have been overlooked in part because statistical methods for treating such results have not been available. The few studies explicitly considering intermediate responses demonstrate that this phenomenon occurs across a broad range of taxa, e.g. *Oedopoda miniata*, Orthoptera (Pener & Orshan, 1980); *Riptortus clavatus*, Heteroptera (Numata & Hidaka, 1982); *Leptinotarsa decemlineata*, Coleoptera (de Wilde *et al.*, 1959); and *Culex tritaeniorhynchus*, Diptera (Kawai, 1969). We demonstrate here that the rate of gonadal maturation in the Mexican bean beetle (Coccinellidae: *Epilahna varivestis* (Mulsant)), which has an adult diapause, responds in a graded fashion to photoperiods intermediate between those that lead to the fastest gonadal maturation and those that lead to diapause. The rate of maturation is reduced by a few to several days at day lengths corresponding to dates after the summer solstice and these delays in reproduction may be of ecological and evolutionary importance. In addition, we demonstrate the use of a new method (Schrader & Taylor, unpublished observations) for dealing with the general problem in which there is a range of responses to photoperiodic signals and at any particular level of signal a sample may contain more than two categories of individuals.

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The standard method for dealing with response curves that can be fitted by a cumulative normal distribution, such as photoperiodic response curves, is *probit analysis (Finney,* 1971). It is usually assumed that there is one threshold, for example, in the transition from non-diapause to diapause condition. This leads to a binary or dichotomous classification. As noted above, it is natural in many cases to consider individuals showing intermediate responses giving rise to a polychotomous classification with multiple thresholds. In the former case, the probability of an individual falling into one of the two classes is described by the binomial distribution and, in the latter case, the probability of falling into one of several classes is given by the multinomial distribution with the constraint that the classes are appropriately ordered. A special case of this form of probit analysis was treated by Finney (1971), but it had not been treated in a general manner in the statistical literature until we did so recently (Schrader & Taylor, unpublished observations) to solve the problem presented in this paper.

Methods

Rearing procedures

In the experiments below, we used the Mexican bean beetle from Los Lunas, Valencia County, New Mexico, U.S.A. To start all experiments, egg batches that had been laid within a 24 h interval were taken from eight lines of a stock population. The eggs were surface sterilized in a 1% aqueous solution of bleach (5.3% sodium hypochlorite) for 10 min (Gerhardt, 1981; p. 501). The eggs were maintained in the appropriate experimental conditions. On day 4, these eggs were placed on Fordhook lima bean leaves inserted in water pics (as used by florists) in 25×150 mm Petri dishes. From day 8 until pupation, larvae were maintained in groups of forty in plastic shoe boxes (31×17×9 cm deep) with clear bottoms and two 11 cm windows in the top. Generally no more than one or two larvae or pupae died in a box and usually none died. As pupae, the beetles were transferred to Petri dishes in which humidity was maintained with moist cotton wool.

On the day after emergence, adults were transferred to short-day (LD, 13:11 h) conditions *in all* experiments. This procedure was adopted because, as has been shown for another species in the subfamily Epilachninae (Kono, 1980), the Mexican bean beetle has a sensitive stage during early adulthood in which long-day conditions can reverse diapause but short-day conditions will not induce diapause in non-diapause individuals that have developing gonads (Taylor, 1985). Therefore, by placing all adults in the same short-day conditions, we avoid the reversing effect of long-days and provide all beetles with uniform conditions as adults. All adults were frozen at the appropriate time (see below) after emergence and subsequently dissected to determine the level of gonadal development. Dissections were done in physiological saline (0.7% NaCl, Humason, 1972; p. 534) to reduce distortions in gonadal size caused by osmotic imbalance.

Experiments

The general procedures outlined above were used to carry out the following two experiments. To estimate photoperiodic response curves, beetles were reared at six different day lengths, 13, 13.5, 14, 14.5, 15 and 15.5 h, at 25°C. The longest day length corresponds to the longest day of the year (or the summer solstice), including civil twilight, at the latitude at which the beetles were collected (34°N). In this experiment, adults were dissected on day 4 after eclosion and gonadal development was assessed as described in the next section. Each treatment contained *c.* 120 beetles reared in three boxes, i.e. sixty of each sex.

To assess the degree to which reproduction is delayed by different levels of gonadal development, in a separate experiment we dissected on days 1–4 after emergence adults that had been reared as juveniles under long-day (15.5 h) conditions. These results provide minimal estimates of the times required to advance through the various stages of gonadal development described below. These estimates were used to assess the effects on development times of the other day length treatments. Again *c.* sixty beetles of each sex were dissected on each day.

Assessing diapause condition

When reared as juveniles under long-day (15.5 h) conditions (see below), by 4 days after adult eclosion almost all females (99%) and all males had matured beyond the diapause state. When reared under short-day (13 h) conditions, all beetles were in diapause (see below). On the other hand, on day 4 it was possible to distinguish intermediate levels of gonadal development under intermediate photoperiods. Thus, this age was used for dissections in the experiment to determine the photoperiodic response curve.

