

A MATERNAL EFFECT THAT ELIMINATES PUPAL DIAPAUSE IN PROGENY OF THE FLESH FLY, *SARCOPHAGA BULLATA*

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Henrich, V.C., and D.L. Denlinger (1982) A maternal effect that eliminates pupal diapause in progeny of the flesh fly, *Sarcophaga bullata*. *J. Insect Physiol.* 28: 881-884.

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Abstract:

Flesh flies that have experienced pupal diapause produce progeny that will not enter diapause even when reared in a strongly diapause-inducing environment. The effect is determined, not by diapause itself, but by the short days previously received by the larvae during the programming of pupal diapause. Reciprocal cross matings indicate that the effect is transmitted solely by the female parent. Though the embryos develop within the uterus of the female, the maternal effect is transmitted prior to the onset of embryogenesis, probably during oogenesis. Only by rearing a generation in long-day (nondiapausing) conditions can the capacity for pupal diapause be restored in the progeny. The effect is likely to provide an adaptive mechanism for preventing an untimely diapause response among the progeny of overwintering females that emerge early in the spring.

Key Word Index: *Sarcophaga*, pupal diapause, maternal determinant, photoperiod

Article:

INTRODUCTION

Among flesh flies of the genus *Sarcophaga*, exposure of embryos and larvae to short days results in the capability for subsequent pupal diapause (DENLINGER, 1971; SAUNDERS, 1971; OHTAKI and TAKAHASHI, 1972; VINOGRADOVA, 1976). Though the embryo resides within the uterus of its mother, the mother does not mediate or process the photoperiodic cues during embryogenesis. Instead, the embryo receives and responds to the stimuli directly (DENLINGER., 1971). In all these previous experiments with pupal diapause, the parental generations were reared under long-day conditions prior to adult eclosion. Consequently, the adults had no history of diapause. We now find that the results are dramatically different if, instead, the flies have a history of pupal diapause. When flies that had previously been in diapause are mated and their offspring reared in a strongly diapause-inducing environment, virtually none of the progeny enter pupal diapause. In the present study we define the events leading to elimination of diapause and evaluate the roles of both the male and female parents in transmitting this effect.

MATERIALS AND METHODS

The colony of *Sarcophaga bullata* originated from flies collected in Lexington, Massachusetts in 1974. A strain of *S. crassipalpis* isolated in Illinois (DENLINGER, 1972a) was used in one experiment. Rearing methods (DENLINGER, 1972a) and the technique for in vitro culture of embryos (DENLINGER, 1971) were described previously.

Flies with no diapause history were raised before eclosion at 15L:9D (light:dark cycle) and 25°C (non-diapause conditions) unless otherwise indicated. Flies with a diapause history were produced by exposing them as embryos to 12L:12D and 25°C and then rearing the larvae at 12L:12D and 20°C. Parents of flies with a diapause history were reared at 15L:9D and 25°C until adult eclosion. To test the capacity of the flies for pupal diapause we exposed the gravid adult flies to 12L:12D, 25°C, and after larviposition larvae were placed at 12L:12D, 20°C.

Diapause incidence was scored approx. 40 days after larviposition using the criteria established by FRAENKEL and HSIAO (1968). To break diapause, pupae were transferred from 20 to 25°C 40-60 days after larviposition. By switching individual pharate adults between the two temperatures, the development of several flies was co-ordinated to permit synchronous adult eclosion.

Table 1. Pupal diapause incidence at 12L:12D, 20°C among the progeny of individuals with a diapause history (D) and individuals with no diapause history (N)

Cross (♀ × ♂)	Replication	No. of females	No. of larvae	Diapause incidence (%)
N × N	a	5	265	70.6
	b	6	148	46.0
D × D	a	3	148	0
	b	2	110	0
N × D	a	4	231	80.9
	b	5	217	54.4
D × N	a	4	231	0
	b	5	229	0

RESULTS

Reciprocal cross matings

When individuals reared under long-day conditions (no history of pupal diapause) were mated and the progeny reared in diapause-inducing conditions, a substantial proportion entered pupal diapause (Table I). Single-pair matings among individuals with a history of pupal diapause produced progeny that did not enter pupal diapause. Reciprocal crosses between a parent with no diapause history and one with a diapause history can determine whether only one or both' parents contribute to the reduction in diapause among the progeny. All crosses involving a female with diapause history showed no diapause in the next generation, while reciprocal crosses indicated that the male parent exerts no reducing influence upon diapause. Reduction in diapause incidence is strictly a maternal effect, In addition to the results shown in Table 1, over 70 additional crosses (n > 2900 progeny) have consistently verified the elimination of diapause in the progeny of females having a diapause history.

