Hereditas 119: 31–38 (1993)

Meiosis in *Haematopinus suis* and *Menacanthus stramineus* (Phthiraptera, Insecta)

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TOMBESI, M. L. and PAPESCHI, A. G. 1983. Meiosis in *Haematopinus suis* and *Menacanthus stramineus* (Phthiraptera, Insecta). — *Hereditas 119*: 31–38. Lund, Sweden. ISSN 0018-0661. Received October 6, 1992. Accepted January 19, 1993

Haematopinus suis (Anoplura) and *Menacanthus stramineus* (Mallophaga) have been cytogenetically analyzed. Both species have 2n = 10, holokinetic chromosomes, and achiasmatic male meiosis. Bivalents orientate with their long axis perpendicular to the spindle fibers at metaphase I, and first anaphase is reductional. As in other species of Phthiraptera, male gametogenesis follows a particular pattern: each cell entering meiosis results in a cyst of 64 (in *H. suis*) and 32 (in *M. stramineus*) spermatozoa and 64/32 non-functional cells (=pycnotic nuclei).

The results are compared with those previously reported for Phthiraptera, and a new terminology for the different stages of male gametogenesis is proposed. The low chromosome number together with the achiasmatic nature of male meiosis and the mitotic divisions that follow meiosis may restrict the potential for genetic variability. This might be related to the high host specificity of these parasites.

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The first cytogenetic studies in the order Phthiraptera were done at the beginning of the century by FOOT (1919), DONCASTER and CANNON (1920) and CANNON (1922). It became evident that chromosome characteristics and male gametogenesis differed from those found in most insects. At present, the chromosome number of very few species is known and although the chromosome behaviour has been described in most of them, many points are still controversial.

Mallophaga and Anoplura (Phthiraptera) have low diploid chromosome numbers (4 to 24) (Table 1) and holokinetic chromosomes, i.e., without a localized centromere. Spermatogenesis follows a peculiar pattern since each cell entering meiosis divides also mitotically and gives rise to a cyst of 32 or 64 active spermatozoa (according to the species) and 32/64 non-functional cells (Fig. 1) (BAYREUTHER 1955; HINDLE and PONTECORVO 1942; SHARMA and MALIK 1953; SCHOLL 1955). Other interesting features of male gametogenesis are achiasmatic bivalents and the fact that sex chromosomes are apparently missing (BAYREUTHER 1955; CANNON 1922; SHARMA and MALIK 1953; SCHOLL 1955). Spermatozoa of Phthiraptera are also remarkable since they have two axial filaments (DONCASTER and CANNON 1920; CANNON 1922; SHARMA and MALIK 1953).

In order to get a better understanding of gametogenesis in this order, we have studied the spermatogenesis in *Menacanthus stramineus* (Mallophaga) and in *Haematopinus suis* (Anoplura). The results are analyzed together with previous reports in species of the order.

Materials and methods

Specimens of *H. suis* (pig parasite) were collected by hand from 4 pigs. Adults and third stage nymphs were collected from the back and rear (BYNUM and WARD 1978), and first and second instar stages were obtained from the head and ears; to get eggs a bundle of hair was cut. A total number of 247 specimens were analyzed (19 eggs, 57 first instars, 61 second instars, 65 third instars, 43 adult males and 2 adult females).

Specimens of *M. stramineus* (chicken parasite) were collected from 3 chickens. The chicken was put in a plastic bag, leaving only its head outside; 10 cc