Five levels of development are readily identified in females. The Mexican bean beetle belongs to the suborder Polyphaga, which has telotrophic ovarioles (Wigglesworth, 1972). In small (S) females, the germarium has a small diameter and there is no apparent development of the oocytes. For both sexes (see below), the S classes constitute the diapause condition. This was determined by dissecting non-reproductive adults several days after these beetles normally start reproducing, and finding them always to be in the S class. In medium-sized (M) females, the diameter of the germarium has increased as has one oocyte. In medium to large-sized (ML) females, three oocytes are observed with the penultimate one enlarged as shown. Large females with little yolk (L1) have advanced in vitellogenesis to the point that the egg is a translucent yellow. Large females with much yolk (L2) have eggs that appear to have completed vitellogenesis and are probably chorionated.

In males, testis size varies continuously with-

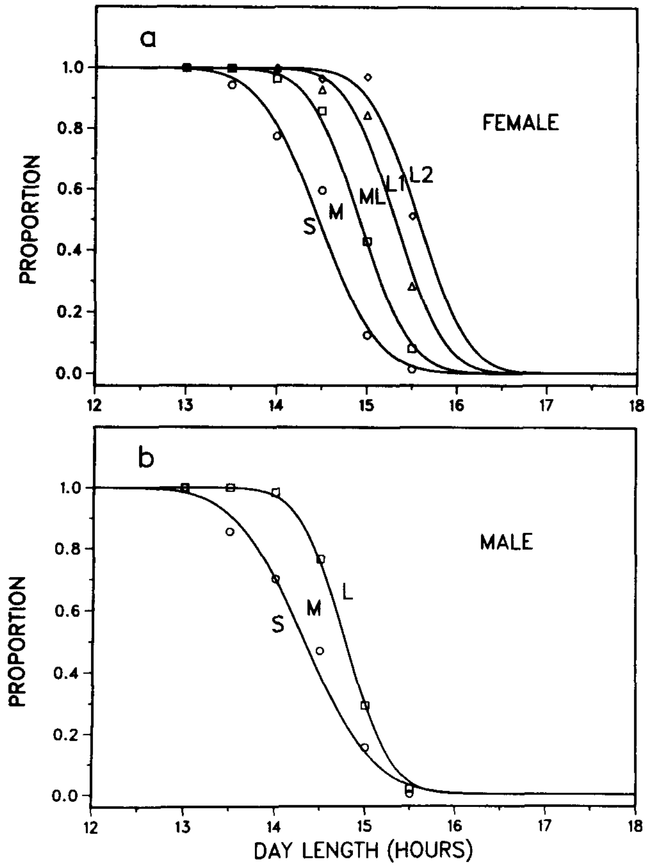

FIG. 1. Photoperiodic response curves resulting from exposure of groups of beetles to various day lengths during the juvenile period. (a) A series of four cumulative normal distributions separates the five levels of female development. The observations for the proportion of S females in a treatment are indicated by circles, S+M by squares, S+M+ML by triangles, and S+M+ML+L1 by diamonds. Each curve represents the best fit of a cumulative normal distribution to the data. (b) Two curves separate the three levels of male development. The observations for the proportion of S males in a treatment are indicated by circles, and S+M by squares.

out the relative size change of various parts as in the females and, therefore, is more difficult to categorize without resorting to excessively time-consuming measurements. Thus, only three categories were recognized. The diameter of each testis was assessed relative to the width of the projection from the first abdominal sternite, which acted as a natural micrometer ($\bar{x}$=0.814 mm, SD=0.19 mm, n=20) that takes into account the likelihood that smaller males have smaller testes. Small (S) males have testes measuring approximately one width of the projection, or less, in diameter. Medium-sized (M) males have testis diameters *c.* 1.5 times the width of the projection, and large-sized (L) males about twice the width, or larger. Few males fell into an ambiguous range between two of the three categories.

Results

Photoperiodic response curves

Each curve in Fig. 1 indicates the transition from one level of gonadal development to the next. For example, when reared at 15 h day length, 12.5% of the females (Fig. 1a) had S ovaries, 30.5% had M (the difference between S+M=43% and S=12.5%), 42% had ML (85%−43%), 12% had L1 (97%−85%), and the remainder (100%−97%=3%) were L2. The curves represent the best fit of cumulative normal distributions to the data for all the day length treatments given the constraint that for each treatment the proportions in all the categories of gonadal development must sum to 1.

The results for females (Fig. 1a) are fitted well by the multinomial probit model (as indicated by a low goodness of fit χ^2=20.75, P=0.188, df=16). The mean day length causing a transition from L2 to L1 females is 15.6 h while that causing S females is 14.5 h, or 1.1 h shorter (Table 1). The latter day length is usually referred to as the critical photoperiod at which 50% of the population exhibits diapause behaviour. (Note that the mean equals the median in the normal distribution.) The mean day lengths are all significantly different (quadratic forms test, χ^2=211, $P\approx0$, df=3, Schrader & Taylor, unpublished observations), but the standard deviations of the curves are not (likelihood ratio test, χ^2=2.47, P=0.481,

TABLE 1. Summary statistics for the multiple threshold analysis of the photoperiodic response curves in Fig. 1. The curves represent thresholds between classes of response, e.g. S refers to the threshold between small-sized gonads and all larger classes. The standard deviation is related to the slope of the curve around the mean; a higher value indicates a shallower slope.

