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Sperm Transfer and Sperm Activation in Tasar Silkmoth, *Antheraea Mylitta*

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ABSTRACT

Two types of sperm, apyrene and eupyrene, are identified in *A. mylitta*. The sperm in the adult moth are motionless in seminal vesicles. At the time of ejaculation they received a secretion from male ejaculatory duct that renders them motile. The dissociation of eupyrene bundles, apyrene, eupyrene sperm motility and the sequence of events of sperm migration in both sexes are described in the present paper.

Key words : Spermatozoa, Sperm activation, Tasar Silkmoth

INTRODUCTION

The nature of sperm, their activation and the fate after mating are known in many insects (Leopold, R.A. 1976, Shepherd, J.G. 1974, Osanai, M. 1989, Nabi, H.N. & Harrison, R.A. 1983). Sperm activation and migration seem to differ considerably among insects and it is not clear how the two types of spermatozoa, apyrene and eupyrene common to Lepidoptera to be activated (Leopold, R.A. 1976). While a great deal of work has been done in the reproductive physiology of *Bombyx mori* and the results are being effectively used in sericulture industry, our knowledge on the sperm biology and related events in tasar silkworm is scanty. Studies on sperm biology generally help to attain a better pupal preservation schedule that in turn enhances fertility of eggs (Katsuno, S. 1991).

Recently we have redescribed the male reproductive system of *A. mylitta* (Rarikumar, G., Rajeswary, H. & Thangarelu, K. 1983). During pharate stage, the spermatozoa from the testes migrate down through vasdeferences to get stored in the seminal vesicles. The secretion of paired accessory gland with sperm is transferred to the female reproductive tract during copulation (Rarikumar, G., Rajeswary, H. & Thangarelu, K. 1983). In the present study we describe the apyrene and eupyrene sperm, their activity patterns and migration.

MATERIALS AND METHODS

Healthy adult bivoltine races (DABA) of *A. mylitta* of both sexes were used in the study. Sperm were dissected out from seminal vesicles, bursa copulatrix, and the spermatheca counted in insect saline and stained according to the WHO manual (WHO 1980) with slight modification.

Sperm activity was observed by the method described by Shepherd (Shepherd, J.G. 1974). The dissected ejaculatory duct and bursa copulatrix were homogenised in a few drops of 0.03 M ammonium bicarbonate acetic acid buffer(pH 7). This homogenate was centrifuged for 10 min, at 5000 rpm and the supernatant was mixed with sperm taken from the seminal vesicles(sperm were diluted in few drops of 0.15 M HEPES KOH buffer, pH 7 before assay). Equal volumes of the extract and sperm were in-

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cubated in moist medium and examined under phase contrst microscope.

RESULTS

Two types of sperm have been identified, nucleated eupyrene and a nucleated apyrene. The eupyrene sperms were easily quantified in the seminal vesicles as they remain there in bundles(Figure 1B) until they are transferred to bursa copulatrix during mating, while the apyrene ones remain loose in form (Figure 1A). The total number of sperm estimated in a seminal esicle was 6.8 million, and about 20% of them were eupyrene in bundles. Each bundle contained approximately 243 sperm. Fresh spermatozoon measures 654 µm in length. The pH of the undiluted semen was 6.9 to 7.0. Both types of spermatozoa were nonmotile in the seminal vesicles and became motile (weak gyrating eupyrene) during copulatin or after deposition in the bursa copulatrix.