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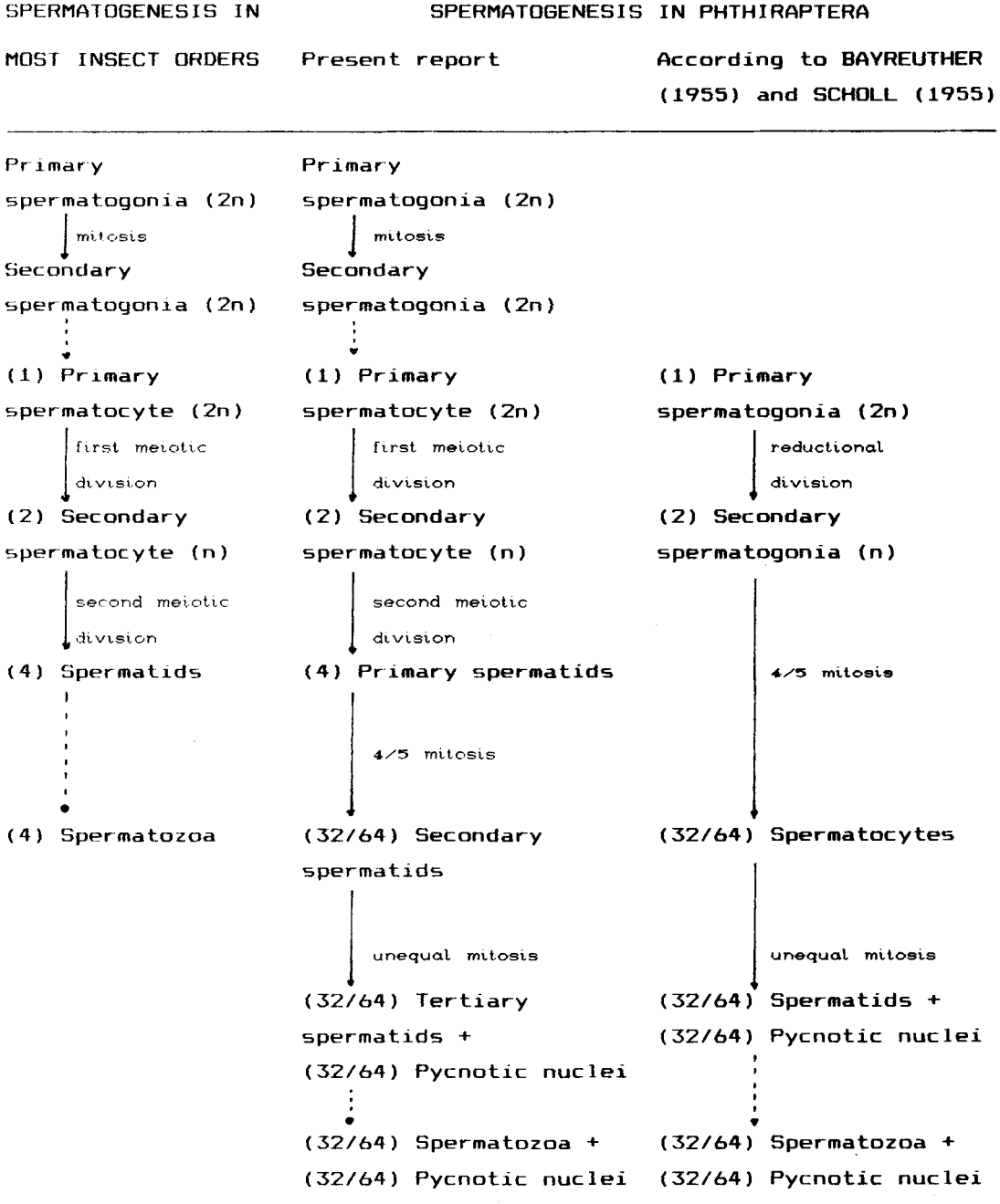

Fig. 1. Comparison of the spermatogenesis in Phthiraptera and in most insect orders. ——→ cell division. - - -→ cell growth or differentiation.

of sulphuric ether was injected into the bag to release the ectoparasites. A total number of 80 specimens were analyzed (1 second instar, 36 third instars, 29 adult males and 14 adult females).

