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# The bipotentiality of lepidopteran primary spermatocytes questioned

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In *Pieris brassicae* L. first signs of eupyrene spermatogenesis are found in one-day-old fifth instar larvae, four days before the first apyrene meiotic divisions. Unlike reports from most other species, both types of spermatogenesis continue side-by-side throughout adult life. No spatial separation in the testes of the two types of spermatocysts could be found. It must, therefore, be concluded that the seemingly bipotential, morphologically undifferentiated, primary spermatocytes are in fact two different types of cells.

## INTRODUCTION

Since the discovery of the "atypical" sperm of the snail, *Paludina vivipara* (V. SIEBOLD, 1836), numerous studies have been carried out on sperm polymorphism in such different groups as echinoderms, molluscs and insects. Even human semen were found to consist normally of different types of sperm (MACLEOD, 1970). Most of the "atypical" sperm are hyperpyrene (FAIN-MAUREL, 1966), e.g. containing an excess of the normal haploid chromosomal mass, while others are oligopyrene of apyrene, respectively, with reduced chromosomal numbers in the nucleus or completely anucleated. The term "atypical" is in so far inaccurate as that in many cases these sperm are regularly produced in large numbers.

In Lepidoptera two types of spermatozoa are found: the normal eupyrene (nucleate) and the "atypical" apyrene (anucleate). Both are produced in more or less equal numbers in different cysts. This dichotomous spermatogenesis was found in primitive species as well as in the higher Lepidoptera, but only eupyrene spermatozoa were found in Trichoptera, the most closely related order (FRIEDLÄNDER, 1983).

In all the lepidopteran species studied eupyrene spermatogenesis was found to preceed apyrene spermatogenesis. In fact an obvious switch over from eupyrene to apyrene spermatogenesis usually takes place in the pupa. The presence of an apyrene spermatogenesis inducing factor (ASIF) responsible for the change in commitment from eupyrene to apyrene spermatogenesis was postulated by FRIEDLÄNDER & BENZ (1981).

Although, ultrastructural studies of spermatozoa in the testes of *Pieris brassicae* L. have been carried out (ZYLBERBERG, 1963, 1965, 1969), no detailed account on the exact timing of the different stages of spermatogenesis was given. A recent analysis of adult *P. brassicae* showed that in this species both types of spermatogenesis seem to continue side-by-side. Similar observations have also been reported for *Calpodes ethlius* (LAI-FOOK, 1982). Since these observations would seem to contradict the ASIF hypothesis, more detailed investigations were carried out.

## MATERIALS AND METHODS

A culture of the white cabbage butterfly, *P. brassicae,* is being held at the Entomological Institute of the SFIT Zurich since more than ten years under variable conditions of light and humidity, and temperature ranges of 20–25 °C. In the course of these investigations *P. brassicae* from a culture of the Glasshouse Crops Research Institute in Sussex, England were also analysed.

Squash preparations were made of the testes of accurately aged larvae, pupae and adults. The testes were either fixed and stained in lactic acid-orcein (CAMENZIND, 1966), or left unfixed in a drop of saline. The latter method proved more effective for analysing elongating spermatids. The preparations were then observed with phase contrast under a light microscope.

## RESULTS

In *P. brassicae,* as in other Lepidoptera, cysts containing eupyrene or apyrene spermatocytes, spermatids, or developing spermatozoa are easily distinguished on the basis of shape, size, and order of the nuclei. The earliest stage at which the two kinds of cysts could be differentiated was the spermatocyte undergoing meiotic division. Eupyrene meiotic divisions are regular with conspicuous metaphase plates, distinct anaphase chromosomes moving towards the poles of the spindle, and well separated chromosomal masses at telophase. The spermatocytes elongate forming pearshaped spermatids with spherical nuclei. Further elongation of the flagella and of the nuclei follows. Apyrene metaphases are characterized by clumps of condensed chomosomes that never form a real equatorial plate. During anaphase the chromosomal masses split irregularly forming bridges. These bridges are especially conspicuous in late anaphase and telophase and were used as a sure indication of apyrene meiosis. Later on, the bridges break-up leaving chromosomal fragments lying outside the newly forming cells. In the apyrene spermatids only the flagella elongate. The nuclei remain as irregular clumps (micronuclei) that travel down the length of the tails and are finally eliminated from the cells.

