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Photoperiodic sensitivity and diapause induction during ovarian, embryonic and larval development of the flesh fly, *Sarcophaga argyrostoma*

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Abstract. Sensitivity to the daily photoperiod, particularly with respect to pupal diapause induction, was studied during ovarian, embryonic, and larval development of the flesh fly *Sarcophaga argyrostoma.* Large flies were shown to have a greater number of primary follicles in their ovaries and to be capable of limited ovarian maturation in the absence of exogenous protein (autogeny). Such ovarian development occurred independently of photoperiod. However, long days experienced during embryogenesis caused more rapid development, and earlier larviposition, than short days. Short days during embryonic and subsequent larval development also induced pupal diapause, whereas long days led to continuous or non-diapause development of the pupae. Pupal diapause could not be induced by photoperiods during the vitellogenic phase of ovarian development. In *Sarcophaga argyrostoma,* a maternal effect preventing pupal diapause among the progeny of files with a diapause history was not observed.

Keywords. Photoperiod; *Sarcophaga argyrostoma;* pupal diapause; diapause induction.

1. Introduction

Flesh flies (Diptera, Sarcophagidae) are ovoviviparous; mature eggs pass from the ovaries to an expanded common oviduct or uterus where embryonic development is completed. First instar larvae are then deposited on carrion or other suitable material. lf flies and their larvae are exposed to long day length the larvae develop into pupae and adults without arrest, but in autumnal short days, flesh flies form puparia which enter a firm diapause in the pupal instar shortly after pupal head eversion (Fraenkel and Hsiao 1968; Denlinger 1971a). In most species (*Sarcophaga bullata* for example) photoperiodic sensitivity is strongly associated with the intra-uterine embryos (Denlinger 1971a, 1972) whereas in *S. argyrostoma* (Saunders 1971, 1980) the larvae, particularly the newly deposited larvae, are also sensitive. In this species there is evidence for a declining sensitivity to the diapause-inducing effects of short days from the embryo to the third instar larva, with photoperiodic sensitivity effectively coming to an end at puparium formation (Saunders 1971, 1980).

The use of an artificial uterus enabled Denlinger (1971a) to demonstrate that the apparent sensitivity to photoperiod exhibited by the adult fly was in the intra-uterine embryos rather than being strictly maternal as in blowflies (Ring 1967; Vinogradova and Zinovjeva 1972; Saunders 1987). Nevertheless, a true maternal effect is seen, at least in S. *bullata,* in which the progeny of flies with a diapause history

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(equivalent to the first post-diapause or spring generation) are unable to enter pupal diapause in response to short days (Henrich and Denlinger 1982; Rockey and Denlinger 1986). The unidentified maternal factor involved was found to enter the offspring during ovarian rather than embryonic development.

This paper further addresses the question of which stages in the development of S. *argyrostoma* (ovarian, embryonic or larval) are sensitive to photoperiod, particularly with respect to the induction of pupal diapause. However, since ovarian development cannot be studied without consideration of those factors that affect vitellogenesis itself, such as body size and the timing of protein meals, these aspects of the biology of *S. argyrostoma* are also included.

2. Materials and methods

2.1 *Stock and experimental cultures*

Stock populations of *S. argyrostoma* (R-D) adults were maintained at 25 ± 1°C under continuous illumination (LL) in large (1 m × 1 m × 1 m) gauze-covered cages. The flies were supplied with water and sugar *ad libitum,* and with meat (beef muscle) for ovarian development and for the deposition of first instar larvae. Newly deposited larvae were then transferred, on pieces of meat, to a supplementary larval diet made from dried milk, yeast and agar, set in plastic dishes. Mature larvae were allowed to wander in dry sawdust to form puparia (Saunders 1971).

Experimental cultures of flies were kept in smaller, cages (20 cm × 30 cm × 25 cm) individually housed in light-tight wooden cabinets within temperature-controlled rooms at either 21° or 17°C. Each cabinet was fitted with a Philips 4 W striplight, water-jacketed to reduce temperature increases when the light was on, and controlled by a commercially available 24 h timer to regulate the daily light–dark cycle.