The material was fixed in 3:1 absolute ethanol: glacial acetic acid and then transferred to ethanol 70 % and kept at 4°C. Different developmental stages of *M. stramineus* were classified according to

Table 1. Chromosome numbers in Phthiraptera

Species	2n(♂)	n(♂)	References
Anoplura			
Haematopinus asini	–	9	CANNON 1922
H. consobrinus	–	7	CANNON 1922
H. suis	10	5	BAYREUTHER 1955
	10	5	Present report
Lignognathus tenuirostris	–	6(♀)	RIES 1932
Pediculus capitis	12	6	DONCASTER and CANNON 1920
	–	6	SHARMA and MALIK 1953
Pediculus corporis	12	6	DONCASTER and CANNON 1920
	12	6	HINDLE and PONTECORVO 1942
	–	6	SHARMA and MALIK 1953
Pediculus vestimenti	10	5	FOOT 1919 (in CANNON 1922)
Mallophaga			
Goniodes stylifer	24	12	PERROT 1934
Gyropus ovalis	4	2	SCHOLL 1955
Lipeurus baculus	11–12(♀)	–	RIES 1932
Menacanthus stramineus	10	5	Present report

the setae of the gular plate (CICCHINO, personal communication).

The material was dissected in a drop of acetic acid 45 % and once the gonad had been isolated, it was transferred to a drop of 2 % iron propionic haematoxylin. Slides were made by squash method. The chorions of the eggs were removed, and the slides were made with all their contents.

Results

Haematopinus suis

The diploid chromosome number in this species is 10 in both sexes (Fig. 2a, b). In oogonial and spermatogonial prophase it is evident that chromosomes lack a primary constriction, and that one pair is slightly larger (Fig. 2b). At mitotic metaphase chromosomes orientate with their long axis perpendicular to the spindle fibers, and at anaphase chromatids migrate parallel to each other, although sometimes both telomeres seem to precede migration.

During spermatogenesis, cells at leptotene and zygotene are not recognized. At pachytene (Fig. 2c) five bivalents and a conspicuous nucleolus are observed. From this stage up to prometaphase I, bivalents gradually condense without modifying their morphology (Fig. 2d–h) while the nucleolus disappears; no typical diplotene or diakinesis stages are observed, and chiasmata are completely absent. Although chromatin connections between homologues can sometimes be detected, they are clearly nonchiasmatic in origin (Fig. 2i). Cells at metaphase I are difficult to analyze because of the high degree of condensation of the chromatin and the close proximity of bivalents at the equatorial plate. However, some cells at metaphase I show that bivalents orientate with their long axis perpendicular to the spindle fibers, four of them arranged in a circle while the fifth lies a little apart (Fig. 2j). The few cells observed at anaphase I reveal that chromosomes migrate parallel to each other and perpendicular to the spindle fibers (Fig. 2j). Telophase I (Fig. 2k) is followed by the second meiotic division (Fig. 2l) without an apparent interkinetic stage. Afterwards, four typically mitotic divisions take place (Fig. 2m), giving rise to a cyst of 64 secondary spermatids (see Discussion). These spermatids go through a last mitotic division which is unequal with respect to the cytoplasm, and results in a cyst of 64 tertiary spermatids (which will finally develop into active sperm) and 64 pycnotic nuclei (which will degenerate) (Fig. 3a, b). The latter are very frequently observed among the tails of active spermatozoa (Fig. 3b). Tertiary spermatids as well as pycnotic nuclei show two centriole adjuncts, of which two axial filaments originate in the former (Fig. 3c–e).

Individuals in different developmental stages (from egg to adult) show that spermatogenesis takes place from first instar up to the adult stage, but with variable frequency. Embryos have only diploid dividing cells, which can be ascribed to the somatic as well as to the germ line. At first instar, testes are small, and dividing spermatogonia ($2n = 10$) as well as primary spermatocytes at prophase I are found. At the following developmen-

