Selection for late pupariation affects diapause incidence and duration in the flesh fly, Sarcophaga bullata

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Henrich, V.C., and D.L. Denlinger (1982) Selection for late pupariation affects diapause incidence and duration in the flesh fly, *Sarcophaga bullata*. *Physiol. Entomol.* DOI: 10.1111/j.1365-3032.1982.tb00316.x

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Abstract:

This artificial selection study with the flesh fly, *Sarcophaga bullata* Parker, tested the hypothesis that phenotypic variability in the length of the larval stage (under non-diapause conditions) is largely a consequence of genetic variability. Selection for late pupariation resulted in a line that pupariated significantly later and also developed more slowly during other stages of the life cycle. In a diapause-inducing environment, the selected line pupariated later, showed a higher incidence of pupal diapause, and remained in diapause longer than the unselected line. This is the first experimental evidence in *S.bullata* to show that diapause incidence and duration are related. The relationship between developmental rate and diapause traits may stem from the pleiotropic effects of genes associated with late pupariation, or from one or more genes associated with late pupariation being closely linked to genes that affect diapause.

Key words: Photoperiodism, pupal diapause, *Sarcophaga bullata*, blowfly, selection.

Article:

Introduction

A variety of insect studies suggests that diapause incidence and duration are highly heritable traits that are not controlled independently (Hoy, 1978; Tauber & Tauber, 1981). Bivoltine and univoltine strains of *Ostrinia nubilalis* show differences in both incidence and duration of diapause in controlled environmental conditions (McLeod, 1978) and hybrids show characteristics for both traits that resemble those of the univoltine strain. Strains derived from northern populations of *Acronycta rumicis* enter diapause at a longer critical daylength and remain in diapause longer than strains isolated from more southern clines (Danilevskii, 1965). These observations lead to the inference that the factors which alter capability for diapause also affect diapause duration.

In this study we tested the association between prediapause developmental time, diapause incidence and diapause duration, using the flesh fly, *Sarcophaga bullata* Parker. This species, like other temperate zone flesh flies (Denlinger, 1981), relies on short daylength and oool temperature to provide the primary signals for induction of pupal diapause (Denlinger, 1972a, b). Larvae destined for diapause tend to pupariate later under controlled conditions than those not entering diapause (Saunders, 1971, 1973; Denlinger, 19T2a). Even in non-diapausing conditions, duration of larval development shows some variability.

We used an artificially selected strain to test the hypothesis that the observed variability in the larval developmental rate is genetically based and that genetic factors influencing developmental rate also affect diapause incidence and duration.

TABLE 1. Comparison of mean length of larval stage, length of life cycle, diapause incidence, and diapause duration between an original strain of *S.bullata* and a strain selected for late pupariation.

	Selected		Unselected	
	$\bar{x} \pm$ SD	*n*	$\bar{x} \pm$ SD	*n*
Under LD 15:9 at 25°C				
Duration of larval stage (days)	5.5 ± 0.6	219	6.3 ± 0.7	232
Duration of life cycle (days)	26.7		30.5	
Under LD 12:12 at 20°C				
Duration of larval stage (days)	21.5 ± 1.6	203	22.5 ± 2.4	216
Diapause incidence (%)	63.9	202	95.6	182
Mean duration of diapause after transfer to 25°C, 60 days after laviposition (days)	8.73 ± 7.5	82	34.7 ± 9.0	70

Materials and Methods

The strain of *S.bullata* originated in Lexington, Massachusetts, in 1974 and was maintained in the laboratory under non-diapausing conditions (15 h light:9 h dark (LD 15:9) cycle, 25°C). Rearing procedures were as described previously (Denlinger, 1972a).

Approximately 600 larvae were reared together in a non-diapause environment (LD 15:9, 25°C) and the last twenty-five larvae that pupariated were selected and raised to adulthood. These adults were mass-mated and the selection procedure repeated for six successive generations. Another strain was reared for seven generations in the same environment but members were not selected on the basis of any phenotype.