		Mean (h)	Standard deviation (h)
Female	S	14.46±0.15*	0.52±0.04
	M	14.92±0.04	0.45±0.04
	ML	15.31±0.05	0.43±0.06
	L1	15.57±0.07	0.44±0.07
Male	S	14.33±0.05	0.62±0.05
	M	14.78±0.04	0.41±0.03

* Standard deviation of estimate.

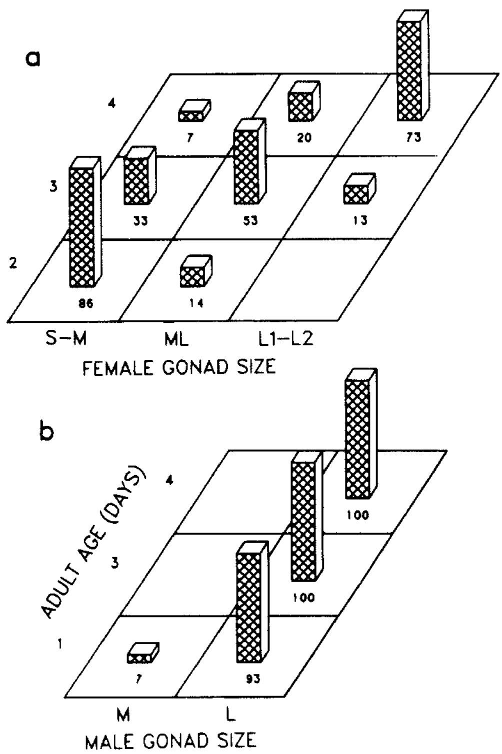

FIG. 2. Development of gonads in adults reared under long-day (15.5 h) conditions. Samples of adults were dissected on days 1–4 after eclosion and their levels of gonadal development assessed. (a) Results for females on days 2, 3 and 4. S and M classes are combined as are L1 and L2. The number under each bar indicates the percentage of the sample showing that level of development. (b) Male development on days 1, 3 and 4.

df=3, Schrader & Taylor, unpublished observations), indicating that the curves are parallel. The results for males (Fig. 1b) are also fitted well by the model (χ^2=7.9, P=0.443, df=8). The mean day length causing M males is 14.8 h while that causing S males (the critical photoperiod) is 14.3 h, or 0.45 h less (Table 1). The mean day lengths differ significantly (χ^2=81.9, $P\approx0$, df=1), and, in this case, the standard deviations of the curves are significantly different (χ^2=19.27, $P\leq0.00001$, df=1) indicating that the variance of the M to S threshold is greater than that for the L to M threshold.

Time course of adult gonadal development

When reared as juveniles under long days, adults advance rapidly through the successive classes of gonadal development. Females, on day 1 of adulthood, are 75% S and 25% M. By day 2, 86% are S or M (Fig. 2a) of which 89% are M. On day 3, 53% are ML and on day 4, 68% are large, of which 61% are L2. Thus, it takes about 1 day to make the transition between the classes S, M, ML and large, while it takes less than a day to move from L1 to L2. Males, in contrast, are already almost all L (93%, Fig. 2b) on day 1 when reared under long-day conditions. Thus, a male that is M on day 4 is retarded by at least 3 days and will take an unknown time to produce L testes, but possibly as little as 1 day.

Discussion

The Mexican bean beetle shows a continuous response to photoperiodic conditions between those leading to the fastest gonadal development and those inducing diapause, with intermediate conditions leading to retarded gonadal development. Even though it is difficult to relate these results to natural conditions where both temperature and day length are changing over time, it is clear that the rate of gonadal development will be reduced in many individuals that are destined to attempt reproduction in the current season. Importantly, the greatest retardation occurs at short day lengths corresponding to dates later in the growing season when beetles have the least time to reproduce before the onset of winter or the deterioration of their hostplant.

The levels of delayed maturation observed in these beetles are on the order of a few to several days. A female with M ovaries on day 4 would require about 2 days to reach the L2 size when reared under long-day conditions. This delay is likely to be greater for a female experiencing short-day conditions. A male with M testes on day 4 is delayed by 3 days and would require at least 1 day to produce L testes. In this species, all males, including those with S testes, have sperm in their seminal vesicles on day 4 and can mate successfully (Taylor, 1985). Thus an M male may be able to copulate as soon as an L male, but may not be able to produce sperm at a maximal rate until he has L testes. These delays in reproduction may be of evolutionary consequence, although to determine this would require detailed studies of natural populations.

Other aspects of diapause physiology to which multinomial probit analysis may be applied include the effect of photoperiod on the duration or intensity of diapause (Dingle, 1978) and the rate of juvenile development (Saunders, 1982; see Appendix 5 for numerous examples). In both cases, populations may show a graded response to photoperiod. In the latter instance, for example, one could expose samples of insects to a series of day length treatments and categorize individuals according to their time to complete development. Multinomial probit analysis would then permit the quantification of differences in day length needed to cause different development times.

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