Spermatogenesis chronology in *Achroia grisella* Fabricius (Lepidoptera, Pyralidae) comparing eupyrene and apyrene spermiogenesis

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Abstract
*Achroia grisella* exhibit dichotomous spermatogenesis producing two types of sperm, one is eupyrene that possesses nucleus, and the other is apyrene that lacks it. Transmission electron microscopy of spermatogenesis morphology is described considering sperm type which will appear at some point during insect development, and differences that mark the two types of sperm formation. The differences between them are only really visible during spermiogenesis even though they were determined before meiosis. Both forms were seen in the larval stage, but there is a little difference in the time of their appearance. Eupyrene cysts were seen from the 8th larval stage, whereas apyrene were only found after the 10th stage. In early insect development stages, eupyrene cysts predominate, but as the insect ages, they are overtaken by apyrene. Although some eupyrene cysts are still present in young adult testis, the majority are apyrene. As eupyrene sperm is formed, bundles migrate to the seminal vesicle therefore in early pupae eupyrene sperm are already present there whereas apyrene cells arrive later. The exact mechanism and determining factors responsible for apyrene sperm origin are still to be clarified. The probable causes of apyrene sperm appearance are discussed as well as its role in the sperm competition.

Keywords
Morphology; ultrastructure; spermatogenic phases; sperm dichotomy; temporal differences

Introduction
Spermatogenic polymorphism is not a rare phenomenon in arthropods where it is found in myriapods (Jamieson, 1987), spiders (Rosati et al., 1970), and insects (Sivinski, 1980). In insects the variations may be morphological or just in gamete size (Jamieson, 1987; Jamieson et al., 1999).

In Lepidoptera, except for species of the genus *Micropterix* (Micropterigidae), which is considered a basal group, all species studied up to now present dichotomic spermatogenesis (Medeiros, 1996), which gives rise to eupyrene spermatozoa, with genetic...
material located in the head, and apyrene spermatozoa which are devoid of nucleus or present an acrosome consisting of only a dense cap, therefore without genetic material. The morphology of lepidopterous spermatozoa (França and Báo, 2000; Friedländer and Gitay, 1972; Friedländer and Miesel, 1977; Kubo Irie et al., 1998; Lai-Fook, 1982; Leviatan and Friedländer, 1979; Mancini and Dolder, 2001; Medeiros and Silveira, 1996; Phillips, 1971; Rieman, 1970), cytochemical components of spermatic cells (França and Báo, 2000; Friedländer, 1976; Friedländer and Gershon, 1978), and the modifications that these cells suffer in the male genital tract, after leaving the testis and inside the female genital tract (Garvey et al., 2000; Kubo Irie et al., 1998; Lai-Fook, 1982; Phillips, 1971; Rieman, 1970; Riemann and Thorson, 1971), are relatively well-known. However, the determining mechanisms of these variations and their biological meaning remain largely obscure. Spermatogenesis in holometabolous insects, as for Lepidoptera, is initiated at the end of the larval phase and generally concluded during pupation or soon after adult emergence. Generally, in adult holometabolous insects, the spermatozoa are stored in seminal vesicles and the testes are in regressive phase (Wigglesworth, 1965).

Cystoblasts (corresponding to primary spermatogonia) and cystocytes (corresponding to secondary spermatogonia) that produce eupyrene and apyrene spermatozoa appear to be similar in the early stages of spermatogenesis, but several reports have shown that apyrene meiotic division begins in the last larval stage or at the beginning of pupation (Friedländer and Miesel, 1977; Friedländer and Benz, 1981; Holt and North, 1970; Katsuno, 1997; Kawanura and Sahara, 2002; Leviatan and Friedländer, 1979; Riemann and Thorson, 1971), although there is some variation between species. So in most species, the two kinds of spermatozoa appear at different times in the course of development.

Although the factors that determine spermatic dichotomy in Lepidoptera are not completely understood, it has been shown, that one characteristic of apyrene spermatogenesis is its late occurrence. Larval and pupal stages are marked by hormonal changes, specifically a decrease in juvenile hormone and an increase of ecdysone titers during larva to pupa transition. Therefore, one possibility is that apyrene spermatozoa appearance is under hormonal control (Silberglied et al., 1984). In the present study, the spermatogenesis in *Achroia grisella* larvae, pupae, and adults was studied in order to determine the time-point where each type of spermatozoon is fate to be eupyrene or apyrene.

**Materials and methods**

Male larvae, pupae and adult *Achroia grisella* were obtained from artificial laboratory breeding. Larval stages were determined by applying Dyar’s law to measurements of larval cephalic width.

Testis from the 8th, 9th, and 10th larval stages, pupae, and new emerged adults were fixed overnight in Karnovsky fixative (2% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4, containing 4% paraformaldehyde). After fixation, the material was rinsed in buffer