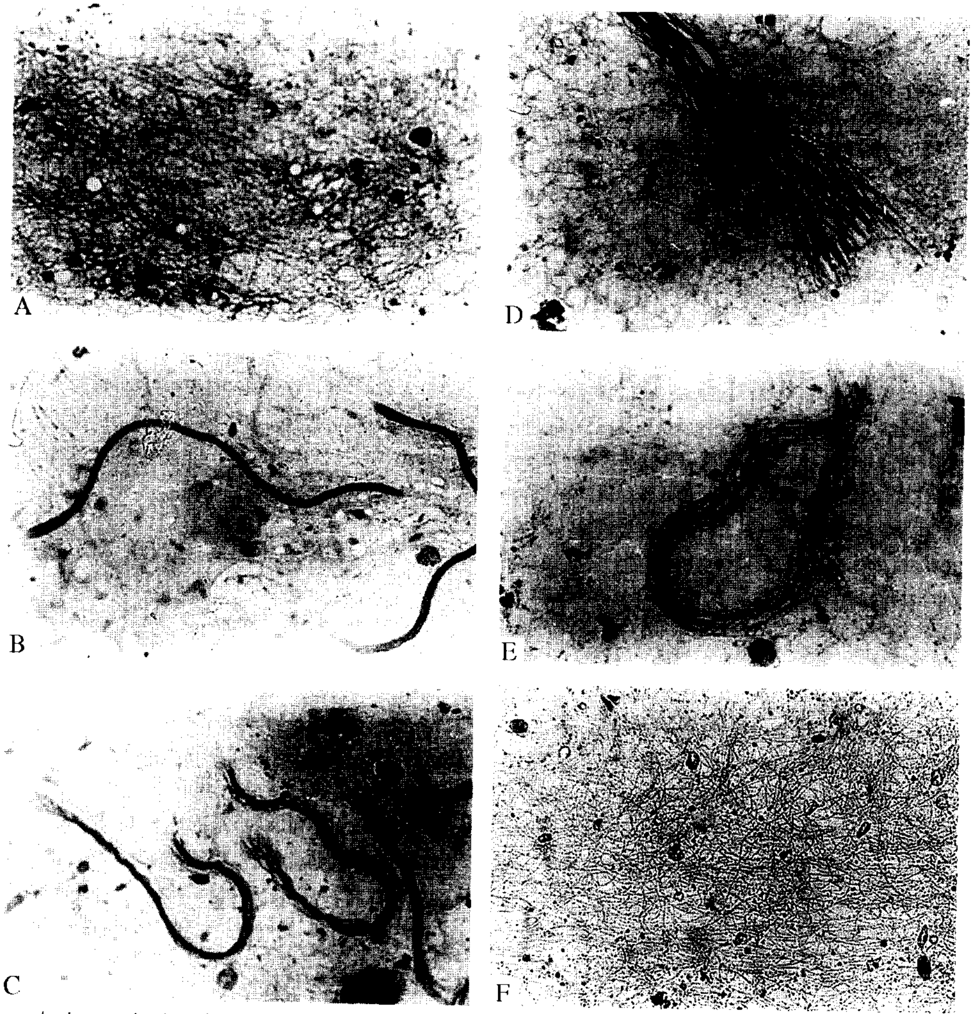

Fig. 1. *Microphotograph showing eupyrene and apyrene spermatozoa in A. mylitta. A) Apyrene spermatozoa in the seminal vesicle ×200, B) Eupyrene sperm bundles in the seminal vesicle ×200, C) Eupyrene dissociation in the bursa copulatrix ×200, D) Magnified view showing that the dissociation starts in the tail end of the eupyrene bundle in bursa copulatrix ×500, E) An advance stage of eupyrene dissociation in the bursa copulatrix ×312.5, F) Dissociated eupyrene spermatozoa in the spermatheca ×200.*

Apyrene sperms were vigorously motile in the bursa copulatrix. Strong and rhythmic movement of bursa was observed just after copulation. The eupyrene sperm were dissociated in bursa and this began at the tail end of the bundles. The above events are histologically presente in Figure 1(CDE). After 10 to 15 min., the loose spermatozoa of both types moved to the spermatheca and remained there. In the spermatheca both types of sperm were active but the apyrene movement ceased in a short while. During oviposition the eggs, permit the entrance of spermatheca and is exposed to eupyrene spermatozoa (Fig. 2). The micropyle part of eggs are positioned in a way to allow the entry of eupyrene sperm. Eupyrene remained active throughout the egg laying period which lasted for 7-8 days.

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+---------------------------+
|          Testis           |
|    Apyrene + Eupyrene     |
|          Bundles          |   --
+---------------------------+     |
              |                   |
+---------------------------+     |
|        Vasdeferens        |     |
|    Apyrene + Eupyrene     |     |
|   (loose)    (Bundles)    |     |
+---------------------------+     |   Male
              |                   |
+---------------------------+     |
|          Mating           |     |
|  Sperm + Accessory Gland  |     |
|Secretion + Sperm Activator|     |
|from the Ejaculatory Duct  |     |
+---------------------------+     |
              |                 --
+---------------------------+
|     Bursa Copulatrix      |
|    Apyrene + Eupyrene     |     --
|   (loose)    Dissociation |       |
| Vigorously     of eupyren |       |
|    Motile     bundles     |       |
+---------------------------+       |
              |                     |
+---------------------------+       |
|        Spermatheca        |       |
|    Apyrene  + Eupyrene    |       |
|Motility ceases motile and |       |
|    slowly        stored   |       |
+---------------------------+       |
              |                     |
+---------------------------+           Female
|      Median Oviduct       |       |
|     Entry of eupyrene     |       |
|        to the egg         |       |
+---------------------------+       |
              |                     |
+---------------------------+       |
|     Collaterial Gland     |       |
|         Secretion         |       |
+---------------------------+       |
              |                   --
+---------------------------+
|        Oviposition        |
+---------------------------+
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Fig. 2. *Sequence of events of sperm migration in A. mylitta.*

