

## Diapause in the gypsy moth : environment-specific mode of inheritance

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### SUMMARY

Preliminary investigation of the genetic basis of diapause in the gypsy moth, *Lymantria dispar*, involved a comparison of reciprocal crosses between wild-type moths and a selectively bred 'non-diapause' strain with the parental stocks, where half of the eggs resulting from each of the four mating types were exposed to one month of chill at 5 °C. The presence of chill dramatically altered the phenotypic expression of diapause-dependent characteristics. Early hatching was completely recessive in unchilled eggs, while hatching time was intermediate in chilled eggs, and there was no difference between reciprocal hybrids. Proportion of eggs hatching and, therefore, number of larvae produced was also influenced by chill. Unchilled hybrids did not differ substantially from wild-type eggs, while chilled hybrids were closer to the performance of the selected line. In this case, a significant reciprocal difference indicated some involvement of sex-linkage in the inheritance of diapause.

### 1. INTRODUCTION

The gypsy moth, *Lymantria dispar*, has become an important pest species in the United States, following its introduction from Europe in the mid-nineteenth century. Defoliation of trees by larvae is especially severe during years of major outbreaks (Kulman, 1971). Obligatory diapause permits overwintering of the egg stage, which normally requires at least 3 months of low temperatures to break diapause before hatching can occur (Masaki, 1956). However, selection for rapid hatching of unchilled eggs has resulted in a 'non-diapause' strain of gypsy moth, in which most eggs hatch within a few days of the completion of embryonation, which requires about 25 days on average (Hoy, 1977). This selected strain provided an unique opportunity to investigate the mode of inheritance

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of diapause in this species. The only previous studies of the genetic control of diapause in the gypsy moth are those of Goldschmidt (1932, 1933) who examined crosses between geographical races. He concluded that several genes were involved, and found some evidence of a maternal effect. However, his experiments are not directly comparable to ours because of the different populations sampled, the complexity of temperatures to which eggs from his crosses were exposed, and the limited numbers of eggs resulting from many of his matings.

## 2. MATERIALS AND METHODS

Reciprocal crosses between laboratory-reared wild-type and selected-line (generation 8) gypsy moths were compared to matings within the two lines. Twenty-five days after deposition, egg masses from each of the four types of matings were halved; one half was exposed to a 'token' one-month chill at  $5 \pm 1^\circ\text{C}$  (then returned to  $21 \pm 1^\circ\text{C}$ ) while the other half was maintained constantly at  $21^\circ\text{C}$ . We observed 20, 50, 50 and 100 whole egg masses respectively from the selected line, each reciprocal cross, and the wild type, and standard errors were based on the variance among egg masses. All groups received a photoperiod of 18:6 light:dark. Since the eggs do not hatch at  $5^\circ\text{C}$ , the period of chill was subtracted from the time taken to hatch for chilled egg masses. All eggs were observed for 6 months, and hatching was recorded three times a week. After this time, the remaining eggs were deaired, and the numbers of embryonated and unembryonated eggs were recorded. The data were analysed according to the least-squares procedures of Harvey (1960) for analysis of variance with unequal subclass numbers.

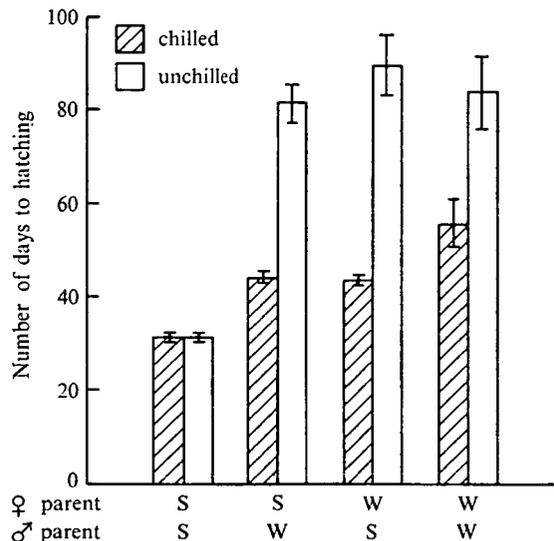


Fig. 1. Mean number of days until the first egg hatched in each half egg mass of selected 'non-diapause', wild-type, and reciprocal-hybrid gypsy moths which were either unchilled or exposed to  $5^\circ\text{C}$  for 1 month. Thin lines indicate standard errors; S = selected strain, W = wild type.

