Gametogenesis in Amphimictic and Parthenogenetic Populations of Aphelenchus avenae[1]

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Abstract: The female reproductive system of *Aphelenchus avenae,* studied in orcein-stained material, showed a peculiar structural pattern not yet reported in other nematodes. Chromosome morphology and behavior during gametogenesis could be studied in more detail than in other tylenchid or aphelenchid species investigated to date. In a bisexual population from Australia, gametogenesis was by normal meiosis and reproduction by amphimixis. The haploid chromosome number was n=8 in both males and females, and no sex chromosomes were detected. Three monosexual populations from Australia, California, and North Carolina underwent oogenesis by meiosis but reproduced by parthenogenesis. The haploid chromosome number was n=8 in the Australia and the North Carolina populations, but n=9 in the California population. Spermatogenesis in temperature-induced males of the California population was by normal meiosis, and sperm had n=9 chromosomes. Most chromosomes consisted of a central euchromatic section and two characteristic heterochromatic ends. No centromere was observed in any chromosome. The relationship between the California population with n=9 and all the other populations with n=8 chromosomes is not well understood. *Key Words:* oogenesis, spermatogenesis, reproduction.

Aphelenchus avenae Bastian is a favorable nematode for experimental work because of its cosmopolitan distribution, short life cycle, and its adaptibility to being reared on different fungi (19). It can also be cultured axenically in chemically defined media supplemented with various extracts (8), a characteristic which has opened many avenues for studying its nutritional requirements, physiology, and various developmental aspects, including the effects of physical and chemical factors on sex differentiation (1, 2, 9, 10). Biochemical studies have dealt with determinations of protein and enzyme patterns (3), and attempts have been made to elucidate the physiological or biochemical mechanism of molting (4). *Aphelenchus avenae* is often used as a test species in evaluating and studying the mode of action of nematicides (13, 14). Findings from such investigations may contribute to the understanding of various aspects of the biology of plant-parasitic nematodes which generally are more difficult to study.

Cytogenetic information about *A. avenae* would be very helpful as a supplement to these biological studies, particularly in view of the taxonomic complexity of this species which is comprised of at least two races, a

Received for publication 20 November 1975.

[1] Paper No. 4840 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, N. C. 27607. This study was supported in part by National Science Foundation Grant No. BMS 73-00900 AO3. We thank Dr. Eder Hansen for supplying the California population of *A. avenae.* Thanks are also due to Mr. Eugene F. McCabe for valuable technical assistance.

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monosexual and a bisexual one. The monosexual race is assumed to be parthenogenetic (5) or hermaphroditic (12), whereas the male is necessary for reproduction in the bisexual race (5). Nothing is known about the cytology of these races, although one monosexual population has been reported to undergo meiosis and to have n=8 chromosomes (21).

In the present study, gametogenesis and the chromosomal complements of one bisexual and three monosexual populations of *A. avenae* were analyzed and compared. The mode of reproduction was also elucidated cytologically. Because the meiotic chromosomes of *A. avenae* were found to be more favorable for cytological analysis than those of any other tylenchid nematode examined thus far, a detailed description of meiosis with representative illustrations from the bisexual race is included.

