Eur. J. Entomol. **94**: 177–182, 1997
ISSN 1210-5759

Deuterium oxide prevents expression of a diapause maternal effect in the flesh fly, *Sarcophaga bullata* (Diptera: Sarcophagidae), and alters development and fecundity

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Diapause, deuterium oxide, maternal effect, development, fecundity, Sarcophagidae, *Sarcophaga bullata*

Abstract. A maternal effect that operates in *Sarcophaga bullata* normally prevents expression of pupal diapause in progeny of females that are reared under short days. Thus, only the progeny from females reared under long daylength can respond to short daylength by entering pupal diapause. When 20% deuterium oxide (D_2O) was added to the larval diet, the diapause fate of the flies was reversed: progeny from long-day females failed to diapause in response to short days, and some progeny from short-day females entered diapause. The diapause fate of the subsequent generation was also affected. Progeny from females reared on the 20% D_2O diet had a high capacity for diapause, thus implying that D_2O exerted an effect similar to that of rearing the mothers under long daylength. Several additional effects were noted in flies on the D_2O diet: duration of the larval feeding period increased, differentiation of the adult abdomen was sometimes incomplete, and fecundity decreased. While an alteration of the timekeeping mechanism can account for some of these responses, the effects of D_2O are pervasive and can influence numerous systems.

INTRODUCTION

Deuterium oxide (D_2O or heavy water), a nonradioactive isotope of water, exerts an interesting effect on biological systems. The substitution of deuterium for hydrogen increases the activation energy of the bond and thereby decreases the rates of reactions (Thomson, 1963). This has consequences for the transport of ions across cell membranes and for membrane polarization. The increased viscosity of D_2O also slows the rate of diffusion. One of the most conspicuous physiological effects of D_2O in a range of organisms is lengthening of the circadian period (Bruce & Pittendrigh, 1960; Dowse & Palmer, 1972; Pittendrigh et al., 1973; Masaki & Watari, 1989), an effect which has interesting implications for insect diapause. In both *Ostrinia nubilalis* (Lepidoptera: Pyralidae) (Beck, 1980) and *Sarcophaga crassipalpis* (Rockey & Denlinger, 1983) the incidences of diapause were dramatically reduced when the photosensitive stages were exposed to D_2O.

In this study we examine the potential for D_2O to alter the expression of a diapause maternal effect in *S. bullata*. In this species of flesh fly, the mother's photoperiodic history determines whether her progeny can respond to short daylength by entering pupal diapause (Henrich & Denlinger, 1982). If the mother is reared under long daylength her progeny can enter pupal diapause if they receive the appropriate short daylength during their photosensitive period. But, progeny from mothers reared under short daylength (and usually this means that the mother herself experienced pupal diapause) lack the capacity to enter diapause, even when reared under strong diapause-inducing conditions of short daylength and low temperature. This maternal effect thus enables the overwintering flies that emerge in

early spring to give birth to progeny that will not enter diapause in response to the short daylengths that prevail at that time of year. We now test whether expression of the maternal effect in *S. bullata* can be altered by D_2O. In addition, we monitor other developmental and reproductive consequences of D_2O exposure.

MATERIALS AND METHODS

Insects

The flesh fly, *Sarcophaga bullata* Parker, was collected in Lexington, Massachusetts in 1974 and reared in the laboratory as described (Denlinger, 1972). Adult flies that emerged on the same day were kept in a cage and fed beef liver for the first five days; sugar and water were supplied throughout adult life. Females deposited their first brood of larvae on the eleventh day. Larvae were fed on beef liver and then crawled into sawdust for pupariation. Long day history (LH) flesh flies were reared at 15L : 9D, 25°C and none entered pupal diapause. When LH flies were transferred to short daylength (12L : 12D) and 25°C at adult eclosion, the LH females produced progeny with a high incidence of pupal diapause when the progeny were maintained at short days (12L : 12D) and low temperature (20°C) during larval development. The short day history (SH) strain was established by maintaining progeny of females with a diapause history under continuous short day conditions. Pupal diapause was averted in these flies due to the maternal effect.

Deuterium oxide treatment

The D_2O diet was prepared as follows. Beef liver was homogenized in a blender for 3 minutes, then 40 g samples were placed in plastic containers and placed in an air-flow hood until the weight was reduced 20% by evaporation of water from the homogenized liver. The liver in each container was restored to its original weight by addition of D_2O (20% of total weight) (Sigma Chemical Co., St Louis, MO). Distilled water was added to the controls.

Larvae (approximately 80) from individual females were placed in separate aluminum foil packets containing 40 g homogenized liver with 20% D_2O or distilled water and maintained at 12L : 12D, 20°C. The incidence of pupal diapause was recorded 40 days after pupariation. The next generation was provided with normal liver and maintained continuously at 12L : 12D, 20°C.

RESULTS

Diapause in the D_2O-treated generation

Larvae deposited by LH females (females that were reared at long daylengths) and reared under diapause-inducing conditions of short daylength and low temperature readily

TABLE 1. The effect of deuterium oxide (D_2O) on the incidence of pupal diapause in the treated and subsequent generations.