Protein meals (beef muscle) were provided for the experimental flies on various days (2, 4, 6 or 8) after eclosion. Resulting larval cultures were maintained in similar conditions as the adults. Newly formed puparia Were removed from the sawdust daily and incubated in darkness at 20°C. About 14 days later, or after the first flies had emerged, the puparia were opened to ascertain whether they contained diapausing pupae (still white in colour) or developing pharate adult flies (pigmented) according to the criteria described by Fraenkel and Hsiao (1968).

2.2 *Measurement of the size of flies and their developing egg follicles*

Flies of different sizes were reared by raising larvae in cultures of different density, small flies developing from overcrowded cultures and large flies from cultures with an excess of food. Flies were assessed for body size by measuring their head width with a calibrated eyepiece micrometer.

To assess ovarian development, female flies were removed from the cages at 4, 6 or 12h intervals every day from eclosion until ovulation. Samples of 5 to 10 flies from each collection were dissected in 0.9% NaCI, the ovaries teased apart, and the lengths of 5 randomly selected egg follicles from each fly measured by means of an eyepiece micrometer. Each follicle was also assigned to a developmental stage

according to a scheme described by Pappas and Fraenkel (1977). Vitellogenic stage 4A denotes a follicle which is less than 1/4 full of yolk, 4B 1/2 full, 4C 3/4 full, and 4D completely full of yolk but still unchorionated; stage M denotes a "mature" or chorionated egg. Yolk deposition starts when the follicle reaches about 0.35 mm in length.

2.3 *Monitoring intrauterine development*

After ovulation, the development of the embryos was monitored according to the following categories: early stages of embryogenesis (E), the appearance of tracheae within the embryo (T), colouration of the posterior larval spiracles (S), and the first appearance of movement of the first instar pharate larvae within the chorion (M).

In studies involving the use of an artificial uterus (Denlinger 1971a), fully developed and ovulated eggs were squeezed from the abdomens of pregnant females, and enclosed in small Petri dishes between layers of filter paper moistened with sterile saline solution or insect Ringer. Removal of embryos during the dark phase of the photoperiod was conducted under a photographic red "safe light". The Petri dishes were then incubated in the appropriate conditions of temperature and photoperiod. When the larvae hatched they were transferred to a piece of meat placed on a bed of the supplementary diet in a culture dish, again under the appropriate conditions. Puparia were later collected, incubated and the contents assessed for diapause of non-diapause development as described above.

2.4 *Photoperiodic experiments*

Earlier work with *S. argyrostoma* from a latitude of 55°N (Saunders 1971) established that the critical day length separating the diapause and non-diapause pathways was about 14 h light per day. For this reason, a strongly diapause inductive photoperiod of LD 12:12 was chosen as a "short day" and a strongly non-diapause inductive photoperiod of LD 18:6 was chosen as a "long day". The various developmental stages of *S. argyrostoma* (vitellogenic adult females, embryos within the maternal uterus or in an 'artificial uterus', and feeding larvae) were therefore exposed to either LD 12:12 or LD 18:6 at 21°C to determine their sensitivity to photoperiod. The diapause or non-diapause status of the pupae was determined 14 days after pupation in dry sawdust (§ 2.1).

3. Results

3.1 *Body size and the number of primary follicles*

Newly emerged females of different sizes were dissected in saline to determine the number of primary egg follicles in their ovaries. Figure 1 shows that the number of follicles at eclosion correlates well with head width. In the sample dissected here, the smallest flies (head width 2·41 mm) contained about 20 primary follicles, whereas the largest (head width 4·15 mm) contained more than 80.