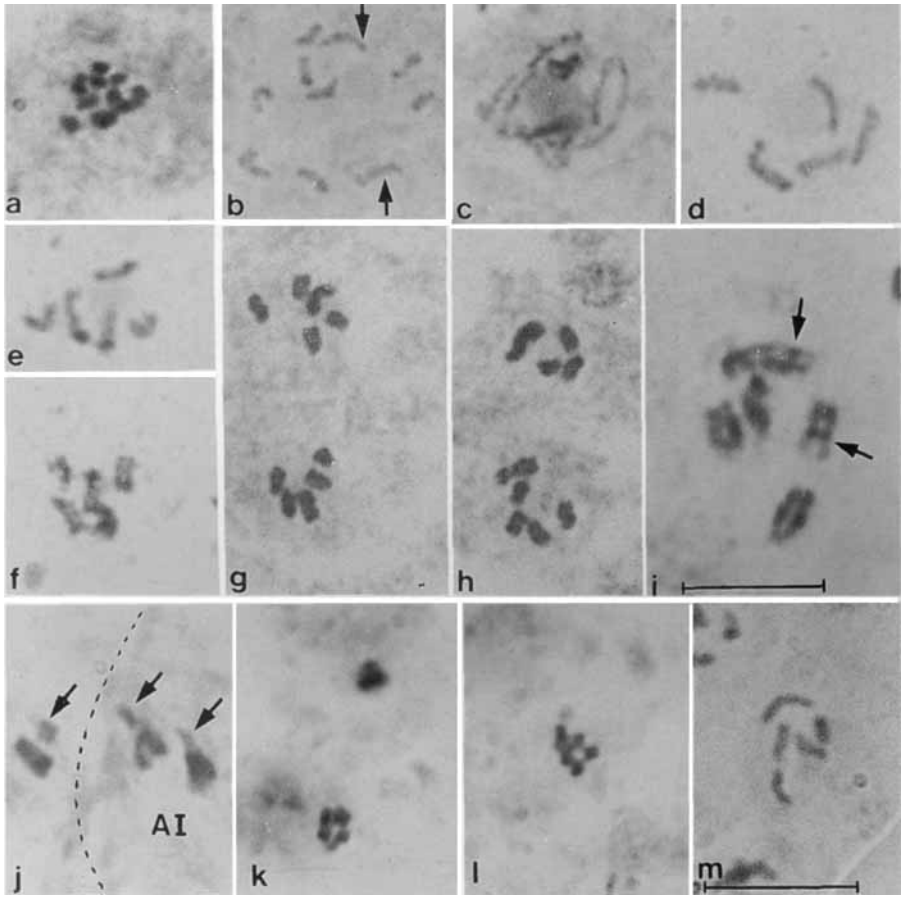

Fig. 2a–m. *Haematopinus suis* (2n = 10). **a** Oogonial prometaphase. **b** Spermatogonial prophase. **c** Pachytene. **d–h** Prophase I; bivalents with different degrees of condensation. **i** Detail of a prophase I cell; arrows point to non-chiasmatic associations between homologues (Bar = 5 μm). **j** Metaphase I and Anaphase I; arrows point to the bivalent (at metaphase I) and the chromosomes (at anaphase I) slightly apart. **k** Telophase I nuclei. **l** Metaphase II. **m** Haploid mitotic prophase (secondary spermatid). Bar in (a–h) and (j–m) represents 10 μm.

tal stage, testes are well developed and with active division (abundant primary and secondary spermatocytes, cysts with dividing secondary spermatids); some individuals also showed cysts of tertiary spermatids and pycnotic nuclei. Finally, third instars and adults are characterized by the presence of few primary spermatocytes and abundant cysts of secondary and tertiary spermatids, pycnotic nuclei, and bundles of spermatozoa.

Menacanthus stramineus

The diploid chromosome number is 10 in both sexes (Fig. 4a). Prophase and prometaphase chromosomes show clearly the absence of a primary constriction. All specimens showed very few dividing cells, spermatids, and spermatozoa. As in *H. suis* no early meiotic stages are detected and the five bivalents show no chiasmata (Fig. 4b–d). As described for *H. suis*, at metaphase I, bivalents arrange themselves in a circle and one of them lies a little apart (Fig. 4e). At anaphase I (Fig. 4f) this configuration persists and chromosomes migrate with their long axis perpendicular to the spindle, a fact that evidences that bivalents were equatorially orientated at metaphase I. After telophase I (Fig. 4g) the second meiotic division takes place. The resulting haploid cells (primary spermatids) divide mitotically (Fig.

