Gametogenesis and Reproduction of Seven Species of *Pratylenchus*[1]

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Abstract: Three bisexual *Pratylenchus* species, *P. penetrans*, *P. vulnus* and *P. coffeae* have n = 5, 6 and 7 chromosomes, respectively, and reproduce by cross-fertilization. The monosexual *P. scribneri* comprises two chromosomal and reproductive forms. One has n = 6 chromosomes and reproduces by meiotic parthenogenesis, the other has a somatic chromosome number of approximately 25 and reproduces by mitotic parthenogenesis. The monosexual species *P. zeae*, *P. brachyurus* and *P. neglectus* have somatic chromosome numbers of approximately 21 to 26, 30 to 32, and 20, respectively, and reproduce by mitotic parthenogenesis. All mitotic parthenogenetic forms probably are polyploid. The phyletic relationships of some species are discussed briefly.

No information is available about gametogenesis and chromosomes in the genus *Pratylenchus* Filipjev. Also, the mode of reproduction has never been studied, although the absence of males in some species indicates parthenogenesis may be operating in this genus (5). To gain a better understanding of the true biological relationships of members of this genus, a cytological study of seven species was undertaken. Three of these species are bisexual, with males and females occurring in approximately equal numbers, and four are monosexual, with either few or no males. A special effort was made to determine the mode of reproduction of each species.

MATERIALS AND METHODS

The nematode populations used in this study (Table 1) were propagated on suitable host plants in the greenhouse at 24 to 30 C. Some of them were also maintained monoxenically on alfalfa callus tissue grown in nutrient medium at 27 C in the dark (2). Adult nematodes were obtained from both types of cultures during a period of high egg production and processed for cytological study.

Oogenesis was studied in gravid females stained *in toto* with acetic orcein (1). Most observations, however, were made on eggs dissected from the nematode body in distilled water or deposited by the nematodes in tap water. These were treated 1 min in 1.25% sodium hypochlorite, then stained with propionic orcein as described for eggs of *Anguina tritici* (Steinbuch) (6).

Spermatogenesis was studied in young males which were smeared on slides and processed by the method used for males of *Anguina tritici* (6).

OBSERVATIONS

OOGENESIS: The morphology of the female reproductive system of various *Pratylenchus* species has been studied and illustrated (3). It consists of a single outstretched ovary, oviduct, spermatotheca, uterus, vagina and postvulvar uterine branch. The ovary can be subdivided into an anterior germinal zone and a posterior growth zone.

Oogonial divisions take place in the germinal zone of the ovary of fourth stage larvae and, probably, of young females, but have not been observed in mature, egg-laying

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TABLE 1. Chromosome numbers and mode of reproduction of various populations of seven *Pratylenchus* species.

Species	Population source	Chromosome No.[a] (♀)		Mode of reproduction
		n	*2n*	
P. penetrans	Canada	5	10	Amphimixis
	Holland	5		Amphimixis
	Japan	5		Amphimixis
P. vulnus	Va., USA	6		Amphimixis
	N. C., USA	6		Amphimixis
	N. C., USA	6		Amphimixis
P. coffeae	Fla., USA	7		Amphimixis
	P. Rico	7		Amphimixis
	P. Rico	7		Amphimixis
P. scribneri	Fla., USA	6	(12)[b]	Meiotic Parthenogenesis
	Cal., USA		(25–26)	Mitotic Parthenogenesis
P. zeae	N. C., USA		(24–26)	Mitotic Parthenogenesis
	N. C., USA		(21)	Mitotic Parthenogenesis
	Holland		(26)	Mitotic Parthenogenesis
P. neglectus	Canada		(20)	Mitotic Parthenogenesis
P. brachyurus	N. C., USA		(30–32)	Mitotic Parthenogenesis
	Cal., USA		(32)	Mitotic Parthenogenesis

[a] At least ten chromosomal figures were scored from each population studied.
[b] Chromosome numbers in parentheses are estimates.

females. Oogonial divisions are unfavorable for cytological analysis, mainly because the chromosomes are very small and indiscrete.

In advanced oogonia, located at the junction of the germinal and the growth zone, the chromatin of each nucleus condenses into a densely staining network. This probably indicates that synapsis of homologous chromosomes takes place. However, similar chromatin behavior is observed in species in which maturation of the oocytes consists of a single mitotic division. In the growth zone the oocytes increase in size, but no chromosomes are visible in their nuclei. Fully grown oocytes pass one by one through the oviduct into the spermatotheca and the anterior part of the uterus. At this stage the chromosomes condense and become visible again. Further chromosomal behavior in bisexual species differs from that of monosexual forms, thus, the two groups are treated separately.