MATERIALS AND METHODS

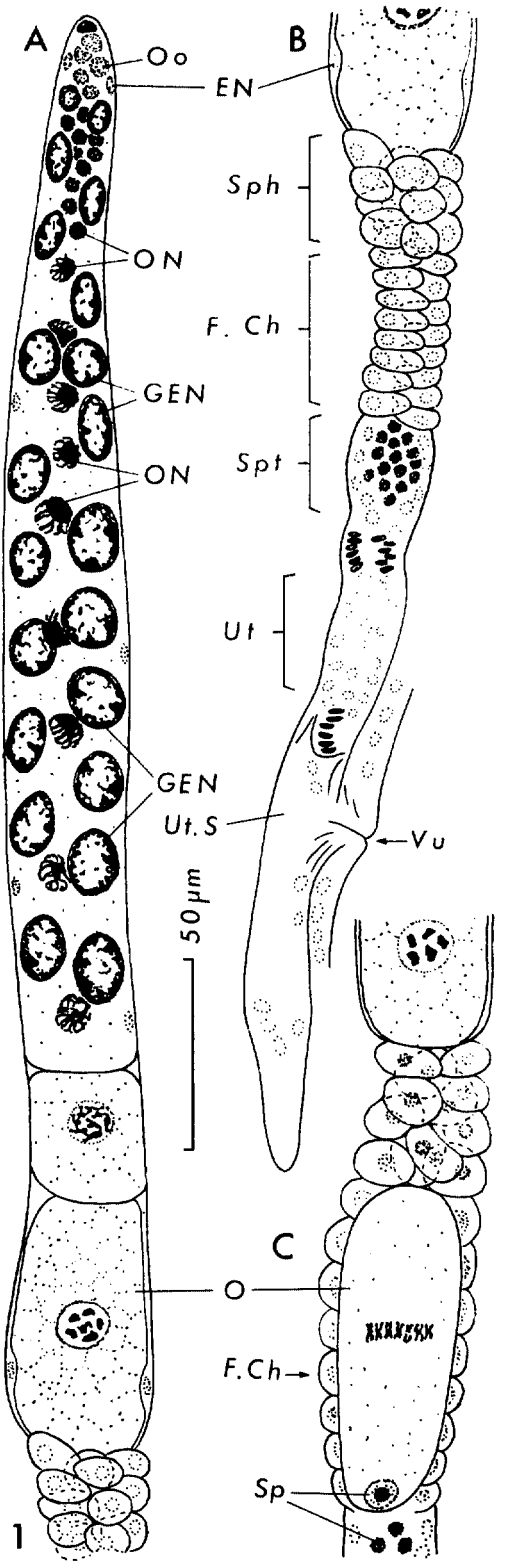
Four populations of *A. avenae* were selected for this study. Two populations from Australia, one bisexual and one monosexual, were those studied earlier by Fisher (5). The third population (from California) was monosexual and had been studied by Hansen et al. (9, 10) with regard to sex expression. The fourth population (from North Carolina) had been examined cytologically by Triantaphyllou (21). All populations were maintained on cultures of *Rhizoctonia solani* Kühn at 26 C in the dark. For observation of gonad structure and detection of insemination, adult females were stained *in toto* with 1.0% acetic orcein (11). For cytological study, adult males and females were smeared on microscope slides which then were processed for fixing and staining by the method described for females of *Heterodera* species (22).

RESULTS

Female gonad structure: The cellular structure of the gonad of *A. avenae* has been studied in detail by Geraert (6). Because it shows certain peculiarities not yet reported in this or other nematodes, it will be briefly redescribed here from orcein-stained material.

The female reproductive system of *A. avenae* is structurally similar in the bisexual and the monosexual races. It consists of the ovary, which is followed posteriorly by a well differentiated gonoduct leading to the vagina and vulva (Fig. 1-A, B). The apical, germinal zone of the ovary, which is only 15-25 μm long, is formed by a thin epithelium containing a distinct cap cell and several flat epithelial cells with small nuclei (Fig. 1-A). Less than 10 oogonial cells with large nuclei, which faintly stain with orcein, are usually present in this region. Posterior to the germinal zone, the ovary extends into a long growth zone which is very conspicuous because of two or three rows of large epithelial cells with very large nuclei that stain heavily with orcein (Fig. 1-A, 2). The chromatin of these "giant epithelial nuclei" is much condensed; it forms a thick network which is distributed unevenly at the periphery of the nucleus. The number of cells with giant nuclei varies from 10 in young females to 25 or 30 in egg-producing females and probably decreases in very old females. Also, the size of giant nuclei may vary considerably among females, probably depending on the cycle of egg production. Along the axis of the growth zone of the ovary, there is a series of oocytes with distinctly smaller nuclei and differently arranged chromatin (Fig. 1-A, 2).

The posterior part of the ovary is lined with about 10 flat, epithelial cells with small nuclei. It is very short in young females, but stretches to become approximately one-half the length of the ovary in egg-laying females with many developing oocytes. The ovary is followed posteriorly by a sphincter of 12 globular cells (Fig. 1-B, C, 3). This sphincter prevents posterior passage of the oocytes before they are fully grown (Fig. 3). A duct, which is continuous with the sphincter, is formed by two rows of seven or eight thick, glandular cells. In egg-producing females, these cells contain much granular material that stains deep red with orcein. The duct appears to function as a "fertilization chamber." Fully grown oocytes pass singly through the sphincter into this chamber. Each oocyte stays there until a spermatozoon enters its posterior end, which partially protrudes into the adjoining spermatotheca (Fig. 1-C) where sperm are stored in inseminated females of the bisexual race (Fig. 3). The spermatotheca is a cylindrical compartment of the