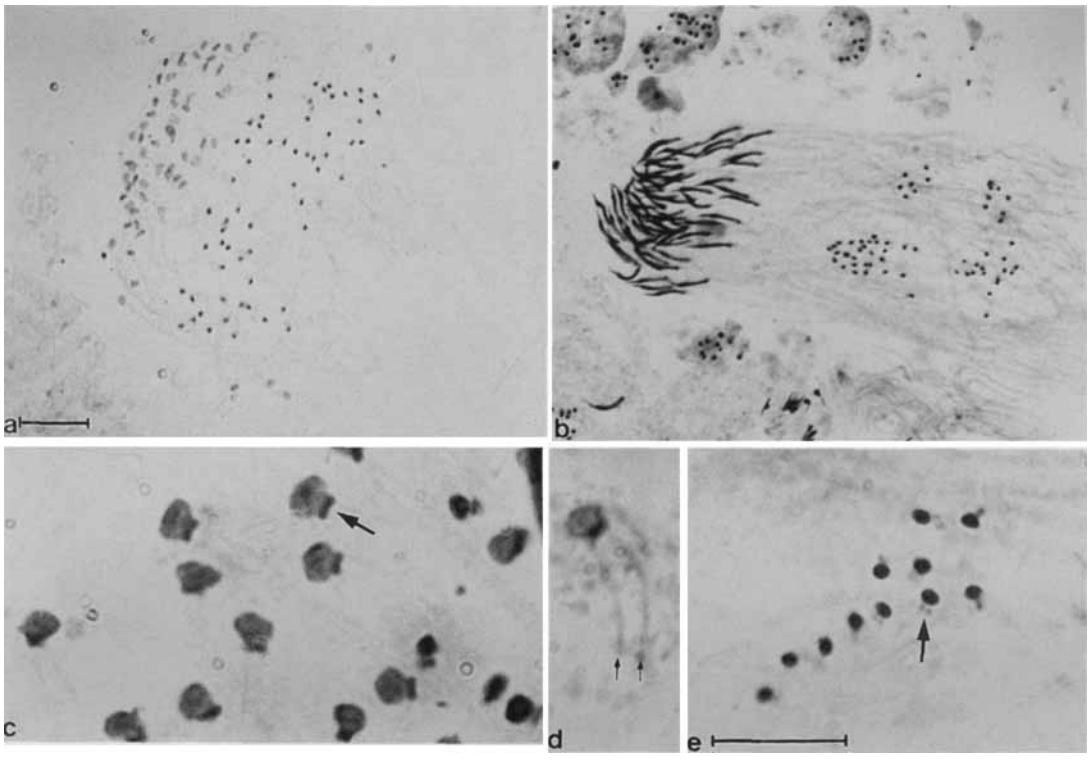

Fig. 3a–e. *Haematopinus suis.* **a** Cyst of 64 tertiary spermatids and 64 pycnotic nuclei. **b** Cyst of 64 spermatozoa and 64 pycnotic nuclei. **c** Tertiary spermatids with two centriolar adjuncts (arrow). **d** Detail of tertiary spermatid; arrows point to the two flagella. **e** Pycnotic nuclei with two centriolar adjuncts (arrow). Bar in (a–b) represents 20 μm and in (c–e), 10 μm.

4h) and give rise to cysts of 32 secondary spermatids. A last unequal division produces 32 tertiary spermatids and 32 pycnotic nuclei.

Discussion

The holokinetic nature of the chromosomes of the Phthiraptera, evidenced by the absence of a primary constriction and the behaviour of chromosomes at mitotic anaphase, has been recognized in almost all cytogenetic studies of the order. Our observations in *Haematopinus suis* (Anoplura) and *Menacanthus stramineus* (Mallophaga) agree completely with the features of holokinetic systems. Experimental evidence has been obtained in *H. suis* by BAYREUTHER (1955), who after irradiating first and second instar individuals observed a regular behaviour of "chromosome fragments" during cell division.