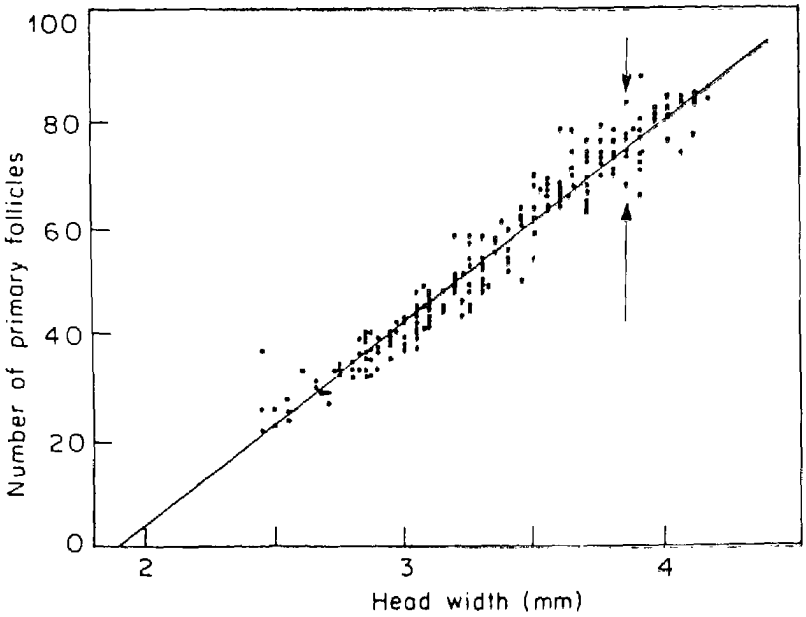

Figure 1. The total number of primary ovarian follicles in the ovaries of newly emerged *S. argyrostoma* as a function of body size (head width) ($y = 38{\cdot}3x - 72{\cdot}5$), Vertical arrows: mark size (head width 3·85 mm) above which flies are partially autogenous, *i.e* able to produce some mature eggs without a protein meal.

3.2 *Ovarian development in large flies (3·5-4·15 mm head width) deprived of meat*

Table 1 shows that even the smallest flies examined in this series (3·5 to 3·8 mm) contained oocytes with some yolk after 11 days (stage 4A, oocyte length about 0·37 to 0·41 mm), but mature or chorionated eggs (1·66 to 1·8 mm) were only observed in the largest flies (head width greater than 3·85 mm). In flies containing mature eggs the number of such eggs was considerably less than the expected number for that particular size class (see figure 1),

3.3 *Ovarian development following a protein meal*

Figure 2 compares the rate of follicular growth of large flies (> 3·5 mm head width) with that of small flies (< 3·5 mm head width) at 25°C and long days (LD 18:6). Both groups were first fed with a protein meal on day 4 post-eclosion. On an initial diet of sugar and water, the primary follicles of the larger flies showed a slow rate of growth to about stage 4A. Following the protein meal, follicular development accelerated rapidly to give mature eggs by day 7 when ovulation occurred, In the smaller flies, however, the initial rate of growth was less, only reaching stage 3 (0·2 mm) by day 4. After the protein meal, follicular growth occurred later as compared to the larger flies, but still led to mature eggs and ovulation on day 8 or 9. Although the small flies produced fewer mature eggs than the larger flies, the egg size distribution in the two groups was indistinguishable (large flies: 1·91 + 0·08 mm; small flies: 1·89 ± 0·04 mm).

Table 1. The effect of body size (head width) on the number and size of mature (M) and immature (I) primary follicles in S. *argyrostoma* kept for 11 days at 25°C without protein,