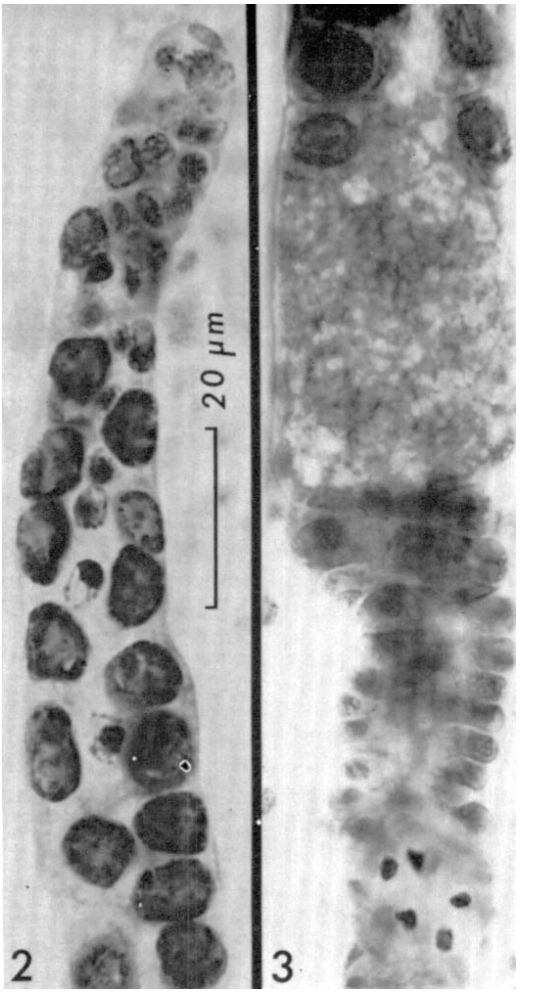


FIG. 2-3. Photomicrographs of female reproductive system of *Aphelenchus avenae* stained with orcein. *2)* Anterior part of ovary showing darkly stained "giant epithelial nuclei" and a central row of smaller nuclei of developing oocytes. *3)* Middle part of gonad including posterior part of ovary with a fully grown oocyte, sphincter, fertilization chamber, and spermatotheca containing sperm. (For identification of homologous parts compare with Fig. 1). Scale for Fig. 3 as in Fig. 2.

FIG. 1-(A-C). Female reproductive system of *Aphelenchus avenae* as seen in orcein-stained nematodes. *A)* Ovary with "giant epithelial nuclei" and a central row of nuclei of oocytes in various stages of maturation. *B)* Posterior part of same gonad as in A illustrating structure of the gonoduct. *C)* Fertilization chamber region with an oocyte penetrated by a spermatozoon. EN, epithelial nucleus; F.Ch, fertilization chamber; GEN, giant epithelial nucleus; O. oocyte; ON, oocyte nucleus; Oo, oogonium; Sp, sperm; Sph, sphincter; Spt, spermatotheca; Ut, uterus; Ut.S, uterine sac; Vu, vulva.

gonoduct; it is formed by small flat epithelial cells and is delimited posteriorly by a constriction of two rows of six cells each with flat, darkly staining nuclei (Fig. 1-B). Behind the spermatotheca, the gonoduct differentiates into a second cylindrical compartment (uterus) which is lined with flat, epithelial cells with small nuclei and confined posteriorly by a second constriction of two rows of six to eight cells each with heavily staining nuclei (Fig. 1-B). The uterine sac formed by the flat epithelium connects ventrally with the vagina and extends posteriorly into a long post-vulval uterine sac (Fig. 1-B).

Oogenesis in the bisexual race and the nature of the meiotic chromosomes: Oogonial divisions take place in the short germinal zone of the ovary of young females but, as in most tylenchids and other aphelenchids, such divisions are not favorable for cytological analysis. Metaphase chromosomes of oogonia could be observed in only a few cytological preparations. The somatic chromosome number could not be determined precisely in any case because some of the chromosomes were always fused with others (Fig. 4).