Spermatogenesis in Phthiraptera differs from most insect orders. Even though this fact has been pointed out by all researchers in the group, it has been interpreted in different ways; in addition, controversy about the terminology employed for the different stages of gametogenesis and about the type of meiosis (pre or post-reductional) has also arisen. First reports by DONCASTER and CANNON (1920), CANNON (1922), PERROT (1934), and SHARMA and MALIK (1953) described the presence of "diploid" and "haploid spermatogonia". Each "haploid spermatogonium" divides mitotically and gives rise to a cyst of 64 cells. After a growth period, these 64 "spermatocytes" go through only one meiotic division, which is unequal with respect to the cytoplasm. The final result is a cyst with 64 spermatids, that will differentiate into active spermatozoa, and 64 "polar body-like cells", that will degenerate (DONCASTER and CANNON 1920).

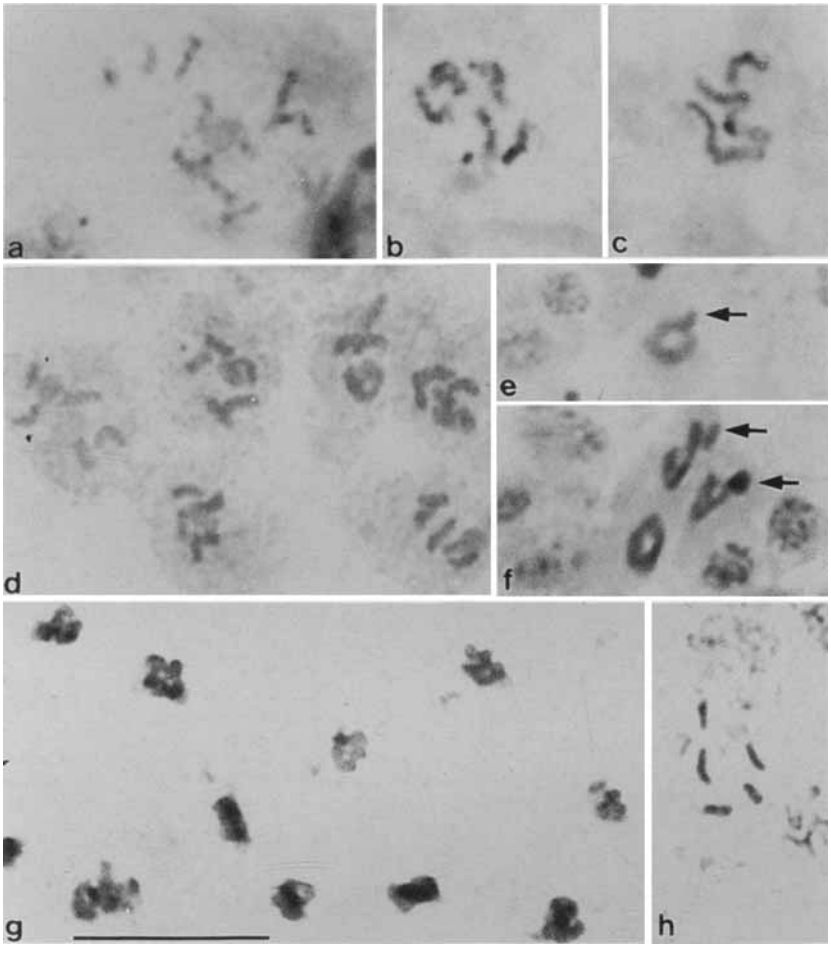

Fig. 4a–h. *Menacanthus stramineus* (2n = 10). **a** Spermatogonial prophase. **b–d** Prophase I; bivalents with different degrees of condensation. **e** Metaphase I. **f** Metaphase I and anaphase I; arrows point to the chromosomes that are slightly apart. **g** Telophase I. **h** Haploid mitotic prophase (secondary spermatid). Bar = 10 μm.

A different description was given by BAYREUTHER (1955) and SCHOLL (1955) (Fig. 1). According to them each "primary spermatogonium" (2n) experiences a "reductional division" which results in two "secondary spermatogonia" (n). These cells divide then mitotically four or five times (according to the species) and originate a cyst of 32/64 cells. As a result of the "maturation division", which is unequal for the cytoplasm, each cyst has 32/64 spermatids and 32/64 non-functional cells ("pyknotische Knospenzellen" or "pyknotische Körper").

