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PUPAL DIAPAUSE IN THE FLESH FLY, SARCOPHAGA DUX

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ABSTRACT

Blow flies (family-Calliphoridae) and flesh flies (family-Sarcophagidae) are amongst the first wave of insects which arrive on a corpse for feeding and breeding purposes. Development rates of these flies are frequently used to determine post-mortem interval (PMI) in forensic entomological investigations. The rate of larval growth is directly affected by environmental conditions such as photoperiod and ambient temperature. In the flesh fly, *Sarcophaga dux*, short-day photoperiods and low temperature induce pupal diapause. Diapause is a form of dormancy in insects which confer survival during unfavourable environmental conditions. Distinct genetic traits and endocrinological factors are found responsible for the induction and regulation of diapauses in different insect species. The life stage at which the insect exhibits diapause is also species specific. Present study investigates the incidence of diapause in the laboratory reared populations of *S. dux* and define further the factors responsible for induction and termination of pupal diapause in flesh flies.

Keywords: Forensic entomology, Post-mortem interval, Flesh fly, S. dux, Diapause

INTRODUCTION

Adverse winter conditions in the temperate regions have channelled the evolution of a pupal diapause in flesh flies of the genus *Sarcophaga*. Diapause in *Sarcophaga* is generally found in the young phanerocephalic pupa, it is the pupal stage in which adult development has not been initiated [1]. Various studies have been conducted on the incidence of diapause in the genus *Sarcophaga* [1-8]. Effect of cold temperature and short photoperiod on pupal diapause induction in *S. argyrostoma* was studied by many workers [3,7], whereas in *S. bullata* larval photoperiod was not shown to influence diapause. Denlinger revealed the importance of photoperiod received by developing embryos within the uterus of the ovoviviparous females in the *S.crassipalpis.* [5, 6] If the larvae are exposed to a short photophase at 25^0 C, the pupae will not enter diapauses [5,6]. In various groups of *S.crassipalpis* males enter diapause at a higher rate than females, experiments revealed a similar pattern in the other species of *Sarcophaga* [1]. In the *Pieris napi*, *Pararge aegeria* and *P. c-album* a higher tendency of males to enter diapause is also found[9].

Quality and quantity of the food provided may also be a factor in diapause influence in some insects. The role of water in diapause termination has been well studied.[10]Addition of 10% water to larval medium increases diapause incidence10%[5], however opposite effect of moisture content in *Lucilia sericata* has been observed, incidence of larval diapause was increased when the larvae were fed dry meat.[11,12] Mature third instar larvae are inhibited to become pupae by contact with a little water.[13] The adults of *S. argyrostoma* are capable of producing viable eggs without an adult protein diet, the incidence of diapause in this species is not affected due to presence or absence of protein diet.[14] Ecdysone is found to act as an accelerator for diapause termination in *Sarcophaga.*[4]

If the flesh flies *S. bullata* having history of pupal diapause are mated and their larvae reared in a strongly diapause-inducing environment, the offspring do not enter pupal diapauses. It is due to maternal effect transmitted by the female parent prior to ovulation and therefore directly affects the germ line.[15] In *S. bullata* probably gamma amminobutyric acid (GABA) and octopamine are involved in the transfer of information from mother to next generation. Other experiments on flesh flies, *S. crassipalpis* and *S. bullata* also confirm the influence of maternal effect in the induction of diapauses. [5, 16] The progeny of silkmoth *B. mori* will enter embryonic diapause or not is determined by the photoperiod received by the female silkmoth during her embryonic and larval development.[17, 18] A neurohormone from the suboesophageal ganglion of the female silkmoth mediates the maternal effect. [19]

At the hormonal level pupal diapause is characterized by failure of the brain to stimulate the prothoracic gland to secrete ecdysone [20-23].Juvenile hormone (JH) profile is also associated with the incidence of pupal diapause in flesh flies. Flies destined for direct development lack JH activity while flies programmed for pupal diapause show major pulses of JH activity [23].

MATERIALS AND METHOD

Stocks were maintained in an insectary at room temperature in normal day night conditions. Environmental cabinets were used to maintain a temperature of 20±0.5°C, 17±0.5°C, 15±0.5°C. Groups of about 100 adult flesh flies were kept in cages. Sugar and water were provided *ad libitum* throughout adult life and chicken liver was provided as a source of protein. The species *S. dux* are ovoviviparous, embryonic development occurs within a sac-like uterus. Larvae were reared on fresh chicken liver, when the third instar larvae cease feeding they crawl out and pupariate in the vermiculite. Pupae were examined every day to record the time required for diapause termination.

RESULT S AND DISCUSSION

Table 1: Influence of Larval Photoperiod and Temperature on Incidence of Pupal Diapause in *S. Dux*.