Photoperiodic history	Maternal generation			Progeny			
	Diet	Total No. pupae	% diapause	Diet	No. females	Total No. pupae	% diapause
LH	normal	743	93.8 ± 0.4^a	normal	15	953	1.6 ± 0.6^a
LH	20% D_2O	279	16.3 ± 0.9^b	20% D_2O	11	407	67.2 ± 2.7^b
SH	normal	1573	1.5 ± 0.1^a	normal	68	1512	2.0 ± 0.3^a
SH	20% D_2O	1022	25.7 ± 0.9^b	20% D_2O	72	2901	36.1 ± 1.6^b

LH – the previous generation was reared under long daylength, thus their progeny have the capacity to respond to short daylength by entering diapause.

SH – the previous generation was reared under short daylength; this promotes a maternal effect that prevents the female's progeny from responding to short daylength by entering diapause.

In each couplet, values followed by different letters are significantly different. T test, $P < 0.01$.

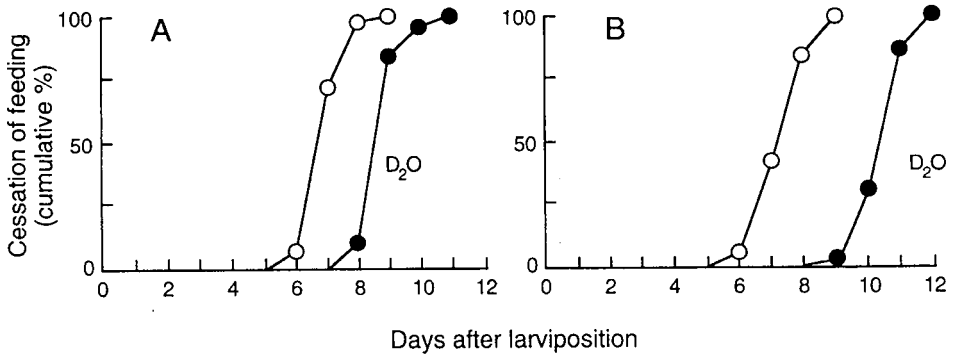

Fig. 1. Effect of deuterium oxide (D_2O) on duration of the larval feeding period in flies with either a short day history (A) or long day history (B). Open circles represent larvae reared on normal beef liver and solid circles represent larvae reared on beef liver containing 20% D_2O.

entered pupal diapause (93.8% in Table 1), but such larvae reared on a 20% D_2O diet exhibited a low diapause incidence (16.3%). This dramatic reduction of diapause in *S. bullata* is thus similar to the response observed in a closely related species, *S. crassipalpis* (Rockey & Denlinger, 1983).

Larvae deposited by SH females (females that were reared at short daylengths) do not diapause (Henrich & Denlinger, 1982) or have a very low diapause incidence (1.5% in Table 1) when reared under short daylength. It is this diapause-suppressing response that we refer to as the maternal effect. Rearing such larvae on a 20% D_2O diet boosted the diapause incidence to 25.7%. Thus, the maternal effect characteristic of SH flies was partially prevented by rearing the larvae on the D_2O diet.

Diapause in progeny of the D_2O-treated generation

The D_2O diet also exerted an effect on the diapause status of the next generation. SH females reared on a normal diet failed to enter diapause, as observed above, and their progeny, likewise reared on a normal diet, failed to enter diapause (2.0%, Table 1).

This is the typical expression of the maternal effect: as long as each successive generation is reared at short daylength, diapause is not expressed (Henrich & Denlinger, 1982). By contrast, a portion of SH females reared on the D_2O diet entered diapause, as seen above. Progeny of the nondiapausing, deuteriated flies reared on a normal diet at short daylength had an elevated diapause incidence (36.1%, Table 1). Thus, the D_2O received by the mother blocked expression of the maternal effect and allowed a portion of her progeny to enter diapause in response to short daylength.

The same response can be seen in progeny of LH females. LH females exposed to short daylength readily entered diapause, as observed in the LH controls shown in Table 1, but their progeny would not be expected to diapause due to the maternal effect, and only 1.6% did so (Table 1). By contrast, rearing the LH females on the D_2O diet averted diapause, as observed above, but their progeny readily entered diapause when reared on a normal diet under short day conditions. Thus, exposure to D_2O prevented diapause in the exposed generation, and this enabled the subsequent generation to enter pupal diapause in response to

Fig. 2. Undifferentiated abdomen in a flesh fly (*S. bullata*) reared on a larval diet containing 20% D_2O.

short daylength. This response is similar in effect to subjecting the mothers to long daylength. Long daylength, in this case simulated by D_2O, enabled the subsequent generation to respond to short daylength by entering diapause.

