Sperm dimorphism in lepidopteran insects

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Introduction

One of nature's most diverse cell types is the sperm, which plays a prominent role in sexual reproduction in animals. Sperm is the only fundamental reproductive unit in the male reproductive system that is transferred from male to female. A gamete (sex cell), also known as a sperm cell or spermatozoa, is formed in the male reproductive system. It is a cell that moves about with only one purpose: to fertilize a female egg.

Sperm dimorphism in insect is scientifically a process known as dichotomous spermatogenesis; in all the males of a species produce two main types of contemporaneous spermatozoa with distinct differentiation paths, structures, and DNA compositions. The usual haploid sperm that fertilizes the eggs is one type. It is conceptually comparable to the generalized type of spermatozoa seen in most animal species (Baccetti, 1991). The other kind, sometimes referred to as parasperm (Jamieson, 1987), is different in form and function, either being anucleated or possessing an imbalanced set of chromosomes. This type of spermatozoa does not fertilize the egg, and its function in many systematic groupings is unknown. There are only these two types of spermatozoa produced by dichotomous spermatogenesis; there are no intermediate morphs.

The structure of insect sperm (as ancestral type of hexapod sperm) consists of an elongated cell with a three- or two-layer acrosome on top of the nucleus and a posterior functioning flagellum with an unmodified 9+2 axoneme. Two mitochondria are found in the flagellum in addition to the axoneme (Dallai *et al*., 2016). The condition of male ejaculates containing two or more different types of sperm is known as sperm polymorphism. Insect species belonging to the orders Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Coleoptera have four different types of sperm polymorphism (Jamieson *et al*., 1999; Presgraves *et al.,* 1999; Swallow and Wilkinson, 2002; Chawanji *et al*., 2005).

Differences in the sperm structure among insect orders

The research conclusion, is based on phylogenetic interpretation of sperm characteristics and their constituent parts, indicates that structural differences have been seen in the sperm of various insects, which are

arranged according to acroneme and axoneme of flagellum. Order-wise the number of axoneme varies in flagellar portion *viz.,* Collembola (9+2 axoneme), Diplura (9+9+2 axoneme), Paraneoptera (9+9+2 axoneme), Ephemeroptera (9+9+0 axoneme), Strepsiptera (9+9+2 axoneme) and Polyneoptera (9+9+2 axoneme) (Dallai *et al*., 2016).

Sperm polymorphism in different insect orders

The polymorphism is occurrence of various forms within a population, colony, or within a single organism's life cycle. In almost all taxa, sperm exhibit strikingly different evolutionary morphologies. Some organisms that reproduce sexually have variations in their male solitary sperm. Sperm polymorphism is a specific sort of variation or polymorphism found in special group of insects. The phenomena of male ejaculates containing two or more different types of sperm are known as sperm polymorphism.

Four different forms of sperm polymorphisms, which vary depending on different reproductive and chromosomal features, have so far been identified in several insect orders. Sperm polymorphism is a type of physiologically adapted behaviour. Below is a list of the different types of sperm polymorphism.

1. *Drosophila* fruit flies (Beatty & Burgoyne, 1971), Diopsidae stalk-eyed flies (both

Diptera) (Presgraves *et al*., 1999), and cicadas (Chawanji *et al*., 2005) (Hemiptera) are examples of the first kind. Some species of these insects produces spermatozoa in more than two sizes wherein they differ only in the length of the sperm head and/or tail but not in terms of ploidy (i.e. number of chromosomes).

2. Some pentatomid insects (Hemiptera) and carabid ground beetles (Coleoptera) are examples of the second kind, in which a typical haploid and enormous polyploid spermatozoa are documented (Schrader, 1960; Bouix, 1963; Jamieson *et al*., 1999; Swallow and Wilkinson, 2002), although additional studies are necessary to confirm the presence of sperm polymorphism in carabids because a different result also is reported (Takami and Sota, 2007).