Apyrene and eupyrene spermatozoa were easily distinguished in seminal vesicles as they remain there in loose and bundle forms respectively. In the female reproductive tract, apyrene are thinner than eupyrene and the motility of the two have different patterns. It is spiral and rapid for apyrene whereas slow and in wave form for eupyrene.

Sperm activation *in vitro*

Sperm incubated in the extract of ejaculatory duct wee motile while they remain non-motile in the extracts of bursa copulatrix. Dissociation of eupyrene bundles was also observed in the former case.

DISCUSSION

Two types of sperm, apyrene and eupyrene were identified in *A. mylitta*. The latter is regarded as functional sperm as it takes part in fertilization and the former assists the latter in the process of fertilization. Both types of spermatozoa were non-motile in the male reproductive tract but apyrene became vigorously motile when ejaculated into the bursa copulatrix. Moreover, the dissociation of eupyrene bundles was also observed in bursa. Male ejaculatory duct secretion was found to be responsible for the activation and dissocation of sperm. Therefore, female moth has no role in the initiation of sperm motility. The in accordance with reports on *Bombyx mori* and other saturniid moths (Shepherd, J.G. 974) but not with another lepidopteran, *Galleria* (Khalifa, A. 1950) in which the sperm activating substance originated from the walls of bursa. Eupyrene spermatozoa were activated soon after the dissociation of the bundles, but activity remained low in the bursa copulatrix. The activity of eupyrene was completed later in the spermatheoa. Unpublished observation from our laboratory showed that the male accessory glands of *A. mylitta* secreted a substance that enhances the fecundity in females and this substance is stored in the seminal vesicles along with sperm until mating. Viable sperm and accessory secretions are prerequisites for optimum fecundity and fertility and the latter is responsible for initiating and sustaining the powerful contractions in female reproductive tract. As noted above, the transport of spermatozoa from bursa copulatrix to spermatheca was brought about by the contractions of bursa. If the contractions of reproductive tract were prevented by removing accessory glands from the male before mating or by paralysing the female with nitrogen or crbon dioxide after copulation, the spermatozoa did not reach spermatheca (Devey, K.G. 1958). Thus, it appears that the role of apyrene is to assist the eupyrene in their migration from bursa to spermatheca as sugested in many cases (Holt, G.G. & North, D.T. 1970, Fried lander, M. & Gitay, H. 1972) seems secondary. In *B. mori* the role of apyrene is to stir the heterogenous content of spermatophore which act as a reactor for various reactions such as arginine degradation coupled with glycolysis (Osanai, M. & Aigaki, T. 1987).

Apyrene spermatozoa are active just after the copulation and are vigorously motile in the bursa. In spermatheca both the spermatozoa are active but eupyrene movement recedes shortly and eupyrene remain active in spermatheca for several days after mating as the egg laying lasts for 7-8 days in *A. mylitta.*

Motility of activated sperm in Saturniids (Shepherd, J. G. 1974), *Periplaneta* and *Rhodnius* (Davey, K.G. 1965) was maintained in anaerobic conditions but in *Cimex* (Rao, H.V. & Davis, N.T. 1969) oxygen was shown to be needed. It was also shown that the activating factor in the male secretion is a small polypeptide(molecular weight between 1600 to 4500) which functions as a catalyst rather than an external source of energy in certain Saturniids (Shepherd, J.G. 1975). Although we have not purified and characterized the sperm activator. our observations suggest that it is responsible for activating anucleated apyrene and the breakdown of eupyrene bundles to separate them fertilizing the eggs and their partial acquistition of motility which is later completed in the spermatheca.

적 요

인도 작잠에서 2종의 정자형태, 즉 무핵정자와 유핵정자가 존재함이 확인되었으며 인도작잠의 정자는 저정낭 내에 머무를때 까지는 운동성이 없었으나, 사정되기직전 사정란의 분비물과 혼합되면서 부터 운동성을 나타내었다.

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