Head width (mm)	*N*	Number with M/I follicles (%)	Mean number M/I follicles per fly (± 2SE)	Mean follicle length (mm ± 2SE)
⩾ 4·05	6	M 6 (100%)	13·7 ± 4·18	1·69 ± 0·05
		I 0		
4·00	9	M 6 (66·7%)	24·5 ± 3·61	1·70 ± 0·11
		I 3 (33·3%)	73·0 ± 3·40	0·49 ± 0·11
3·95	11	M 6 (54·5%)	25·5 ± 5·39	1·67 ± 0·09
		I 5 (45·5%)	73·7 ± 3·31	0·51 ± 0.15
3·90	10	M 1 (10·0%)	32	1·8
		I 9 (90·0%)	74·0 ± 3·87	0·49 ± 0·09
3·85	11	M 2 (18·2%)	14·5	1·66
		I 9 (81·8%)	72·9 ± 1·92	0·48 ± 0·07
3·80	2	M 0		
		I 2 (100%)	77·5	0·39
3·75	7	M 0		
		I 7 (100%)	68·2 ± 5·22	0·41 ± 0·07
3·70	4	M 0		
		I 4 (100%)	71·5 ± 2·18	0·37 ± 0·04
⩽ 3·65	3	M 0		
		I 3 (100%)	67·3 ± 5·68	0·38 ± 0·04

These results (and those in table 1) suggest that the initial stage of vitellogenesis can occur in the absence of a protein meal. However, full follicular development without additional protein (autogeny) can only occur in the largest flies (> 3·81 mm head width). Flies smaller than about 3·5 mm head width are apparently unable to initiate vitellogenesis without this protein meal.

Follicular growth rates of large flies first fed protein 2, 6 or 8 days after eclosion were compared with those fed on day 4 (figure 3). In each case, vitellogenesis and follicular development were rapid after the first protein meal, suggesting that the protein meal acted as a "trigger" for development, in addition to a food source.

3.4 *Photoperiodic effects on vitellogenic flies, intrauterine embryos and feeding larvae*

Figure 4 shows that follicular development in long and short days was almost identical, reaching stage 4A before the protein meal, followed by rapid development to mature chorionated eggs by day *7*.

Following ovulation, however, the rate of intrauterine development was more rapid in LD 18:6 (figure 4), each developmental sign (appearance of tracheae, colouration of the spiracles, and onset of larval movement) occurring at least 12 h earlier in the long-day flies. When allowed to proceed naturally, larviposition also occurred about 20 h earlier in LD 18:6 than in LD 12:12.

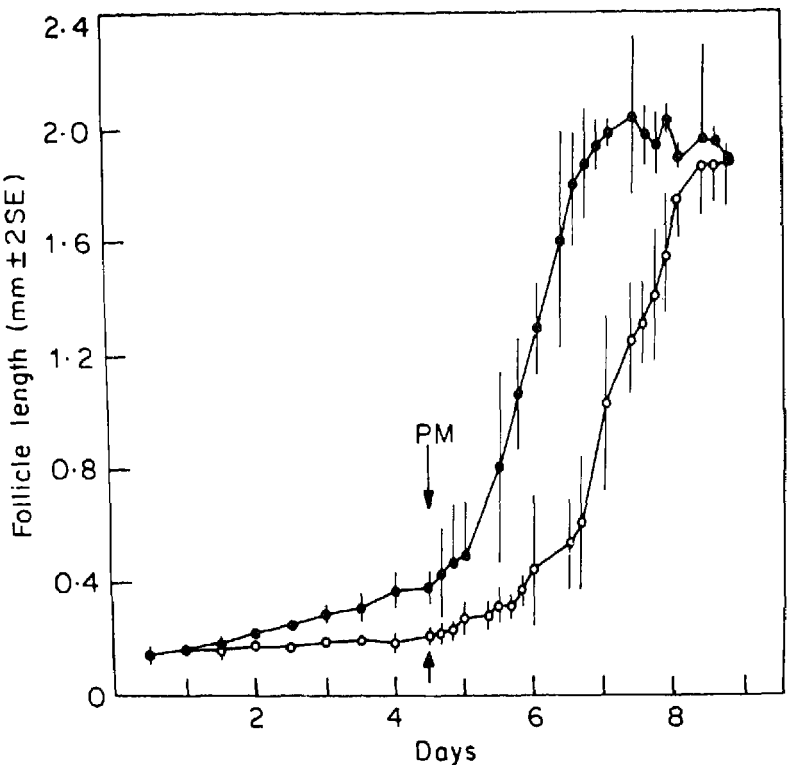

Figure 2. Ovarian maturation in large (●, head width >3·5 mm) and stall (O, head width <3·5 mm) females of S. *argyrostoma* maintained on sugar and water at 25°C, long day length (HD 18·6), and first fed a protein meal (PM) on day 4.