    Other insect species have not been found to exhibit the same kind of sperm polymorphism as like *Scarites terricola* ground beetle. Unlike other instances*, S. terricola's* sperm dimorphism is unusual with the heads of multiple spermatozoa are "glued" together while the tails are free to move in one form. The other form is free as single spermatozoa and has an extended tail and an abnormally huge head. In Ringer's solution, both types are motile (Sasakawa and Toki, 2008).

3. A hymenopteran species that has multiple forms of spermatozoa, including two that differ only in the spiral orientation of the

spermatozoa's helical structure, represents the third type (Lee and Wilkes, 1965).

4. Lepidoptera, which includes butterflies and moths, is the fourth kind. In this type, anucleated shorter spermatozoa (apyrene) are produced in addition to usually nucleated spermatozoa (eupyrene), making up the most well-studied group in terms of sperm polymorphism. (Swallow and Wilkinson, 2002)

Sperm dimorphism in lepidopteran insects

The Lepidoptera is the group of animals with dichotomous spermatogenesis that is most well-known. Meves (1903), in a stunning work hypothesized at the very beginning of the twentieth century, accurately and in great detail the process at the cytological level. He was the first to disclose that moths and butterflies produce two types of sperm. Katsuno (1977) claims that Toyama's (1894) study of spermatogenesis in the commercial silk moth, *Bombyx mori* was the first publication to note the dichotomous spermatogenesis of lepidoptera, but a careful examination of the text and figures of Toyama's paper shows no evidence that he recognized the distinction between eupyrene and apyrene meiosis or the resulting presence of two kinds of sperm.

Sperm polymorphism in lepidopteran leads to formation of two kinds of sperm *viz*., eupyrene (nucleate) and apyrene (anucleate) spermatozoa. Sperm dichotomy is present in all species of lepidoptera with an exception in two species of primitive Micropterigidae (Sonnenschein and Hauser, 1990). In the lepidopteran order, dimorphism with eupyrene and apyrene sperm is distinctive. The former have a prolonged glycocalyx made up of blade-like structures termed laciniate appendages and are functional (eusperm). These appendages and the nucleus are absent in apyrene parasperm. Sperm polymorphisms were described in snails as early as 1836 by von Siebold which was described by Sakai *et al*. (2019) and have subsequently been reported in invertebrates and vertebrates.

Eupyrene sperm bundles have needle-shaped sperm nuclei that are placed in the anterior section of the elongating cells and contain the normal haploid spermatozoa to fertilize eggs. These bundles extrude cytoplasm by a process called "peristaltic squeezing." Sperm bundles from apyrene are shorter and entirely devoid of nuclear material. In the final stages of spermatogenesis, the cytoplasm and spherical micronuclei in the center of the apyrene sperm bundles are squeezed out (Chen *et al*., 2020).