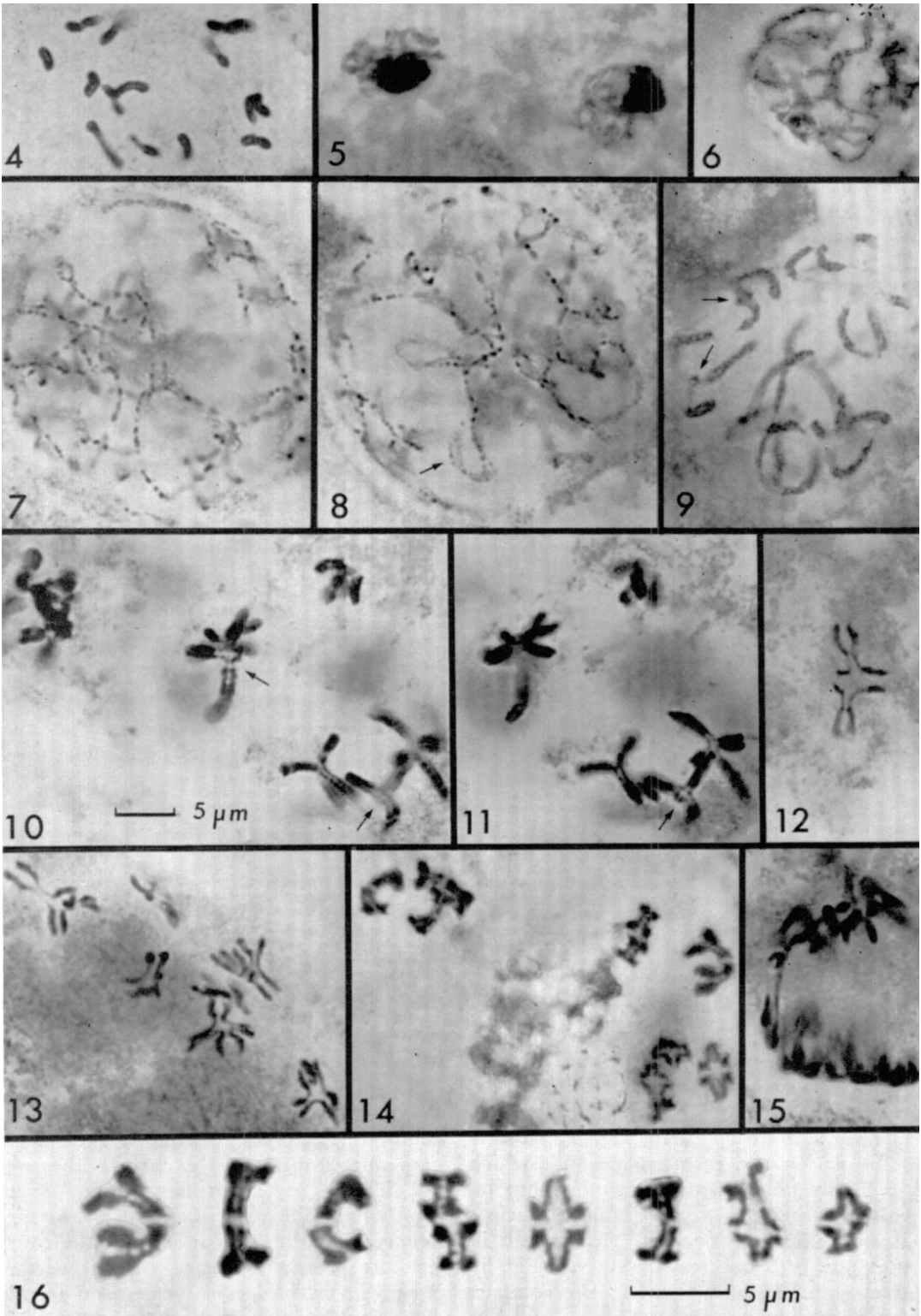
Posterior to the germinal zone, 4 to 15 young oocytes may be arranged in several rows. The chromatin inside their nuclei characteristically forms a dense, heavily staining network. Further posteriorly, the oocytes become arranged in tandem and advance to what could be characterized as the zygotene stage. The chromosomes become visible as thin, knotted, double filaments extending as loops from the compact chromatic network into the nuclear cavity (Fig. 1-A, 2, 5). This arrangement of the chromatin represents the "bouquet" arrangement (synizesis) of leptotene or early zygotene chromosomes commonly observed in many animals and in some plants (18). The compact chromatic network apparently is formed by the chromosome ends which are highly heterochromatic and clump together in an area adjacent to the nuclear envelope. Double loops are clearly seen in more advanced oocytes at zygotene (Fig. 6). As the nucleus grows larger, the compact chromatic network becomes loose and disappears, while the double chromatic filaments condense and fuse to form single, knotted, thick filaments, representing pachytene chromosomes (Fig. 7). Concurrently, pachytene chromosomes split longitudinally (diplotene?) and the homologues become discrete again (Fig. 8-arrow). It is possible, however, that such double filaments may still represent pachytene chromosomes that failed to pair along their entire length during zygotene.