Using Denlinger's (1971a) artificial uterus technique the diapause-inducing effects of photoperiods experienced during ovarian, embryonic and subsequent larval development could be separated. Table 2 shows that exposure to long days during embryonic and larval development led to a very low incidence of pupal diapause, whereas exposure to short days led to high diapause. Exposure of the flies to long or short days during the vitellogenic (ovarian) phase, on the other hand, had no effect.

Table 3 shows the results of further experiments in which the insects were exposed to long days (LD 18:6), short days (LD 12:12) or to continuous darkness (DD) during each of the developmental stages (ovarian vitellogenesis, embryogenesis, and larval development) separately. When vitellogenic flies were exposed to long or short days prior to ovulation, but all later stages to DD, the incidence of diapause in the two groups was almost identical (groups A v B, χ_2. 0·11, d.f.= 1, *P* not significant). This shows that exposure to photoperiod during ovarian development is without effect, and fails to confirm earlier indications to the contrary (Saunders 1980). However, exposing the embryos to long days (groups D to l) consistently produced a negligible incidence of diapause, whereas exposing them to short days (groups J to N) produced a uniformly high incidence of diapause (82 to 95%). Exposure of feeding larvae to long days also reduced the incidence of diapause (L v

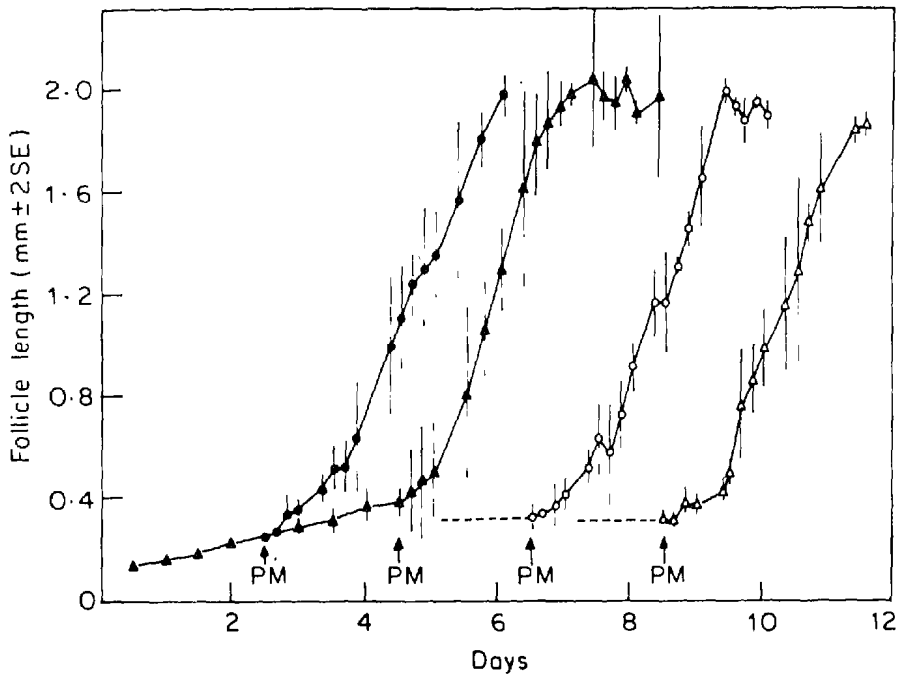

Figure 3. Ovarian maturation in large flies maintained at 25-C, LD 18:6, and first fed protein (PM) on day 2 (●), 4 (▲), 6 (O) or 8 (Δ) after eclosion.