Role of apyrene sperm in fertilization

Eupyrene and apyrene sperms are produced in the same testicular follicles, but eupyrene spermatogenesis occurs before apyrene spermatogenesis. The major function of nucleated eupyrene sperm is to fertilize eggs. Different functions of apyrene sperm is being proposed including their role in
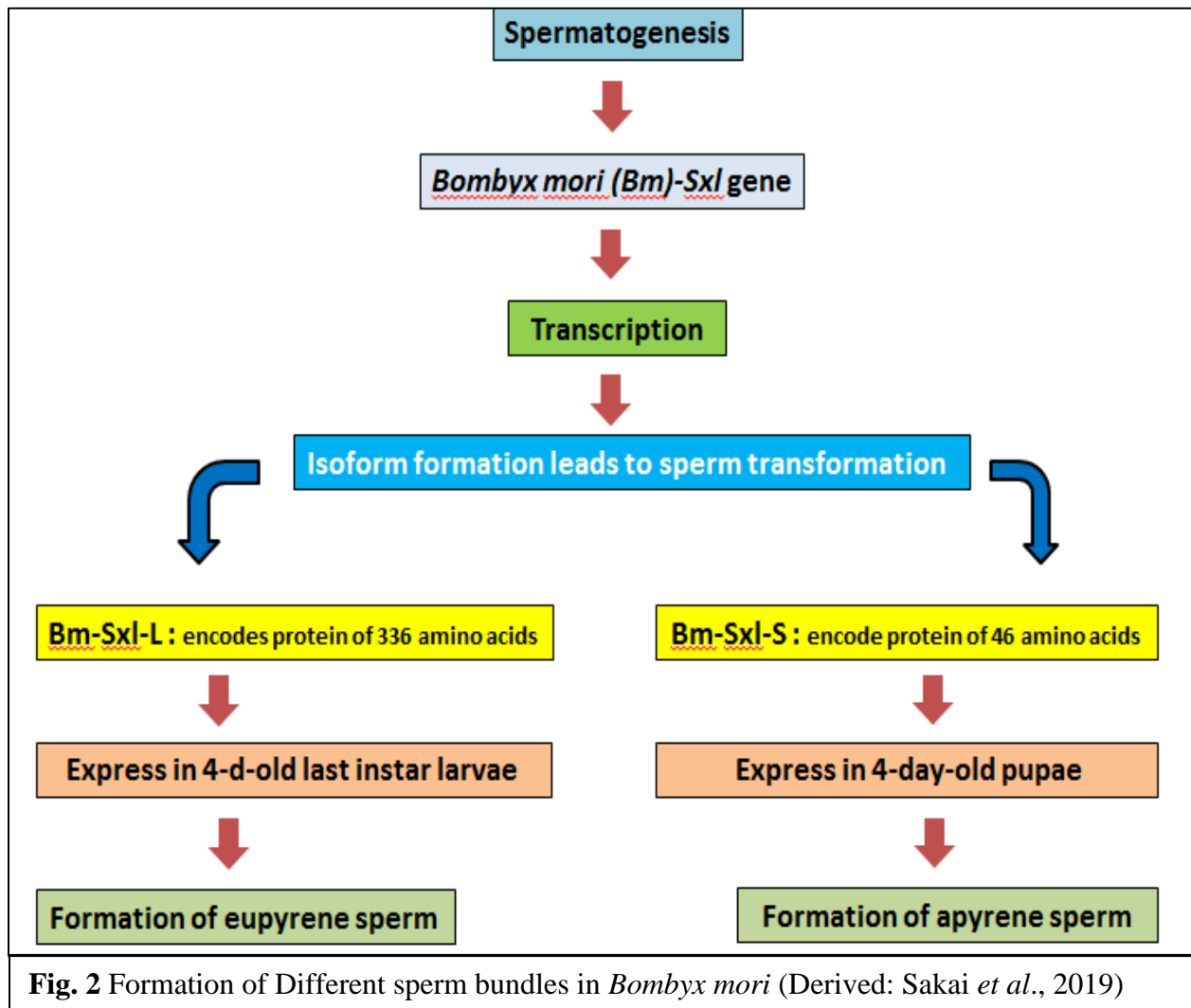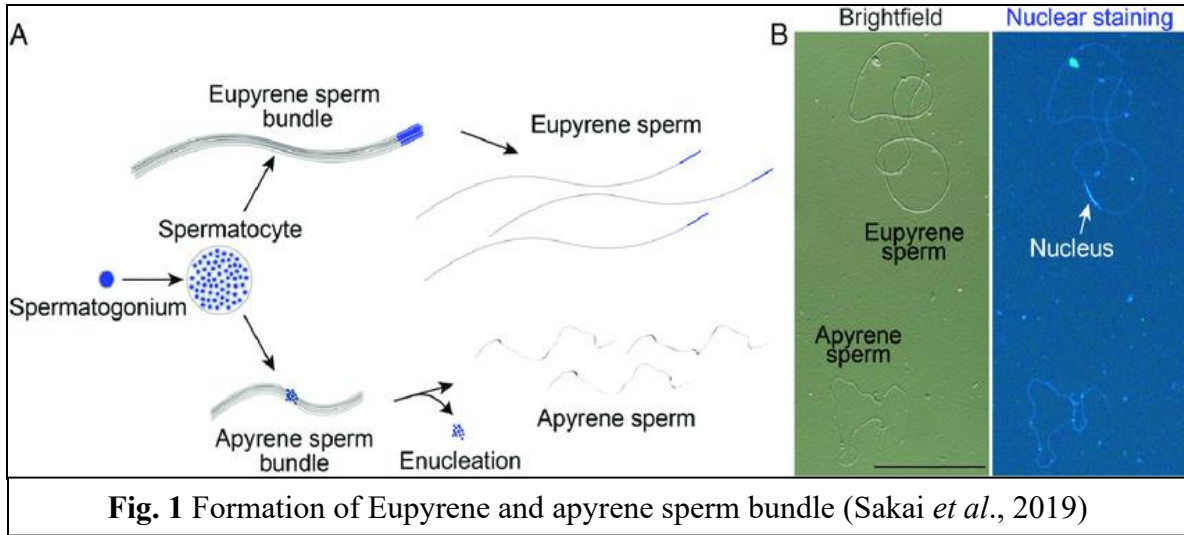
improved fertilization during sperm competition; defense against spermicidal conditions; assisting the eupyrene sperm in spermiation or transport; regulation of sex ratios and the provision of nutrients for females or other sperm; facilitating dissociation of eupyrene sperm bundles; serving as a source of nutrients for the eupyrene sperm, the female, or the zygote (Higginson and Pitnick, 2011) But in many animals, there is still insufficient evidence to support their functions. In addition, it was observed that the apyrene sperm carry out a number of unique functions. Apyrene sperm delayed female remating in *Pieris napi*, protecting male reproductive investments and contributing to improved fertilization success in the context of sperm competition.