Table 1. Fertility and percentage hatch of eggs and egg masses of selected 'non-diapause', wild-type and reciprocal-hybrid gypsy moths exposed to different chill conditions (1 month at 5 °C or none).

Averages represent values per half egg mass ( $\pm$  standard errors). S = selected strain; W = wild type

Treatment ...	Chilled				Unchilled			
	S	S	W	W	S	S	W	W
Female parent ...	S	W	W	W	S	W	W	W
Male parent ...	S	W	S	W	S	W	S	W
Percentage hatch of embryonated eggs	82.2	53.3	62.2	0.9	86.2	12.0	7.5	0.2
Percentage of egg masses which showed any hatching	100.00	92.7	100.0	11.0	100.0	90.0	80.9	3.0
Number of unbryonated eggs	11.8 $\pm$ 5.3	17.3 $\pm$ 4.2	15.1 $\pm$ 8.4	14.9 $\pm$ 3.5	5.1 $\pm$ 1.9	19.1 $\pm$ 5.7	16.6 $\pm$ 8.3	17.4 $\pm$ 3.0
Number of unhatched embryonated eggs	39.8 $\pm$ 8.9	87.4 $\pm$ 14.0	110.8 $\pm$ 13.6	272.7 $\pm$ 79.3	26.5 $\pm$ 5.8	168.4 $\pm$ 17.6	245.7 $\pm$ 18.3	174.2 $\pm$ 8.4
Number of larvae hatched	184 $\pm$ 20	100 $\pm$ 10	183 $\pm$ 13	3 $\pm$ 2	166 $\pm$ 14	23 $\pm$ 4	20 $\pm$ 6	0 $\pm$ 0

## 3. RESULTS AND DISCUSSION

The number of days required for hatching to begin is shown in Fig. 1, egg masses showing no hatching being excluded. Selected-line eggs began to hatch soon after embryonation, whether chilled or not, while chilling accelerated hatching in all other genotypes. The mode of inheritance of hatching times differed substantially depending on whether the eggs had experienced the token chill. Unchilled hybrid eggs showed complete dominance of the wild phenotype, while chilled hybrids were exactly intermediate in phenotype between the selected line and wild type. The difference in relative extent of dominance for hatching time under the two temperature conditions was significant at  $P < 0.001$ .

Chilling also increased the percentage of eggs per egg mass that hatched in all groups except the selected line (Table 1), and in both treatments the inclusion of wild-type genes systematically reduced the percentage. Although hybrid eggs produced rather more hatching without chill than wild-type eggs, their superiority was much more obvious under chill, when they became clearly displaced toward the selected line (Table 1). The number of larvae hatched also showed a significant ( $P < 0.001$ ) switch in the direction of dominance depending on temperature. Data in Table 1 provide additional evidence. Although most hybrid egg masses (representing individual matings) produced some hatching regardless of temperature, the proportion of eggs hatching was low in the unchilled groups, showing dominance of the wild type, and high in the chilled groups, showing dominance of the selected line.

Since in Lepidoptera the male is the homogametic sex (Robinson, 1971), sex linkage is easily distinguished from maternal effects. Therefore, the considerable reciprocal difference ( $P < 0.001$ ) in number of larvae hatched in the chilled hybrid groups suggests environment-specific expression of sex-linked genes. Differences in fertility could also account for reciprocal effects, since selected-line females produce somewhat fewer total eggs than do wild-type females. However, percentage hatching also reflects the reciprocal difference, although to a lesser extent (Table 1). If some of the genes influencing diapause are sex-linked, the excess larvae hatched in the chilled hybrids with selected-line fathers should be primarily females. Since the hybrid larvae were reared to adults, data were available to test this hypothesis. A sample of 2505 adults from chilled hybrid eggs with selected-line fathers yielded a sex ratio of 2.3 females to 1 male, while the sex ratio of 1684 adults from the reciprocal cross (wild-type fathers) was 1:1, the difference being consistent with a sex-linkage hypothesis. The genetic basis of diapause in the gypsy moth may be similar to that in the silk moth, both in the influence of sex-linked genes and the effect of temperature on altering gene expression (Tazima, 1964), that is, the phenotypic expression may be environment-specific. This illustrates the importance of genetic analyses under varying environmental conditions, if relevant ecological or environmental variables may be involved.

Our results further indicate that a non-diapause strain of gypsy moth would

not prove useful in biological control by means of premature hatching, since hybrid eggs still require some chilling in order to hatch. In fact, the dual ability of hybrids to break diapause early while withstanding some cold could lead to the establishment of a multivoltine race in warmer regions.

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