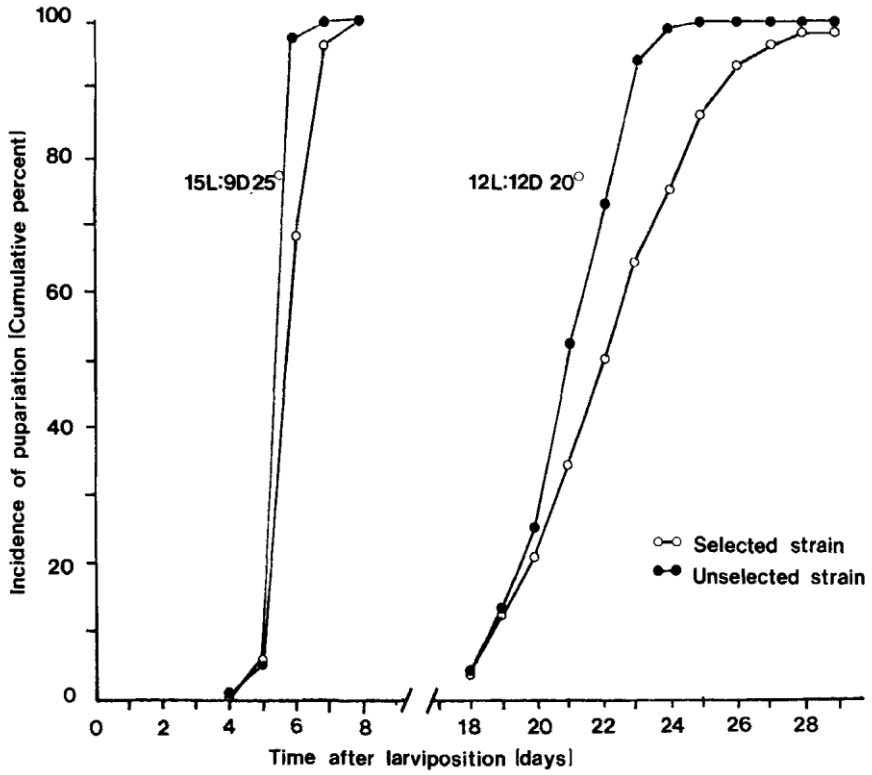

FIG. 1. Time of pupariation for an unselected and selected strain of *S.bullata* held at LD 15:9, 25°C (left-hand curves) or at LD 12:12, 20°C (right-hand curves).

Newly emerged adults of each line were then transferred to LD 12:12 at 25°C and the progeny of individual females were transferred to LD 12:12 at 20°C (strongly diapause inducing conditions) and maintained in these conditions through the onset of pupal diapause. The incidence of diapause was determined 35-40 days after larviposition, according to the criteria established by Fraenkel & Hsiao (1968). Pupae from both groups were

transferred to LD 12:12 at 25°C 60 days after larviposition, and the time of diapause termination (Fraenkel & Hsiao, 1968) was recorded.

Results

As a consequence of six cycles of selection for late pupariation under LD 15:9 at 25°C, the selected line showed a significantly greater variance (*F*-test, P<0.05) and pupariated significantly later than the unselected line (*P*<0.01, *t*-test) in long-day conditions (Table 1). The median and modal length of the larval stage was about 5 days in both groups. The difference in mean duration of the larval stage arose primarily because more individuals in the selected line pupariated 6 or more days after larviposition (Fig. 1).

Selection for a longer larval stage also increased the duration of other stages of the life cycle. The average life cycle in the selected line was 3.8 days longer than the mean life cycle of the unselected line. This difference cannot be fully accounted for by the difference in duration of the larval stage.

In diapause inducing conditions (LD 12:12, 20°C), the larval stage lasts considerably longer in both lines because larval developmental rate is temperature dependent (Fig. 1). Mean duration of the larval stage in the late selected line was about 1 day longer than in the unselected group (Table 1) and again the variance of the selected line was significantly greater than the variance of the original line (*F*-test, *P*<0.05). Likewise, the median (day 23) and mode (day 22) of the selected line was later than in the unselected line (day 21 for both median and mode). Though differences in mean duration of the larval stage are not large, they are statistically significant (*P*<0.01, *t*-test) and give rise to large differences in diapause incidence (Table 1). The diapause incidence of the selected line was the highest recorded for any line derived from our *S.bullata* colony.

Substantial differences also exist between the lines in the amount of time that elapsed before initiation of pharate adult development (Table 1). When transferred to 25°C, most individuals in the unselected line began to develop within 10 days while in the selected group, pupae broke diapause muoh later and over a longer period (Fig. 2).

Discussion

Artificial selection for greater duration of the larval stage in non-diapausing conditions produced a population that tends to pupariate later in both non-diapause and diapause-inducing conditions. The genetic factors that affect larval development rate also affect developmental rate in other stages of the life cycle. Moreover, these individuals are more likely to enter diapause, and they remain in diapause considerably longer than do un-selected individuals. The genetic factors that affect developmental rate therefore appear to influence diapause.

A viable alternative explanation is that gene loci affecting developmental rate are closely linked to loci affecting diapause. While separate loci may account for the relationship, linkage would nevertheless tend to associate the two traits and maintain the adaptive advantages of keeping the two traits together. The linkage model could be tested over several generations by measuring the correlation between duration of the larval stage and diapause characteristics in the absence of any selection. If the relationship between these traits depends upon linkage, the correlation should decrease.