Elimination of maternal influence during embryogenesis

By culturing embryos in vitro, direct interaction between mother and offspring can be eliminated during embryogenesis. Eggs are fertilized at the time of ovulation (5 days after adult eclosion) and embryogenesis ends about 5 days later. Earlier studies using females with no diapause history have shown that diapause incidence does not change as a consequence of extrauterine culturing (DENLINGER, 1971), The same technique was used to determine whether reduction in diapause is the consequence of a factor transmitted from the mother to her progeny during embryogenesis. The results of this experiment (Table 2) show that the mother does not influence diapause incidence among here progeny after ovulation. The effect was already expressed in embryos removed from the female immediately after ovulation. Consequently, the differences between these groups must arise from events occurring prior to ovulation, quite possibly during oogenesis, a time of extensive genetic activity.

Cue for inducing the maternal effect

Pupal diapause occurs in response to short days. Thus, the maternal effect may occur as a result of diapause per se or alternatively, exposure to the short days that are involved in programming diapause. The two possibilities can be experimentally separated. When *S. bullata* is reared in a strongly diapause- inducing regime of short days and 20°C, diapause can be averted by transferring the flies from 20 to 15°C at or before pupariation.

Figure 1 illustrates a variety of environmental regimes to which groups of larvae were exposed. Upon adult emergence, flies were mated and diapause incidence was recorded for the progeny. When reared, under short days and 20°C through the onset of diapause, 43.1% of the larvae entered diapause (Fig, 1A). As expected from previous experiments, none of the pupae in the next generation entered diapause even though they were reared in a strongly diapause-inducing environment of 12L:12D, 20°C. A group of larvae held in strongly diapause-inducing conditions for the first four days after larviposition and then transferred to long day, 25°C until

pupariation did not enter diapause (Fig., 1B). Progeny of females reared in this regime produced a high diapause incidence, indicating that 4 days of diapause-inducing conditions were inadequate to trigger the maternal effect. When females were raised in diapause-inducing conditions until pupariation, only a few pupae entered diapause (Fig. 1C). Progeny of flies that did not diapause show a highly reduced diapause level. Similar results were obtained when the flies received only 10 days of strongly diapause-inducing conditions (Fig. 1D). Even at 25°C, exposure to short days resulted in progeny that were less likely to enter diapause (Fig. 1E). Clearly, larval exposure to short days can induce the maternal effect. Diapause itself is not a prerequisite.

Restoration of diapause among descendants

Crosses involving descendants of a female with a history of diapause consistently failed to yield diapause when the previous generation developed in a short-day regime (Fig. 2). When F₁ larvae of a female with a diapause history developed in a long-day environment, the F₂ larvae were capable of entering diapause when exposed to the proper conditions. Thus, the capacity for diapause can be restored with one intervening generation at long day.

Table 2. Incidence of pupal diapause in *S. bullata* when embryos (12L:12D, 25°C) were removed from the female at daily intervals after ovulation, cultured *in vitro*, and transferred as larvae to 12L:12D, 20°C

Time of removal from mother (days after ovulation)	Mothers with no diapause history		Mothers with diapause history	
	No. of pupae	Diapause incidence (%)	No. of pupae	Diapause incidence (%)
0	29	69.0	10	0
1	126	54.6	59	0
2	81	49.4	375	0.3
3	91	58.2	187	0
4	95	51.6	26	0
5	2177	66.4	885	0.7

Comparison to *S. crassipalpis*

Preliminary investigations with *S. crassipalpis* indicate that a maternal effect may operate in this species as well, but the effect is not as pronounced. Females of this strain, reared as larvae in a long-day environment, produced progeny with a very high diapause incidence (98.1%, n = 256) at 12L:12D, 20°C. Under, the same environmental conditions females with a diapause history produced progeny with a lower incidence of diapause (82.5%, n = 285), Similar results were obtained at 12:12, 25°C: starting with a diapause incidence of 39.1% (n 325) in the parental generation, diapause incidence in the progeny of flies with a diapause history dropped to 25.6% (n = 258) and in the next generation dropped to 9.9% (n = 270).

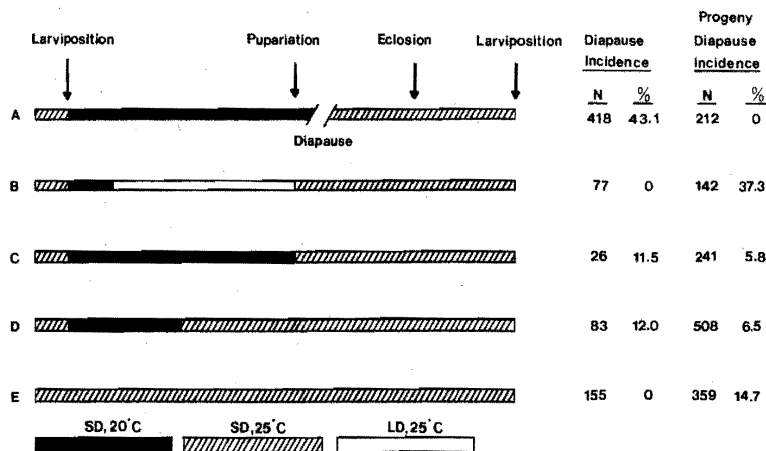


Fig. 1. Pupal diapause incidence among progeny of parents exposed to various photoperiodic and temperature regimes. Except for A, all progeny were collected from females that had not entered diapause.