Predictive pathway for the formation of eupyrene sperm and apyrene sperm

Both nucleated fertile eupyrene sperm and anucleate non-fertile parasperm, also known as apyrene sperm, are produced during spermatogenesis in lepidopteran insects (moths and butterflies). Sex lethal gene (*Sxl*) mutants were used in genetic investigations, which demonstrated the gene's essential role in the correct morphogenesis of apyrene sperm. Similarly, using *Sxl* mutants clearly demonstrates that apyrene sperms are necessary for eupyrene sperm migration from the bursa copulatrix to the spermatheca.

The formation of apyrene sperm occurs after the formation of eupyrene sperm. According the explanation quoted by Sakai *et al*. (2019) the alternative splicing isoforms *Bm-Sxl*-L and *Bm-Sxl*-S are produced during transcription of *Bm-Sxl* gene. 336 amino acid protein, *Bm-Sxl*-L encodes eight exons and an open reading frame (ORF). Contrarily, *Bm-Sxl*-S lacks the second exon and encodes an ORF that is truncated at the N terminus by 46 amino acids. Apyrene sperm meiosis begins prior to the spinning stage, which corresponds to 5- to 6-day-old fifth instar larvae in this strain, and continues throughout the pupal stage, whereas meiosis for eupyrene sperm primarily occurs during the early fifth instar in *Bombyx mori*.

High expression of *Bm-Sxl*-S was observed immediately before the spinning stage (pupal stage), corresponding well with the developmental stage for the transition from eupyrene to apyrene spermatogenesis. Apyrene sperm are considered an apomorphic trait in advanced lepidoptera. Moreover, expression patterns of *Sxl* in five representative lepidopteran species from different families showed that *Bm-Sxl*-S is mainly expressed at the pupal stage (i.e., the apyrene sperm formation period). Therefore, *Bm-Sxl*-S is likely involved in apyrene sperm formation.