Effect of D_2O on development and reproduction

When both SH (Fig. 1A) and LH (Fig. 1B) larvae were reared on the D_2O diet they continued to feed for 2–3 days longer than larvae reared on the normal diet. Most individuals successfully completed development and eclosed as adults, but approximately 10% of the flies displayed developmental abnormalities in the abdomen. In the most severe cases, nearly the entire abdomen remained pupal-like (Fig. 2), while the head and thorax were indistinguishable from normal adults. In flies less severely affected, the black bristles characteristic of the adult extended further toward the posterior of the abdomen. The legs of the affected flies showed some movement, but such flies never successfully emerged from the puparium.

Females that had been reared as larvae on the D_2O diet produced approximately the same number of eggs as the controls (Table 2), but the number of fertile eggs produced by the deuteriated flies was much lower.

TABLE 2. Fecundity of female flies reared on a larval diet containing deuterium oxide (D_2O). Values in each couplet followed by the same letter are not significantly different ($P > 0.05$); values followed by the letters a and b are significantly different at $P < 0.01$ (T test).

Photoperiodic history	Diet	No. females	Eggs/♀ (mean ± SE)	Fertile eggs/♀ (mean ± SE)
LH	normal	63	66.2 ± 1.9^a	63.5 ± 2.5^a
LH	20% D_2O	37	60.3 ± 2.5^a	37.0 ± 2.6^b
SH	normal	82	87.0 ± 1.1^a	82.0 ± 1.1^a
SH	20% D_2O	40	77.2 ± 3.1^a	43.5 ± 2.1^b

DISCUSSION

When the maternal generation of *S. bullata* is reared under long daylength, the progeny can respond to short daylength by entering diapause. But, when the progeny are reared on beef liver containing 20% D_2O, very few enter diapause. This result, like that observed in a closely related species, *S. crassipalpis* (Rockey & Denlinger, 1983), can be interpreted as a disruption in the time-keeping mechanism. By slowing the functioning of the clock,

the period of circadian oscillations may be extended, thus causing a short daylength to be misread as long daylength (Beck, 1980; Rockey & Denlinger, 1983; Masaki & Watari, 1989). This interpretation is also satisfactory in explaining the expression of diapause in the subsequent generation. Such females would not normally produce progeny capable of diapause because the mothers were exposed to short daylength. But, D_2O, by simulating long day conditions, enables those females to produce progeny capable of diapause. In this scenario, the female actually was exposed to short daylength, but the D_2O caused her to interpret the short days as long days. Hence, her progeny did not receive the maternal factor that precludes the expression of pupal diapause.

The effect of D_2O on progeny of females with a short day history is less easy to interpret. Normally, the diapause-suppressing maternal effect is strongly expressed in such flies, but when larvae from the SH flies were reared on the D_2O diet a significant portion entered diapause as pupae. This implies that the maternal effect could be overridden when the larvae were reared on D_2O. Short days can not simply be misinterpreted as long daylengths; if that were the case, one would predict less diapause, not the higher incidence of diapause that was observed. The chemical nature of the maternal effect is still unknown, but apparently D_2O somehow interferes with expression of the maternal effect within the progeny. This implies that the information transferred from the mother to her progeny requires some additional processing within the progeny, and the processing of this information is derailed when the progeny consume D_2O. Other chemical agents including octopamine, picrotoxin and pilocarpine are also capable of partially blocking expression of the maternal effect (Webb & Denlinger, unpublished observations).

As noted in the Introduction, the substitution of deuterium for hydrogen decreases the rate of all reactions. This effect may be responsible not only for disrupting key reactions involved in the maternal effect but also in the prolongation of the feeding period and in the developmental abnormalities noted on the abdomen of the pharate adult. Adult development in flies is initiated anteriorly and progresses posteriorly (Fraenkel & Bhaskaran, 1973; Denlinger & Žďárek, 1994). On the D_2O diet some of the flies failed to complete metamorphosis, and consistently it was the abdomen or in some cases just the more posterior segments of the abdomen that were affected. Possibly the slowing of functions caused by D_2O did not enable the entire body to become responsive to ecdysteroids within the narrow gate of hormone release. In appearance, the undifferentiated abdomens generated by D_2O look much like the abdomens of flies that have been treated with a juvenile hormone analog (Srivastava & Gilbert, 1968; Žďárek & Denlinger, 1975; Sehnal & Žďárek, 1976) or irradiation (Sivasubramanian et al., 1974). In all of these cases, the abdomen is the most severely affected.

D_2O impairment of reproductive success in *S. bullata* was reflected in lower rates of fertility among the eggs produced. Katz et al. (1962) also reported marked effects with D_2O in reproduction of mice. D_2O-treated mice produced no progeny. In mice, the infertility was attributed both to the impaired ability of the female to carry through with a normal gestation and reduced viability of spermatozoa.

Since the chemical properties of water are so crucial for all life processes it is likely that the effects we observed with D_2O can be attributed to a variety of causes. For this reason, D_2O has limited potential for probing specific reactions, yet in this case it has proven useful in establishing the fact that expression of the maternal effect can be altered. What the

mother transfers to her progeny requires further processing within the body of her progeny, and this event is subject to manipulation.

ACKNOWLEDGEMENTS. This research was supported in part by grant no. 94-37302-0502 from the USDA-CRGO.

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Received March 14, 1996; accepted June 21, 1996