DISCUSSION

In response to short-day exposure during their larval stage, females of *Sarcophaga bullata* prevent expression of

diapause in their progeny. The transmission of this maternal effect to the next generation occurs prior to ovulation and therefore directly affects the germ line. The capacity for diapause can be re-sores in a subsequent generation only by rearing an intervening generation under a long-day photoperiod.

The virtual elimination of diapause among the progeny of females with a diapause history suggests that a critical component of the diapause mechanism is turned off or blocked. Genetic activity is extensive during oogenesis and maternally directed activity occurring at this time can effect the fate of post-fertilization developmental events (BERRY, 1982). It is likely that the maternal effect involves an alteration of a genetic regulatory event that occurs during oogenesis as a consequence of an earlier photoperiodically induced event and ultimately affects the organism's capacity to diapause. Studies with *S. argyrostoma* suggest that photoperiodic environment during oogenesis may also be important (SAUNDERS, 1980).

The nature of the maternal determinant operating in flesh flies has not yet been identified, but the regulatory mechanism for the embryonic diapause in the silkworm *B. mori* may provide an insightful comparison. The photoperiod received by the female silkworm during her embryonic and larval development determines whether her progeny will enter embryonic diapause (FUKUDA, 1952; HASEGAWA, 1952). In this species, a neurohormone from the suboesophageal ganglion of the female mediates the maternal effect (YAMASHITA et al., 1981). The situation in flesh flies is quite similar to *B. mori*: in both species a photoperiodic event occurring at an early developmental stage affects the incidence of diapause in the next generation. In several other species of Diptera (CRAGG and COLE, 1952; RING, 1967; ANDERSON, 1968; DEPNER, 1962; VINOGRALXWA and ZINOVJEVA, 1972) and Hymenoptera (SCHNEIDERMAN and HOROWITZ, 1958; JACKSON, 1903; RYAN, 1965; SAUNDERS, 1965), the maternal photoperiodic environment determines the incidence of diapause in the next generation, but in all of these cases the photoperiod acts directly on the adult female rather than on an earlier stage of development. The seasonal response of several species of aphids (LEEs, 1960) represents a close parallel to the observation with flesh flies. Young clones derived from eggs that have overwintered in diapause produce only virginoparae when exposed to short daylength. Only in older clones is short daylength capable of eliciting production of adult form.

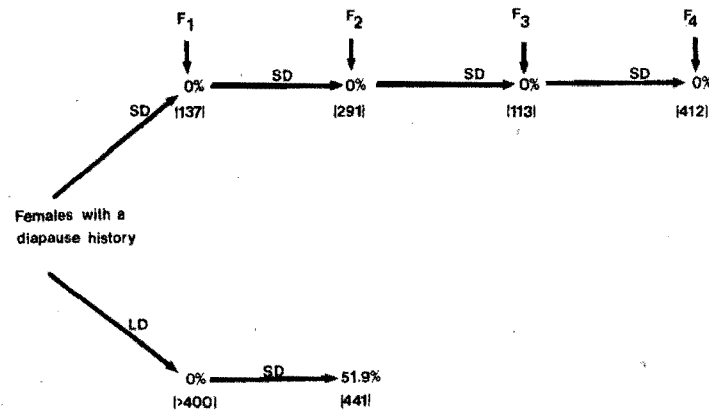


Fig. 2. Diapause incidence among descendents of a cross involving a female with diapause history. SD designates a short day, diapause-inducing environment (12L:12D, 20°C) during embryonic and larval stages. LD designates a long day, nondiapause-inducing environment (15L:9D, 25°C). Sample sizes are indicated in brackets.

The maternal effect observed in *S. bitalata* effectively prevents two successive generations of flies from entering diapause. The adaptive value of such a mechanism can only be appreciated in geographic areas that can support multivoltine populations of the fly. Flies emerging from diapause in the spring are thus assured of producing nondiapausing offspring. The timing of spring emergence is temperature dependent (DENLINGER, 1972a, 1972b), and early in the spring flesh flies can be confronted with short daylengths that are diapause inducing (DENLINGER, 1972b). The maternal effect we now observe would prevent such an untimely entry into diapause. With the developmental consequences of springtime short daylength obliterated by the maternal effect, the species can safely invade a new temporal niche. Such a mechanism that could advance spring

emergence may be of special importance to flesh flies. A long pupal diapause is achieved at a high cost: as the duration of pupal diapause increases, the ultimate reproductive output of the female declines (DENLINGER 1981). The advancement of spring emergence would shorten diapause and hence increase the number of fertile eggs produced by the female.

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