Fig. 1 Formation of Eupyrene and apyrene sperm bundle (Sakai *et al*., 2019)

Fig. 2 Formation of Different sperm bundles in *Bombyx mori* (Derived: Sakai *et al*., 2019)

Some research finding says that the formation of Eupyrene sperm bundle depends upon another gene called as *BmPnldc1* (*B. mori poly (A)-specific ribonuclease-like domain-containing 1*) which regulates the development of eupyrene sperm. When a binary transgenic CRISPR/Cas9 system was employed to create a representative line of BmPnldc1 from BmPnldc1- sg12× nos-cas9, although the generation of apyrene sperm was unaffected, the disruptions of BmPnldc1 were accompanied by a decrease in BmPNLDC1 protein expression in BmPnldc1 testes and deformation of eupyrene sperms (Chen *et al*., 2020).

Evidences of interrelationship between apyrene and eupyrene sperm

A test was carried out using the *Bombyx mori, Bm-sxl* gene. Additionally, mutants were created utilizing the TALENS genome editing method (Sakai *et al*., 2019). The findings showed that, firstly, the eupyrene and apyrene spermatozoa had morphological differences that were not visible in spermatogonia but that can be seen in primary spermatocytes and were clearly visible at the meiotic metaphase. Secondly, *Bm-Sxl*-S may control the transition from eupyrene to apyrene spermatogenesis in primary spermatocytes because it is expressed in these cells' capacity to differentiate into either apyrene or eupyrene sperm.

Thirdly, the male-specific sterility of *Sxl* mutants with defective apyrene sperm was reversed by the insertion of functional apyrene sperm, indicating that apyrene sperm is required for fertilisation in *B. mori*. Additionally, it was shown that *Sxl* mutants ejaculated eupyrene sperm into the bursa copulatrix but none of these sperm appeared in the spermatheca. According to these findings, eupyrene sperm must migrate from the bursa copulatrix to the spermatheca with the help of apyrene sperm.

Another experiment was conducted by the Mongue *et al.,* (2019) by taking the monandrous Carolina sphinx moth and the highly polyandrous monarch butterfly based on population genetic analyses evidence to check the adaptive evolution in fertilizing sperm and results revealed that; Initially, instead of directly influencing the outcome of female remating, non-fertilizing sperm may be utilized to postpone it and lessen the likelihood of sperm competition. The majority of the proteins that were discovered were shared by the two cell types (Apyrene and eupyrene sperm cells), the set of proteins were exclusive to eupyrene sperm, and finally the smallest set of proteins that were only present in apyrene sperm and by delaying female remating, apyrene sperm may play a passive role in lowering the risk of competition.

Conclusion

The physiology of reproduction in lepidopteran insects depends on sperm cell dimorphism in addition to sperm structure. Both of these two sperm cells, eupyrene and

apyrene work together in the reproductive system. Although apyrene sperms do not have the role of fertilizing, they are necessary for the long-term fertilisation of eupyrene sperm, which is why they have an impact on the mobility and survival of eupyrene sperm.

Acknowledgement

References

Beatty, R. A and Burgoyne, P. S. 1971. Size classes of the head and flagellum of *Drosophila* spermatozoa. *Cytogenet. Genome Res.* **10**: 177-189.

Bouix, G. 1963. Sur la speratogenese des *Carabus*, modalite et frequence de la spermiogenese atypique. *C. r. hebd. séances Acad.* **256:** 2698–2701.

Baccetti, B., Burrini, A.G. and Falchetti, E. 1991. Spermatozoa and relationships in Palaeognath birds. *Biol. Cell.* **71:** 209-216.

Chawanji, A. S., Hodgson, A. N. and Villet, M. H. 2005. Sperm morphology in four species of African platypleurine cicadas (Hemiptera: Cicadomorpha: Cicadidae). *Tissue Cell* **37:** 257-267.