Contraction and separation of the homologues (diplotene) proceeds as the oocytes migrate down the growth zone of the ovary and, at the same time, each homologue splits, longitudinally into its chromatids (Fig. 9). At advanced diplotene, the homologues of each bivalent are associated at one point (chiasma) along their central euchromatic region. The four heterochromatic ends are spread in different directions and usually show a cross configuration (Fig. 10, 11). Eight bivalents can be seen clearly at this stage. In some oocytes, one or two heteromorphic bivalents were observed [Fig. 10 (arrows), 11, 12]. With further condensation, the heterochromatic ends of each chromatid dissociate so that each bivalent now has eight club-shaped heterochromatic ends (Fig. 13). At late diakinesis, the chiasmata become terminalized, and the homologues are associated end-to-end as they form different, characteristic configurations (Fig. 14, 16).

Oocytes advance to metaphase inside the fertilization chamber after they are penetrated by a spermatozoon (Fig. 1-C). Anaphase I proceeds while the eggs are in the uterus or after they are laid. Chromosomal bridges were frequently observed in telophase I figures (Fig. 15). The second maturation division and fusion of sperm and egg pronuclei are normal and take place after the eggs are deposited.

Spermatogenesis in the bisexual race: The male gonad is similar to that of other tylenchid nematodes. It consists of the testis, seminal vesicle, vas deferens, and ejaculatory duct leading to the cloaca. The testis is similar in structure to the ovary. Epithelial cells with giant nuclei, as were observed in the growth zone of the ovary, are also present in the testis. Furthermore, arrangement of spermatocytes and nuclear behavior during meiotic prophase are the same as described for oocytes in oogenesis.

As soon as each spermatocyte passes behind the area of the "giant epithelial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nuclei," it undergoes two maturation divisions in close succession. Eight bivalents were observed in many spermatocytes at diakinesis or prometaphase I. At diakinesis, the homologues are associated with one another at a point along their euchromatic regions. Therefore, each bivalent has a central, lightly-staining region with eight heavily-staining arms extending in various directions (Fig. 17). Bivalents with terminalized chiasmata (Fig. 17-arrow) attain characteristic configurations, analogous to those observed in oocytes. At telophase I and metaphase II, the chromosomes consist of two discrete chromatids each (Fig. 18). At anaphase II, the chromosomes represent single chromatids of the original bivalents and move broadside first toward the poles. Eight chromosomes were observed in all telophase II figures, an occurrence indicating that only spermatozoa with eight chromosomes are produced (Fig. 19).

Oogenesis in the monosexual race. The monosexual populations from North Carolina, Australia, and California underwent oogenesis in a manner similar to that described for the bisexual population from Australia. Maturation of the oocytes was by classical meiosis. Eight bivalent chromosomes were observed at prometaphase I in the North Carolina and Australia populations (Fig. 20), but nine were present in the California population (Fig. 21). One polar body was formed as a result of the first maturation division. The second maturation division was abortive and no second polar body was formed. Apparently the two groups of chromosomes at telophase II were included in the same egg nucleus; thus the somatic chromosome number was re-established. Because of limited stainability of the eggs at this stage, this process was not clearly followed. Spermatozoa were not observed inside the oocytes or the female gonoduct, an indication that reproduction was by parthenogenesis.