The normal timetable of eupyrene and apyrene spermatogenesis in *P. brassicae* and its relationship to the different stages of development is shown in Fig. 1. The testes of one-day-old fifth instar larvae contain first signs of eupyrene meiotic divisions. Early spermatids with spherical nuclei appear the next day, the third day from moult to the fifth instar. Elongation of the spermatid nucleus begins early on the sixth day before the two testes fuse. The earliest signs of apyrene meta-, ana-, and telophases are also found at about this time, four days later than the first eupyrene metaphases. The resulting apyrene spermatids with micronuclei appear the next day in the fresh pupa. Elongating apyrene spermatids with the nuclei distributed along the flagella are first found in one-day-old pupae. All stages of eupyrene and apyrene spermatogenesis continue in the adult. The oldest males examined (eight days old) contained cysts in all the different stages of spermatogenesis listed above. Only very few spermatocysts were found, though, containing either eupyrene or apyrene meiotic divisions. Since adult males in culture live for around six to ten days, it can be said that the testes of adult *P. bras-*

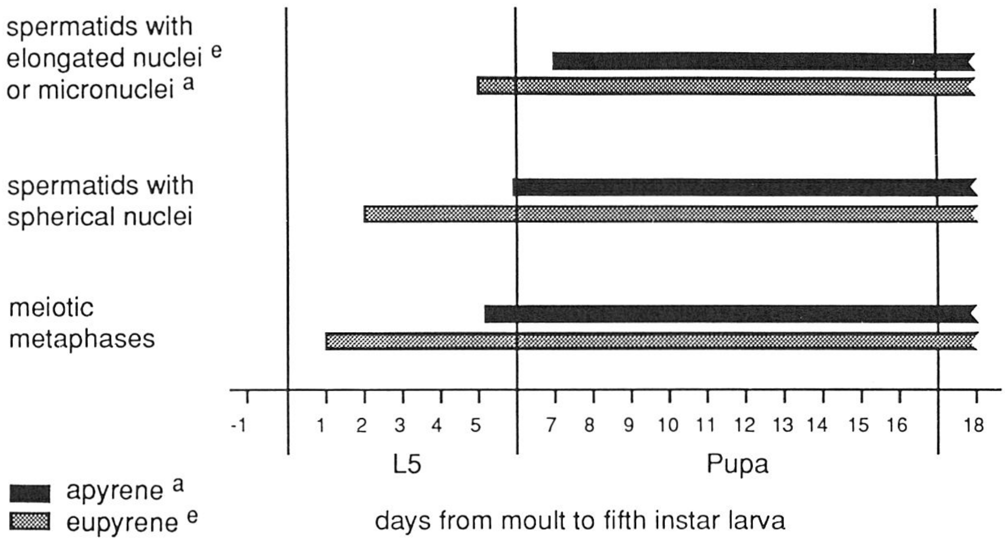


Fig. 1. Timetable of eupyrene and apyrene spermatogenesis and its relationship to the different stages of development of *Pieris brassicae.*

*sicae* males continue to produce both types of sperm side-by-side for as long as they live.

No spatial separation in the testes of the two types of spermatocysts could be found. Cysts undergoing apyrene and eupyrene spermatogenesis were found intermingled. A separation of the two types of spermatocysts could have indicated exposure to different environmental conditions and could have explained how two different types of spermatogenesis coexist. The only spatial separation found, though, was that immature cysts (earlier stages of spermatogenesis) are arranged laterally around the wide edge of the testicular follicles, while the more mature cysts with spermatids and developing spermatozoa are found towards the narrow edge leading to the vasa deferentia.

## DISCUSSION

In *P. brassicae* eupyrene spermatogenesis is first detected in one-day-old fifth instar larvae, four days before apyrene spermatogenesis. ZYLBERBERG (1963) reports for the same species that first signs of apyrene meiotic divisions were observed in one-week-old pupae, although no detailed time-table for the different stages of spermatogenesis is otherwise given. This discrepancy cannot be explained, but it seems important to emphasize that the observations reported here were made in a laboratory strain cultured at our institute, as well as in individuals of a culture from Sussex, England.

Unlike reports from most other species (Tab. 1), except *Calpodes ethlius* (LAI-FOOK, 1982), the dichotomous spermatogenesis of *P. brassicae* continues in the pupa and throughout adult life. Not only are cysts found with signs of eupyrene and apyrene spermiogenesis, but both types of meiotic divisions as well. No actual switch over from eupyrenen to apyrene spermatogenesis occurs. The start of apyrene spermatogenesis is simply delayed by a few days, but both types then continue for at least 20 days.