The higher variance of the selected group relative to the unselected group contradicts the expectation that variance decreases as a consequence of selection. Selection normally decreases phenotypic variability by decreasing heterozygosity and genetic variability. However, if the frequency of alleles that tend to delay pupariation was initially low, then selection for late pupariation might increase heterozygosity at those loci and increase the variance. This explanation also indicates that selection for still later pupariation is possible in the selected lines.

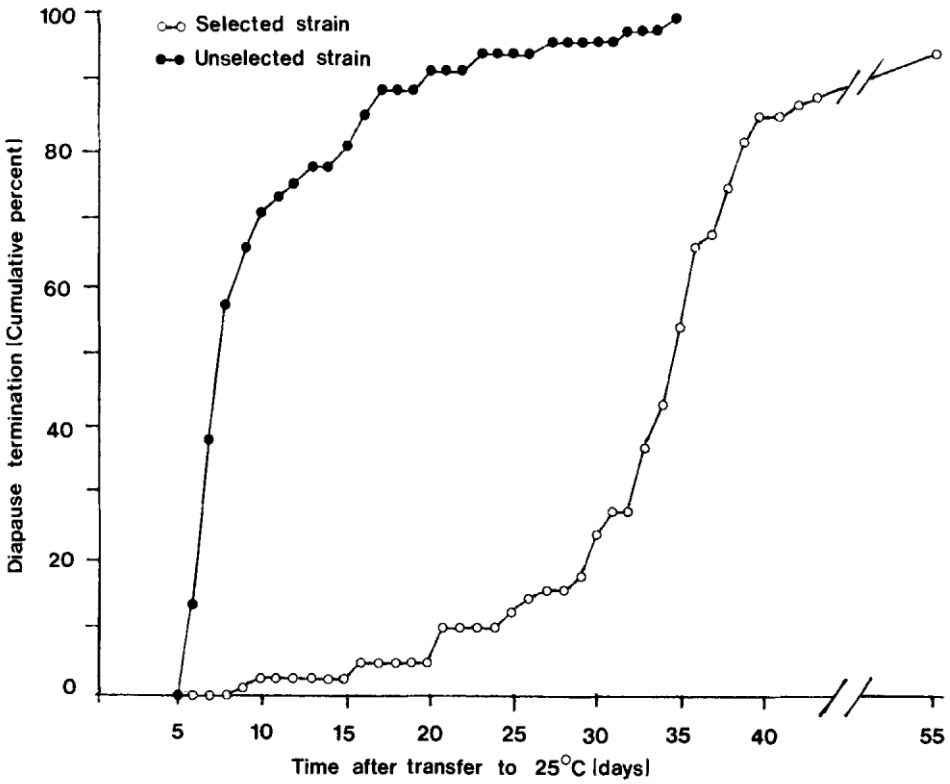

FIG. 2. Termination of diapause when pupae of an unselected and selected strain of *S.bullata* were transferred from LD 12:12, 20°C, to LD 12 : 12, 25°C, 60 days after larviposition.

At present, we cannot be certain how developmental rate might influence the response to diapause. Denlinger (1981) suggest that the embryonic programming for diapause also affects larval developmental rate and thus accounts for the delay in pupariation seen among larvae destined for diapause. Following this line of reasoning, individuals that tend to pupariate later might actually be more likely to diapause because the same genetic factors affect both diapause programming and developmental rate. In contrast, the photoinduction model of Saunders (19T1, 1973) explains the greater likelihood to enter diapause among late pupariating individuals as a consequence of exposure to more photo- periodic cycles. Therefore, those genetic factors that influence developmental rate would influence diapause capability indirectly in an appropriate environment.

Larval developmental rate can be altered by photoperiod even in the absence of diapause, a suggestion that developmental rate and diapause induction involve distinct meohanisms (Saunders, 1976, 1981). If these responses are distinct, they are nonetheless interrelated, because a relatively retarded larval developmental rate always precedes later entry into diapause.

The present results cannot reconcile these issues but they do represent the first experimental evidence that developmental rate and diapause induction and maintenance are components of the same system in *S.bullata*, and that these common elements are genetically based.

Termination of diapause in *Sarcophaga* is a two-part process: completion of a temperature-insensitive phase is followed by a temperature-sensitive phase in which the pupae respond immediately to high temperature. The observation that most unselected individuals break diapause within 10 days after transfer to 25°C suggests that the insensitive phase is already over for most members of this group. Delay in resumption of development in the late line may occur because these individuals still remain in the insensitive stage.

The relationship between diapause induction and duration serves an adaptive role in nature. Northern populations usually enter diapause earlier in the season and must remain in diapause longer. By maintaining

both parameters with common genetic elements, the appropriate diapause phenology could be readily sustained within a population.

Acknowledgments:
V. L. House and W. C. Rothenhuhler kindly reviewed the manuscript. The research was supported by the Science and Education Administration of the U.S. Department of Agriculture under grant no. T800595 from the Competitive Research Grants Office.

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