Spermatogenesis in temperature-induced males: Spermatogenesis was normal in males of the California population recovered from cultures maintained at 30 C. This process followed the same pattern described for males of the bisexual Australia population. Nine bivalent chromosomes were observed in two prometaphase figures. The chromosome number could be determined in many telophase I or metaphase II figures in which nine dyads were clearly visible (Fig. 22). Similarly, telophase II figures included nine single, rod-shaped chromosomes (Fig. 23, 24) which eventually condensed into a loose chromatin mass during the formation of the sperm nucleus. In a few cases, metaphase II figures and spermatids with 8 or 10 chromosomes were also observed. One dyad in some telophase I figures was obviously asymmetric with two chromatids of different size (Fig. 25-arrow).

DISCUSSION AND CONCLUSIONS

The epithelial cells of the growth zone of the ovary and the testis of *A. avenae* increase considerably in size during late post-

FIG. 4-16. Photomicrographs of chromosomes of the amphimictic race of *Aphelenchus avenae* during oogenesis. *4)* Metaphase chromosomes in an oogonial division. *5)* Nuclei of two oocytes at early zygotene; most of the heterochromatin is included in a compact mass, whereas the euchromatin forms single or double loops projecting into the nuclear cavity (bouquet arrangement). *6)* Advanced zygotene; the compact heterochromatic mass has almost disappeared, and the homologues are paired along their entire lengths. *7)* Pachytene stage; the homologues are fused to single thick filaments formed by a long chain of distinct chromomeres. *8)* Early diplotene; the pachytene chromosomes start to split longitudinally, and the homologues become distinct again (arrow). *9)* Mid-diplotene; the homologues have separated from each other except at the points of chiasmata (arrows), and each has split longitudinally into its chromatids. *10)* Late diplotene; there is one chiasma visible along the central euchromatic region in each bivalent. The four heterochromatic ends are spread in different directions and usually form a double-cross configuration. Two bivalents (arrows) in this figure are heteromorphic. *11)* Part of Fig. 10 in different focus to better show one of the heteromorphic bivalents (arrow). *12)* An isolated heteromorphic bivalent at late diplotene. Part of the heterochromatic end of one of the homologues is missing. *13)* Early diakinesis; terminalization of chiasmata proceeds toward the end of the euchromatic regions of each bivalent. The heterochromatic ends of the homologues and of sister chromatids separate completely so that each bivalent has eight free heterochromatic ends. *14)* End of diakinesis; the chiasmata are terminalized and each bivalent attains a characteristic configuration. *15)* Telophase I showing bridges. *16)* The chromosomes of Fig. 14 enlarged and reoriented to facilitate comparison of their morphology. Scale for Fig. 4-15 as in Fig. 10.