FRIEDLÄNDER & BENZ (1981) postulated the presence of an apyrene spermatogenesis inducing factor (ASIF) that becomes active towards pupation and is

Tab. 1. Timetable of eupyrene and apyrene spermatocyte meiosis in different lepidopteran species.

| Species | Meiotic Divisions eupyrene | apyrene | Reference |
|---|---|---|---|
| *Actias selene* Hb. | early L5-? | pupa-? | Friedländer *et al.* 1981 |
| *Bombyx mori* L. | early L5-early pupa | only in pupa | Sado 1963 |
| *Calpodes ethlius* Stoll | early L4-adult | L5-adult | Lai-Fook 1982 |
| *Cydia pomonella* L. | early L5-pupa | late L5-adult | Friedländer & Benz 1981 |
| *Ectomyelois ceratoniae* Zell. | late L4-early pupa | mid L5-adult | Leviatan & Friedländer 1979 |
| *Ephestia kühniella* Zell. | only in larva* | ? | Riemann & Thorson 1971 |
| *Heliothis virescens* F. | L4-pupa | pupa | LaChance & Olstad 1988 |

* not specified in which larval stage(s).

responsible for the change in commitment from eupyrene to apyrene spermatogenesis in bipotential primary spermatocytes. Conclusions are based on the fact that in the codling moth, *Cydia pomonella,* as well as in the carob moth, *Ectomyelois ceratoniae,* eupyrene spermatogenesis preceeds apyrene, and an obvious switch over from one form of spermatogenesis to the other occurs. The short period in which both types of spermatogenesis take place is explained by the fact that the prophase of the apyrene spermatocyte is much shorter than that of the eupyrene spermatocyte (FRIEDLÄNDER & HAUSCHTEK-JUNGEN, 1986). Thus, in the late last larval instar and in young pupae both types of metaphases can be found. Cultured testes of young fifth instar larvae of *C. pomonella* (explanted before the fourth day) in a medium containing mammalian serum, but neither hemolymph nor insect hormones, showed no signs of apyrene spermatogenesis. Only eupyrene spermatids developed, even after 12 days of culture (FRIEDLÄNDER & BENZ, 1981). Since the culture testes from a later stage produced eupyrene and apyrene spermatocytes and spermatids, it was concluded that the ASIF was not yet active at the earlier stage. But since no quantitative analyses were carried out, it could well be that only the predetermined eupyrene spermatocytes had developed in the testes cultured from young fifth instar larvae.

In *P. brassicae* both eupyrene and apyrene spermatogenesis continues in the adult. Thus, although eupyrene spermatogenesis preceeds apyrene by four days both types of meiotic divisions continue side-by-side for at least 20 days until the male dies. Since the two types of spermatocytes are not separated in the testes, but are found intermingled in the same follicle, these observations can hardly be the result of exposure to different environmental conditions.

An alternative explanation concerning the production of two different types of spermatozoa seems necessary. It could well be that the seemingly bipotential, morphologically undifferentiated primary spermatocytes are in fact two different types of cells predetermined at an earlier ontogenetic stage, and that the ASIF is responsible for the onset of apyrene spermatogenesis, not for a switch over from eupyrene to apyrene. This would explain how both types of spermatogenesis can continue side-by-side in the testes of *P. brassicae* and *Calpodes ethlius* pupae and adult males. Biochemical analyses of primary spermatocytes might possibly reveal some interesting information that could help separate the two groups at an earlier stage.

## ZUSAMMENFASSUNG

Lepidopteren produzieren zwei Spermientypen: eupyrene (kernhaltige) und apyrene (kernlose). Die eupyrene Spermatogenese beginnt vor der apyrenen. In der einzelnen Spermatocyste erfolgt entweder eupyrene oder apyrene Spermatogenese (nie beides), doch sind die beiden Spermatocystentypen in den Hodenfollikeln räumlich nicht getrennt, sondern durchmischt. In *Pieris brassicae* L. können die ersten Zeichen beginnender eupyrener Spermatogenese in einen Tag alten letzten Larvenstadien beobachtet werden, vier Tage bevor die ersten apyrenen Meiosen gefunden werden. Im Gegensatz zu den Befunden bei den meisten anderen untersuchten Schmetterlingsarten, bei denen die eupyrene Spermatogenese vor dem Adultstadium beendet ist (Tab. 1), laufen bei *P. brassicae* beide Spermatogenesetypen während des ganzen Adultlebens weiter. Aus den Tatsachen wird geschlossen, dass die morphologisch undifferenzierten primären Spermatozyten nur scheinbar bipotent sind, sondern zwei verschiedene Zelltypen darstellen.

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