Q, χ^2 110·32, d.f.= 1, P <0.001) whereas short days enhanced it (A v P, χ^2 25·09. d.f.= 1, P<0·001). This observation confirms that photoperiodic sensitivity in *S. argyrostoma* extends well into larval life (Saunders 1971, 1980).

3.5 *Incidence of pupal diapause among the progeny of flies with a diapause or non-diapause history*

Working with S. *bullata,* Henrich and Denlinger (1982) showed that the progeny of flies which had undergone diapause (equivalent to the first spring generation) were unable to respond to the diapause-inducing effects of further short days, although this response was reinstated after an intervening generation in long days. The effect was associated with the short days experienced by the maternal generation, during embryogenesis, rather than with the diapause *per se.* The presumed (and unidentified) maternal factor was shown to be transmitted during ovarian development.

Similar experiments were conducted with S. *argyrostoma* during the present investigation (table 4). Flies emerging from either non-diapause (N) or diapause (D) pupae were kept at 25°C under LD 12:12 and their larvae exposed to LD 12:12 at either 17° or 25° C. In all groups, regardless of the diapause or non-diapause history of the parents, the incidence of pupal diapause was high (94 to 99%). Thus the maternal effect observed with S. *bullata* was not seen in S. *argyrostoma.*

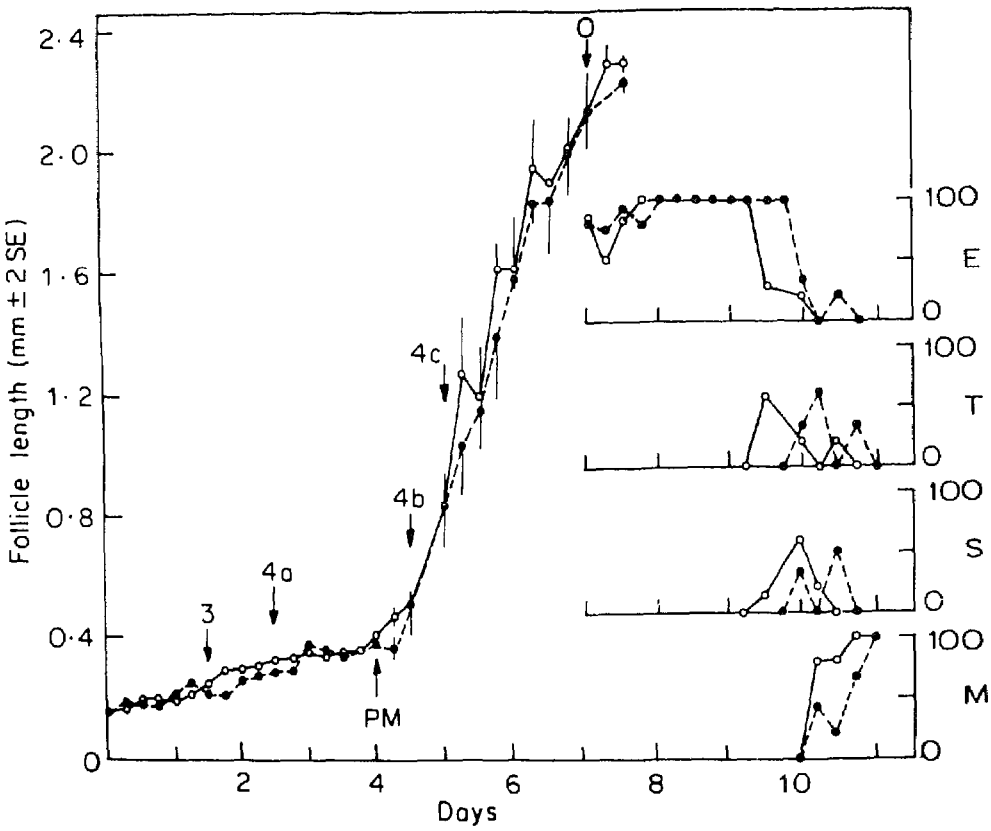

Figure 4. *Left:* Ovarian maturation in large flies at 25°C and short days (HD 12:12, ●) or long days (HD 18:6, O). First protein meal (PB) provided 4 days after eclosion.