Photoperiod	Temp.	No. of pupae	% Diapause
12 : 12	25°C	540	0
12 : 12	20°C	240	0
16 : 8	17°C	225	100
16 : 8	15°C	192	100

Under an LD 12:12 h photoperiod, no pupae enters diapause when the temperature is above 20°C, whereas all the pupae enter diapause below 20°C. The duration of diapause is longer than 90 days. Under experimental conditions the incidence of diapause increases with a decrease in temperature. Thus, the combination of short days and low temperature (17^{0}C and 15^{0}C) caused maximum diapause response. The temperature influences the effect of photoperiod. These results indicate that *S. dux* is sensitive to the direction of change in photoperiod. Different diapause characteristics were observed among different species of *Sarcophaga*, *S. argyrostoma*, *S. crassipalpis*, and three strains of *S. bullata* [1]. A comparatively high incidence of pupal diapause was observed in *S. argyrostoma* at 17°C and 12 hour photoperiod. Significant differences were observed among wild strains and lab strain of *S. bullata* [1].

Pupariation is observed to be delayed in the larvae destined to diapause and occurs over a period of many days, this was also supported by Denlinger and Beck[1,10]. Post feeding or wandering stage is found prolonged. Delay of pupariation is strongly correlated with induction of pupal diapause, as this delay may provide the larvae some more time to find a suitable place to pupariate. This may be a preparative phase and involves in the deposition of additional fat reserves, extra hydrocarbons for water proofing the cuticle and some storage proteins present in the hemolymph may also be synthesized during this phase[24-26]. These reserves may help in survival during dormancy and in the metamorphosis at the termination of diapauses [27]. Larval injections of juvenile hormone [28] and exposure to the moisture can cause the delay in pupariation in *Sarcophaga* [29-31]. By reducing the rearing temperature pupariation is delayed, and incidence of diapause increases [7].

Termination of diapause is temperature dependent. The duration of diapause decreases with an increase in temperature and is terminated at the temperature around 25° C. Since the pupae of *Sarcophaga* are buried under the soil surface, soil temperature rather than photoperiod could provide appropriate information of the favourable season. Ecdysone can terminate pupal diapause in *H. cecropia* [32] and *Sarcophaga* [4]. Immediate termination of diapause can be brought by giving topical treatment of hexane [33].

The phenomenon of diapause can be regulated by many factors; these may be environmental regulators, hormonal regulators as well as molecular regulators. At the molecular level, expression of some genes is silenced, and some others are expressed during diapause. Some upregulated genes are expressed throughout diapause, and others are expressed in early diapause, some in late diapause whereas some genes expressed intermittently. The termination of diapause is brought about by expression of many down regulated genes, while decline in expression of the diapause-upregulated genes is observed during diapause termination [27].

Moribayashi *et al* have studied the physiology of flesh flies, *Boettcherisca peregrina* and *Parasarcophaga similis*, and of blow flies, *Aldrichina grahami* and *Calliphora nigribarbis* [13]. At 20°C larvae of the flesh fly enter pupal diapause on exposure to short photoperiod but do not enter diapause in long photoperiod, at this temperature larvae destined non-diapause develop adults in 4 weeks [13]. Future diapause research may be helpful to have a better understanding of seasonality in insects and applicable in the field of medical and forensic entomology.

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REFERENCES

1- Denlinger D. L. Seasonal phenology of diapause in the flesh fly *Sarcophaga bullata.* Ann. Ento,nol. Soc. Amer. 1972; in press.

2- Roubaud E. Etudes sur le sommeil d'hiver preimaginal des Muscides. Bull. Biol. Fr. Beig. 1922; 56, 455-544.

3- Fraenkel G.and C. Hsiao, Manifestations of a pupal diapause in two species of flies, Sarcophaga argyrostotna and S. bullata. J. Insect Phsiol. 1968a; 14, 689-705.

4- Fraenkel G. and C. Hsiao. Morphological and endocrinohogical aspects of pupal diapause in a flesh fly, *Sarcophaga argyrostoma* (Robineau-Desvoidy). J. Insect Physiol. 1968b; 14, 707-718.

5- Denlinger D. L. Embryonic determination of pupal diapause induction in the flesh fly *Sarcophaga crassipalpis* Macquart. Amer. Zool. 1970; 10, 320-321.

6- Denlinger D. L. Embryonic determination of pupal diapause in the flesh fly *Sarcophaga crassipalpis* Macquart. J. Insect Physiol. 1971b; 17, 1815-1822.

7- Saunders D. S. Temperature- compensated photoperiodic clock 'programming' development and pupal diapause in the flesh-fly, *Sarcophaga argyrostoma.* J. Insect Physiol. 1971; 17, 801-812.

8- Vinogradova E. B. and K. B. Zinovjeva. The photoperiodic and temperature in duction of pupal diapause in flies of the family Sarcophagidae. Proc. Jut. Congr. Ent. Moscow. 1971; 1968, 456-457.