Chen, S., Liu, Y., Yang, X., Liu, Z., Luo, X., Xu, J. and Huang, Y. 2020. Dysfunction of dimorphic sperm impairs male fertility in the silkworm. *Cell Discov.* **6:** 1-15.

Dallai, R., Gottardo, M and Beutel, R. G. 2016. Structure and evolution of insect sperm: new interpretations in the age of phylogenomics. *Annu. Rev. Entomol*, **61:** 1-23.

Higginson, D. M and Pitnick, S. 2011. Evolution of intra-ejaculate sperm interactions: do sperm cooperate?. *Biol.* **86:** 249-270.

Jamieson, B. G., Erseus, C., and Ferraguti, M. 1987. Parsimony analysis of the phylogeny of some Oligochaeta (Annelida) using spermatozoal ultrastructure. *Cladistics*, **3:**145-155.

Jamieson, B.G.M., Dallai, R. and Afzelius, B.A. 1999. *Insects: Their Spermatozoa and Phylogeny*. Science Publishers, U.K. **49:** 555.

Katsuno, S., 1977. Studies on eupyrene and apyrene spermatozoa in the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae): I. The intratesticular behaviour of the spermatozoa at various stages from the 5th-instar to the adult. *Appl. Entomol. Zool.,* **12:** 142-153.

Lee, P. E. and Wilkes, A. 1965. Polymorphic spermatozoa in the hymenopterous

wasp Dahlbominus. *sci.*, **147:** 1445-1446.

Mongue, A. J., Hansen, M. E., Gu, L., Sorenson, C. E and Walters, J. R. 2019. Nonfertilizing sperm in Lepidoptera show little evidence for recurrent positive selection. *Mol. Ecol.* **28:** 2517-2530.

Meves, F. 1903. U¨ ber oligopyrene und apyrene Spermien und ihre Entstehung nach Beobachtungen an Paludina und Pygaera. Arch. Mikrosk. Anat. Entwicklungsmech. 61, 1–84 (with 8 plates).

Presgraves, D. C., Baker, R. H. and Wilkinson, G. S. 1999. Coevolution of sperm and female reproductive tract morphology in stalk–eyed flies. *Proc. R. Soc. B: Biol. Sci.* **266:** 1041-1047.

Sakai, H., Oshima, H., Yuri, K., Gotoh, H., Daimon, T., Yaginuma, T and Niimi, T. 2019. Dimorphic sperm formation by Sex-lethal. *Proc. Natl. Acad. Sci. U.S.A* **116:** 10412-10417.

Sasakawa, K. and Toki, W. 2008. A new record, sperm bundle morphology and preliminary data on the breeding type of the ground beetle *Jujiroa estriata*

Sasakawa (Coleoptera: Carabidae: Platynini). *Entomol. Sci.,* **11:** 415–417.

Sonnenschein, M. and Hauser, C. L., 1990. Presence of only eupyrene spermatozoa in adult males of the genus *Micropterix hübner* and its phylogenetic significance (Lepidoptera: Zeugloptera, Micropterigidae). *Int. J. Insect Morphol. Embryol.* **19:** 269–276.

Schrader, F. 1960. Cytological and evolutionary implications of aberrant chromosome behavior in the harlequin lobe of some Pentatomidae (Heteroptera). *Chromosoma,* **11:** 103–128.

Swallow, J. G. and Wilkinson, G. S. 2002. The long and short of sperm polymorphisms in insects. *Biol.* **77:** 153-182.

Takami, Y. and Sota, T. 2007. Sperm competition promotes diversity of sperm bundles in Ohomopterus ground beetles. *Sci.* **94:** 543-550.

Toyama, K. 1894. On the spermatogenesis of the silkworm. Bull. Coll. Agr. Tokyo Imp. Univ. **2:** 125–157.