Right: Intrauterine embryonic development at 25°C, HD 12:12 (---●---) or D 18:6 (O). E, Early stages of embryonic development after ovulation; T, appearance of tracheae; S, colouration of the larval spiracles; M, movement of the pharate first instar larva within the chorion prior to hatching and larviposition.

Table 2. Effect of photoperiod during ovarian, embryonic and larval development on the incidence of pupal diapause in the flesh fly *S. argyrostorna* at 25°C.

Photoperiod during		Number of	Number of pupae
OV	AU+L	larvae	in diapause (%)
18:6	*18*:6	71	0
18:6	*12*:12	63	56 (88·9%)
12:12	*18*:6	90	0
12:12	*12*:12	82	73 (89·0%)
18:6 normal development *in vivo*		163	3 (1·8%)
12:12 normal development *in vivo*		227	197 (86·8%)

OV, Ovarian development; AU, embryonic development an artificial uterus; L, larval development.

Table 3. The effect of photoperiod during ovarian, embryonic and larval development on the incidence of pupal diapause in S. *argyrostoma* at 25°C.

Culture	Photoperiod during OV	AU	L	Number of larvae	Number of pupae in diapause (%)
A	18 : 6	DD	DD	70	17 (24·3%)
B	12 : 12	DD	DD	78	21 (26·9%)
C	DD	DD	DD	98	46 (46·9%)
D	18 : 6	18 : 6	DD	117	2 (1·7%)
E	DD	18 : 6	DD	158	0
F	DD	18 : 6	18 : 6	108	2 (1·9%)
G	18 : 6	18 : 6	18 : 6	107	0
H	12 : 12	18 : 6	DD	187	0
I	DD	18 : 6	12 : 12	106	0
J	12 : 12	12 : 12	DD	107	102 (95·3%)
K	DD	12 : 12	DD	96	79 (82·3%)
L	DD	12 : 12	12 : 12	57	50 (87·7%)
M	12 : 12	12 : 12	12 : 12	212	182 (85·8%)
N	18 : 6	12 : 12	DD	36	34 (94·4%)
O	12 : 12	DD	18 : 6	63	0
P	18 : 6	DD	12 : 12	94	80 (85·1%)
Q	DD	12 : 12	18 : 6	130	1 (0·8%)

A v B χ_2= 0·1056, df = 1, *P* not significant.
A v P χ_2=25·0886, df=1, *P* <0·001.
J to N χ^2=1·2371, df=4, *P* not significant.
L v Q χ_2=110.3185, df=I, *P*<0·001.
OV, Ovarian development; AU, embryonic development within an artificial uterus; L, larval development.

Table 4. The incidence of pupal diapause among progeny of *S. argyrostoma* adults with a history of diapause (D) or non-diapause (N).

Diapause/non-diapause (D/N) history of adult flies	Larval photoperiod and temperature	Number of larvae	Number of pupae in diapause (%)
N	12:12 17±0·5°C	222	209 (94·1)
D	12:12 17±0·5°C	575	545 (94·8)
D	12:12 25±1°C	162	161 (99·4)

All adult flies were kept at 25°C under LD 12:12.

4. Discussion

4.1 *Ovarian development in S. argyrostoma*

The number of primary egg follicles in newly emerged females of S. *argyrostoma* was strongly correlated with adult body size (as measured by head width), an observation similar to that of Spradbery and Schweizer (1981) for the screw worm *Chrysomyia bezziana.* When subsequently maintained on a protein-free diet, the follicles of small *S. argyrostoma* failed to develop beyond the first stages of yolk

deposition (stage 4A), whereas large flies (head width >3·81 mm) were able to produce a few eggs. Only the largest flies, therefore, were autogenous. In an earlier investigation on flesh flies, Denlinger (1971b) found that females of *S. argyrostoma* were frequently autogenous whilst those of *S. bullata* were not. Later studies on *S. bullata,* however, demonstrated that large flies from well-fed larvae could produce mature eggs without additional protein, whereas smaller flies could not (Baxter *et al* 1973). Denlinger made no mention of size: presumably all of his autogenous flies were "large".