The results in *Haematopinus suis* and *Menacanthus stramineus* here reported agree in general with this last description, i.e., each cell entering meiosis experiences several mitoses in addition to the two meiotic divisions (Fig. 1). We think that it is advisable to refer to the stages of the spermatogenesis in Phthiraptera using the terms employed in the spermatogenesis of most insects. In lice, the four spermatids resulting from meiosis of one primary spermatocyte, here referred to as primary spermatids, divide mitotically to give rise to a cyst of 32/64 secondary spermatids (Fig. 1). As already described, the result of a last unequal mitosis is a cyst of 32/64 tertiary spermatids (which will differentiate into functional sperm) and 32/64 non-functional cells (pycnotic nuclei). This last unequal

mitotic division may increase certain cytoplasmic components, and resembles the general course of oogenesis, in which three of the four meiotic products (polar-bodies) are non-functional. The presence of two centriolar adjuncts both in tertiary spermatids and pycnotic nuclei is also remarkable. Biflagellate spermatozoa have been also described in *Pediculus capitis* and *P. corporis* by DONCASTER and CANNON (1920), and in *Haematopinus asini* by CANNON (1922).

Both in *Pediculus capitis* and *P. corporis* DONCASTER and CANNON (1920) described the presence of "spermatogonia" (=primary spermatocytes) in the first instar individuals, while in the second instars the number of cells which developed to "spermatocytes" (primary spermatids) increased. Later, HINDLE and PONTECORVO (1942) reported that in *P. corporis* meiosis would begin in the embryo before hatching and continue at least during the three nymphal stages. In *H. suis* spermatogenesis starts at the first instar, and is very active at the second and third nymphal stages. At the third instar secondary spermatids differentiate, a process that is at its maximum in the adult stage.

Previous reports on Phthiraptera did not describe the presence of sex chromosomes. We did not observe any heteromorphic pair either in males or females; one may, however, note that one bivalent behaves slightly differently at metaphase I.

Another interesting characteristic of male meiosis is the absence of chiasmata, a fact already suggested in other species of the order (BAYREUTHER 1955; SCHOLL 1955). Bivalents show both homologous chromosomes completely paired from pachytene up to metaphase I, with no signs of chiasmata. They orientate equatorially at metaphase I, i.e., with their long axis perpendicular to the spindle fibers. Consequently, homologous chromosomes will segregate at anaphase I and sister chromatids at anaphase II. Thus, male meiosis of these species appears to be of pre-reductional type. Quite similar kinds of achiasmatic, pre-reductional type of meiosis have been observed to occur in other insect groups with holokinetic chromosomes: in some species of Lepidoptera (SUOMALAINEN et al. 1973), Trichoptera (SUOMALAINEN 1966), Heteroptera (NOKKALA and NOKKALA 1986), and Homoptera (BLACKMAN 1976), and also in *Elays setosa* (Acorina) (KEYL 1957) and some species of scorpions (Buthidae, Scorpionida) (SHANAHAN 1989). BAYREUTHER (1955), considering the behaviour of a supernumerary chromosome in one specimen of *H. suis*, suggested that the first meiotic division should be equational and that the reductional division should take place either in the following haploid mitotic division or at the last unequal mitotic division. The meiotic behaviour of supernumerary chromosomes is, however, very variable, both in monocentric and holokinetic systems; as an example, in Heteroptera, a group of insects with holokinetic chromosomes and chiasmatic meiosis of the pre-reductional type, B chromosomes always divide post-reductionally (PAPESCHI 1992). The achiasmatic nature of meiosis in Phthiraptera, at least in the male sex, implies that recombination between homologues may be very restricted; the low chromosome number also restricts the between chromosome recombination. Spermatozoa result from a variable number of mitoses following meiosis, which also constrains genetic variability in this group of insects. The limited potential for genetic variability can be related to the high host specificity of these ectoparasites, as each species very frequently infests only one host.

Acknowledgments. — We wish to thank Lic. A. Cicchino for identification of the material. We are indebted to Dr. L. Mola for critical reading of the manuscript, and her helpful comments. This research was supported by grants to Dr. C. A. Naranjo from CONICET and CIC, and to Dr. M. Suriano from CONICET.

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