9- Wiklund C., Wickman P.-O. & Nylin, S. A sex difference in reaction norms for direct/diapause development: a result of selection for protandry. Evolution. 1992; 46, 519–528.

10- Beck S. D. Insect Photoperiodism. Academic Press, New York. 1968; 288 PP.

11- Cousin G. Etude experimentale de la diapause des insects. Bull. Biol. Fr. Beig. Sup. 1932; 15, 1-341.

12- Mellanby K. Diapause and metamorphosis of the blowfly *Lucilia sericata* Mg. Parasitology. 1938; 30, 392-402.

13- Moribayashi A., H. Kurahashi., T. Hayashi., Y. Tsuda and M. Kobayashi. Physiological studies of four forensically important flies found in different seasons in Japan, 6th international congress of dipterology 23-28 September 2006; Fukuoka, JAPAN.

14- Denlinger D. L. Autogeny in the flesh fly *Sarcophaga argyrostoma*. Attn. Entomol. Soc. Amer. 1971a; 64, 961-962.

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

16- Webb M-L.Z., Denlinger D. L. GABA and picrotoxin alter expression of a maternal effect that influences pupal diapause in the flesh fly, *Sarcophaga bullata*. Physiol. Entomol. 1998; 23,184- 91.

17- Webb M-L.Z., Denlinger D. L. GABA and picrotoxin alter expression of a maternal effect that influences pupal diapause in the flesh fly, *Sarcophaga bullata*. Physiol. Entomol. 1998; 23,184- 91.

18- Hasegawa K. Studies on the voltinism in the silk worm, *Bombyx mori* L., with special reference to the organs concerning determination of voltinism.

J. Fac. Agric, Tottori Univ. 1952; 1, 83-l24.

19- Yamashita O.,Yaginuma T. and Flasegawa K. Hormonal and metabolic control of egg diapause of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) Ent. gen. 1981; 7, 195-211.

20- Fraenkel G. & hsiao C. Morphological and endocrinological aspects of pupal diapause in a flesh fly. *Sarcophaga argyrostoma*. J. Insect Physiol. 1968; 14, 707-718.

21- Ohtaki T. and Takahashi M. Induction and termination of pupal diapause in relation to the change of ecdysone titer in the fleshfly, *Sarcophaga peregrina*. Jap. J. med. Sri. Biol. 1972; 25, 369-376.

22- Zdarek J. and Denlinger D. L. Action of ecdysoids, juvenoids, and non-hormonal agents on termination of pupal diapause in the flesh fly. J. Insect Physiol. 1975; 21, 1193-1202.

23- Walker G. P. and Denlinger D. L. Juvenile hormone and moulting hormone titres in diapause and non-diapause destined flesh flies. J. Insect Physiol. 1980; 26, 661-664.

24- Chippendale G. M. Role of proteins in insect diapauses. In Endocrinological Frontiers in Physiological ecology, ed. F Sehnal, A Zazba, D L Denlinger. 1988; pp. 331-46. Wroclaw Tech. Univ. Press

25- Adedokun T. A., Denlinger D. L. Metabolic reserves associated with pupal diapauses in the flesh fly, *Sarcophaga crassipalpis*. J. Insect physiol. 1985; 31, 229-33.

26- Yoder J. A., Denlinger D. L., Dennis M. W., Kolattukudy P. E. Enhancement of diapausing flesh fly puparia with additional hydrocarbons and evidence for alkane biosynthesis by a decarbonylation mechanism. Insect Biochem. Mol. Biol. 1992; 22, 237-43.

27- Denlinger D. L. Regulation of diapause. Annu Rev Entomol. 2002; 47, 93-122.

28- Srivastava U. S. and L. I. Gilbert. The influence of juvenile hormone on the metamorphosis of *Sarcophaga bullata*. J. Insect Physiol. 1969; 15, 177-190.

29- Evans A. C. Studies on the influence of the environment on the sheep blow-fly, *Lucilia sericata* Meig. II. The influence of humidity and temperature on prepupae and pupae. Parasitology. 1935; 27, 291-298.

30- Ohtakt T., R. D. Milkman and C. M. Williams. Dynamics of ecdysone secretion and action in the fleshfly *Sarcophaga peregrina*. Biol Bull. 1968; 135, 322-334.

31- Zdarek J. and G. Fraenkel, Overt and covert effects of endogenous and exogenous ecdysone in puparium formation of flies. Proc. Nat. Acad. Sci. USA. 1970; 67, 331-337.

32- Williams C. M. Physiology of insect diapause. X. And endocrine mechanism for the influence of temperature on the diapausing pupa of the cecropia silkworm. Biol. Bull. 1956; 110, 201-218.

33- Denlinger D. L., Campbell J. J., Bradfield J. Y. Stimulatory effect of organic solvents on initiating development in diapausing pupae of the flesh fly *Sarcophaga crassipalpis* and the tobacco horn worm *Manduca sexta*. Physiol. 1980; Ent. 5, 7-15.