After a protein meal on day 4, ovarian growth and vitellogenesis in *S. argyrostoma* accelerated. Both small and large flies produced mature eggs of the same size, but the accelerated vitellogenesis in small flies was delayed because larger flies began to deposit yolk in their oocytes before the protein meal. When flies were provided with their first protein meal 2, 4, 6 or 8 days after eclosion, the accelerated phase of ovarian development was sequentially delayed (day 6 or 8) or advanced (day 2), suggesting that the protein meal may provide a developmental 'trigger' as well as protein needed for full maturation. Large flies maintained in either long days (LD 18:6) or short days (LD 12:12), and fed protein on day 4, showed indistinguishable rates of ovarian growth. Body size and protein availability are therefore important factors in the ovarian development of *S. argyrostoma,* but photoperiod is not.

4.2 *Developmental stages sensitive to photoperiod*

Although the rate of ovarian growth in *S. argyrostoma* was found to be unaffected by photoperiod, this factor was found to be important during later stages of development. Embryonic development may be followed by noting when the tracheae become apparent (T), when the posterior spiracles of the pharate larva become pigmented (S), and when the pharate larva becomes mobile within its egg shell (M). Each of these developmental stages, and final larviposition, was found to occur earlier in LD 18:6 than in LD 12:12, indicating a more rapid intrauterine development in long days.

By using Denlinger's (1971a) artificial uterus technique, possible photoperiodic effects on the embryos may be separated from maternal effects and from the photoperiodic effects on deposited or feeding larvae. Such an approach (tables 2 and 3) produced unequivocal evidence that pupal diapause induction, the most important consequence of photoperiod in *S. argyrostoma* (Saunders 1971), cannot be regulated by photoperiods experienced during ovarian development. Long days experienced by the embryos, however, whether within the uterus or *in vitro,* led to non-diapause development in the pupa, whereas similar exposure to short days causes the pupa to enter a firm diapause. Exposure of feeding larvae to long or short days has a similar effect. These results thus confirm earlier observations on the importance of embryonic (Denlinger 1971a; Saunders 1980) and larval (Saunders 1971) photoperiods for the induction of pupal diapause. More accurate delineation of the 'sensitive period' is difficult, but sensitivity may begin when the embryonic central nervous system is sufficiently developed and continues through larval development and the period of post-feeding 'wandering' to come to an end before or at puparium formation (Saunders 1971, 1992).

4.3 *Diapause incidence in the first past-diapause generation*

Working with *S. bullata,* Henrich and Denlinger (1982) showed that the progeny of flies emerging from diapausing pupae were unable to re-enter diapause under further short days, although those from flies with a non-diapause history were able to do so. More limited data for *S. crassipalpis* indicated a similar but much weaker effect. Data presented in this paper, however, show that this phenomenon does not occur in *S. argyrostoma,* progeny of flies emerging from diapause or non-diapause pupae being equally susceptible to the diapause-inducing effects of short days.

Henrich and Denlinger (1982) suggested that the selective advantage of the phenomenon (in *S. bullata)* might be to avoid untimely entry into diapause in the spring when days are still short, or to allow over-wintering flies to enter the breeding population at the earliest possible date. For more northerly populations, such as the *S. argyrostoma* used in this investigation, insects in their natural environment are probably subjected to a longer winter and a colder spring. The phenomenon may thus be absent because day lengths have already lengthened past the 'critical day length' for pupal diapause induction by the time the first spring generation of flies has completed its post-diapause development.

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