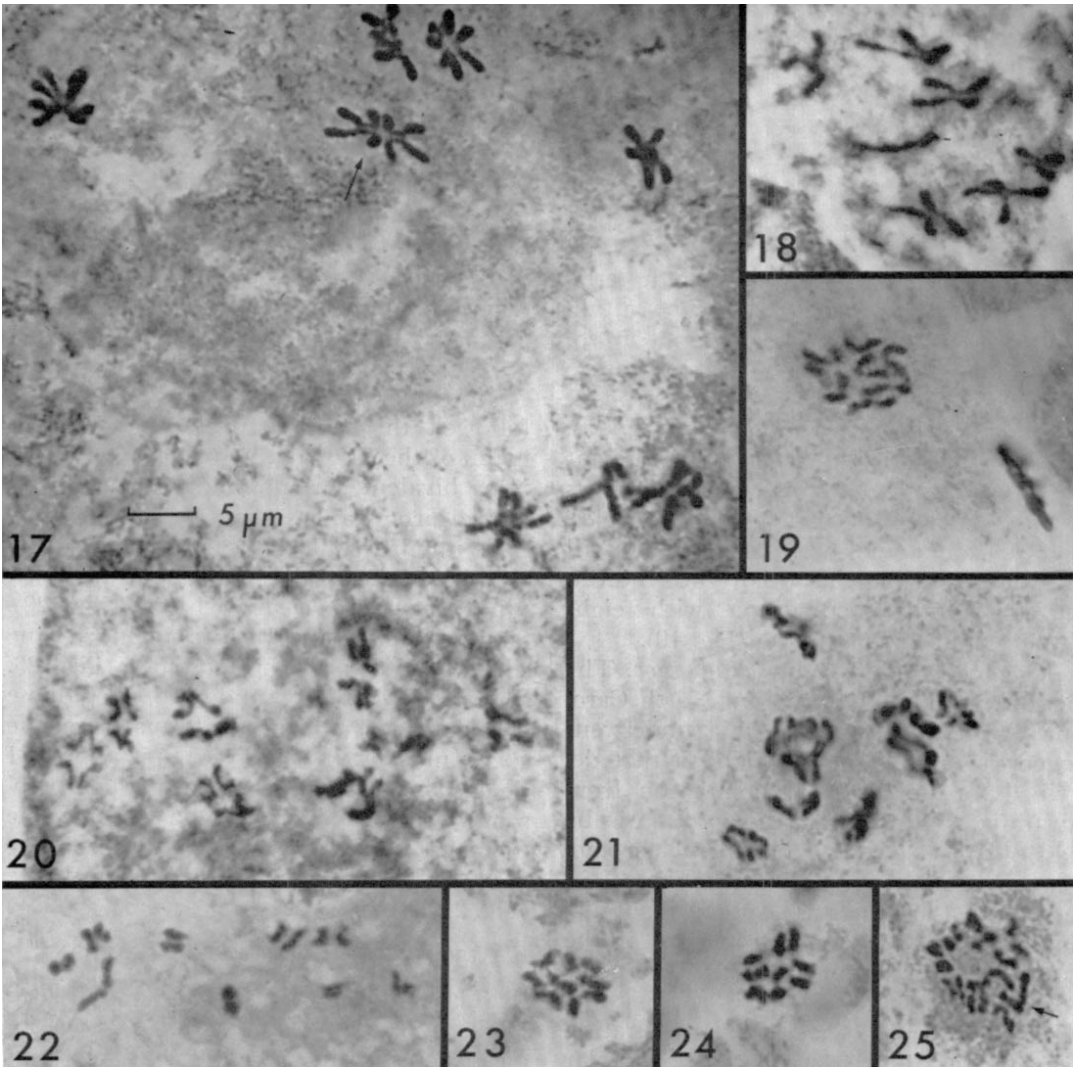

FIG. 17-25. Gametogenesis in *Aphelenchus avenae*. *17-19)* Spermatogenesis in males of the bisexual race from Australia. *17)* Eight mid-diakinetic chromosomes of a primary spermatocyte. The arrow points to a bivalent with terminalized chiasmata and a characteristic configuration similar to that observed in diakinetic chromosomes of oocytes—compare with Fig. 14. *18)* Telophase I chromosomes consisting of two chromatids each. *19)* Telophase II figure with eight simple chromosomes. *20)* Eight prometaphase chromosomes in a primary oocyte of the North Carolina population of *A. avenae*. *21)* Nine prometaphase chromosomes in a primary oocyte of the California population of *A. avenae*. *22-25)* Spermatogenesis in temperature-induced males of the California population of *A. avenae*. *22)* Nine telophase I chromosomes consisting of two chromatids each. *23-24* Telophase II figures with nine simple chromosomes each. *25)* Telophase I figure with one heteromorphic dyad (arrow). Scale for Fig. 18-25 as in Fig. 17.

embryogenesis and have very large nuclei that stain heavily with orcein. To our knowledge, this has not been reported for any other nematode. The structure of the ovary of *Aphelenchoides goodeyi* Siddiqi and Franklin (17) appears to be similar, but the giant nuclei, as illustrated in the ovary of this species, were assumed to represent "developing syngonic spermatozoa." The function of these cells with giant nuclei is not clear, but we suspect that they serve as nurse cells providing nutrients primarily for the development of the nuclear component of the gametocytes. The cytoplasmic component of the oocytes increases rapidly later when the oocytes pass into the posterior part of the ovary which is made up of normal, flat epithelium.

The structure of the female gonoduct of *A. avenae* is rather complicated, as Geraert

has indicated (6). The most important parts of the gonoduct, designated by Geraert by numbers, correspond to the present terminology as follows: part 2 = uterus, part 4 = spermatotheca, part 5 = fertilization chamber, part 6 = sphincter, and part 7 = posterior region of ovary. The posterior region of the ovary is structurally distinct from the anterior region in that it lacks the large epithelial cells with the "giant" nuclei. However, we consider it as part of the ovary and not as an oviduct because it is the area of rapid growth of the oocytes. Its epithelium apparently provides the necessary nutrients for growth of the oocytes, and this we regard as the function of ovarian epithelium and not of epithelium of gonoducts. An oviduct could be defined as a long, narrow tube through which the oocytes pass rapidly after they are fully grown in the ovary. This concept could help to clarify the situation in many tylenchid and aphelenchid nematodes in which an oviduct, well defined structurally or functionally, may be an exception. Discussion of this matter by Geraert (6) points out the inconsistency existing in the literature. Still, Geraert's statement that "oocytes accumulate yolk and attain their full size only while they are in the oviduct" is in contrast to our concept about the function of an oviduct.

The region of the gonoduct that functions as a fertilization chamber is characteristic of *A. avenae*. The fertilization chamber and the anteriorly located sphincter appear as one unit in gonads of young females without oocytes, and, probably for this reason, the two have been regarded as an oviduct by previous investigators (7, 16). However, the two parts are distinctly different functionally in egg-producing females. The glandular cells of the fertilization chamber of egg-producing females are filled with granular material which stains heavily with orcein. We suspect that some of this material is deposited on the surface of the eggs shortly after sperm penetration and the initiation of eggshell formation. The sphincter, on the other hand, functions as a regulatory valve that permits fully grown oocytes to pass one by one into the fertilization chamber. At times, the sphincter itself appears as an elongated narrow tube which structurally and functionally could be characterized as an oviduct.

Although the chromosomes of several species of tylenchid nematodes have been studied in detail (21), the present study of *A. avenae* provides a much clearer picture of the nature and behavior of the meiotic chromosomes of these nematodes. The main characteristic of the meiotic chromosomes of *A. avenae*, observed also in other tylenchid nematodes, is that each homologue splits longitudinally into its chromatids early in prophase I. The chromatids lie parallel to each other and maintain this orientation until they separate completely at anaphase II. Because of this behavior, the individual chromosomes appear to be bipartite (dyads), and the bivalents form characteristic tetrapartite configurations (tetrads). At late diplotene, the homologues of each bivalent are associated along their central euchromatic parts where chiasmata apparently occur. At diakinesis, the chiasmata become terminalized, and the homologues are always associated end-to-end, but often with the adjacent heterochromatic end-portions protruding transversely (Fig. 16, 17-arrow). This special cross configuration, observed also in *Caenorhabditis elegans* (15), probably is due to the large heterochromatic ends of these chromosomes. Terminalization of chiasmata apparently extends to the end of the euchromatic regions, but does not proceed through the heterochromatic end-portions which are directed sideways. Complete terminalization occurs, however, later at metaphase or early anaphase.

The consistent end-to-end arrangement of the homologues at metaphase I favors an interpretation that the chromosomes are acrocentric or subacrocentric. This arrangement, however, does not exclude the possibility that the chromosomes may be holokinetic, a view supported by the lack of morphological evidence for the presence of a centromere. On the other hand, the orientation of the chromosomes during metaphase II and their "broadside first" movement toward the poles at anaphase II suggest a diffuse-kinetochore activity along the entire euchromatic region of the chromosomes, which has been assumed for other tylenchid nematodes (20).

Often one or two bivalents in oocytes of the bisexual race were heteromorphic, i.e.,

they consisted of two homologues of different size. Differences appeared to involve only heterochromatin; usually part of the heterochromatic end of one of the homologues was missing. Such deletions of heterochromatic segments were observed only in the heterozygous state. They may be lethal in homozygous condition.

Bridges encountered frequently in anaphase I figures do not seem to represent classical inversions or breakage-fusion situations but may be the result of stickiness of the large heterochromatic ends of most chromosomes. It should be pointed out, however, that stickiness was not observed during earlier stages, such as diplotene and diakinesis, during which there was actually a strong repulsion of heterochromatic ends.

The amphimictic population from Australia had n=8 chromosomes and the males produced only one kind of sperm. No sex chromosomes could be detected, although an XY sex chromosome mechanism cannot be excluded.

Nothing can be stated at present about the relationship of the 9 chromosomes of the California population to the 8 chromosomes of the other populations. It should be noted that the California population is the one which responds to high temperatures and various chemical treatments with the production of numerous males (10). The other parthenogenetic populations do not produce any males under similar temperature treatment and produce only few males under different circumstances (1). Further studies are needed to elucidate the relationship between the California and the other parthenogenetic populations of *A. avenae*.

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