Maturation of oocytes and fertilization in the bisexual species P. penetrans, P. vulnus and P. coffeae.—Oocytes in the spermatotheca contain a small number of bivalent chromosomes at prometaphase I. The precise number could not be determined. One spermatozoon enters each oocyte, which then rapidly completes the first division. At telophase I the chromosomes that will eventually form the first polar nucleus are rather discrete (Figs. 1–4). Most determinations of haploid chromosome numbers were made from such figures (Table 1). A second maturation division follows rapidly, and the sperm pronucleus is formed (Fig. 9) and fuses with the egg pronucleus to form the zygote nucleus (Fig. 10). Actual fusion of the pronuclei was observed in nondeposited eggs of the Canada population of *P. penetrans* and in laid eggs of *P. coffeae*. Fusion of pronuclei was not observed in *P. vulnus*. In all species, one of the pronuclei, probably

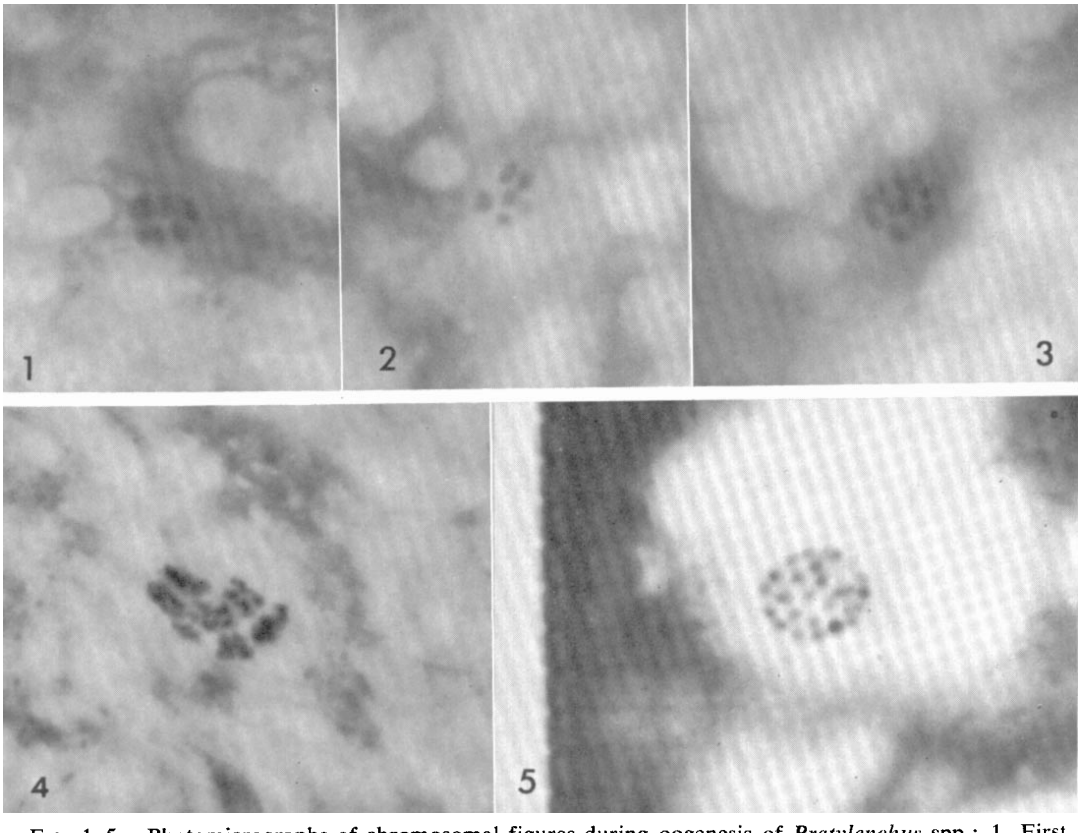

FIG. 1–5. Photomicrographs of chromosomal figures during oogenesis of *Pratylenchus* spp.: 1. First polar nucleus in *P. penetrans* with 5 chromosomes; 2. First polar nucleus in *P. vulnus* with 6 chromosomes; 3. First polar nucleus in *P. coffeae* with 7 chromosomes; 4. Duplication of the chromosomes of the first polar nucleus of *P. coffeae*; 5. Polar nucleus with approximately 26 chromosomes in *P. zeae*. × 3600.

the sperm pronucleus, is larger than the other (Figs. 9, 10). The first cleavage division takes place after egg deposition. The somatic chromosome number ($2n = 10$) was determined in prometaphase figures of the first cleavage division in the Canada population of *P. penetrans* only.

Maturation of oocytes in the monosexual species.—(i) *P. scribneri*: In the Florida population, the young oocytes contain the haploid number of 6 bivalent chromosomes, indicating prior pairing of the homologues, as in the case of the bisexual species. No sperm was observed in the spermatotheca or in the oocytes. Two maturation divisions occur, then the egg pronucleus is formed. The somatic number of approximately 12 chromosomes was observed in prometaphase figures of the first cleavage division. How the somatic chromosome number was reestablished was not determined. Reproduction in this population is considered to be by meiotic parthenogenesis.

In the California population, a large number of univalent chromosomes was observed in young oocytes at metaphase I, indicating that synapsis does not occur, or if it does, the homologues separate before metaphase I. The small size and close association of the chromosomes precluded precise determina-

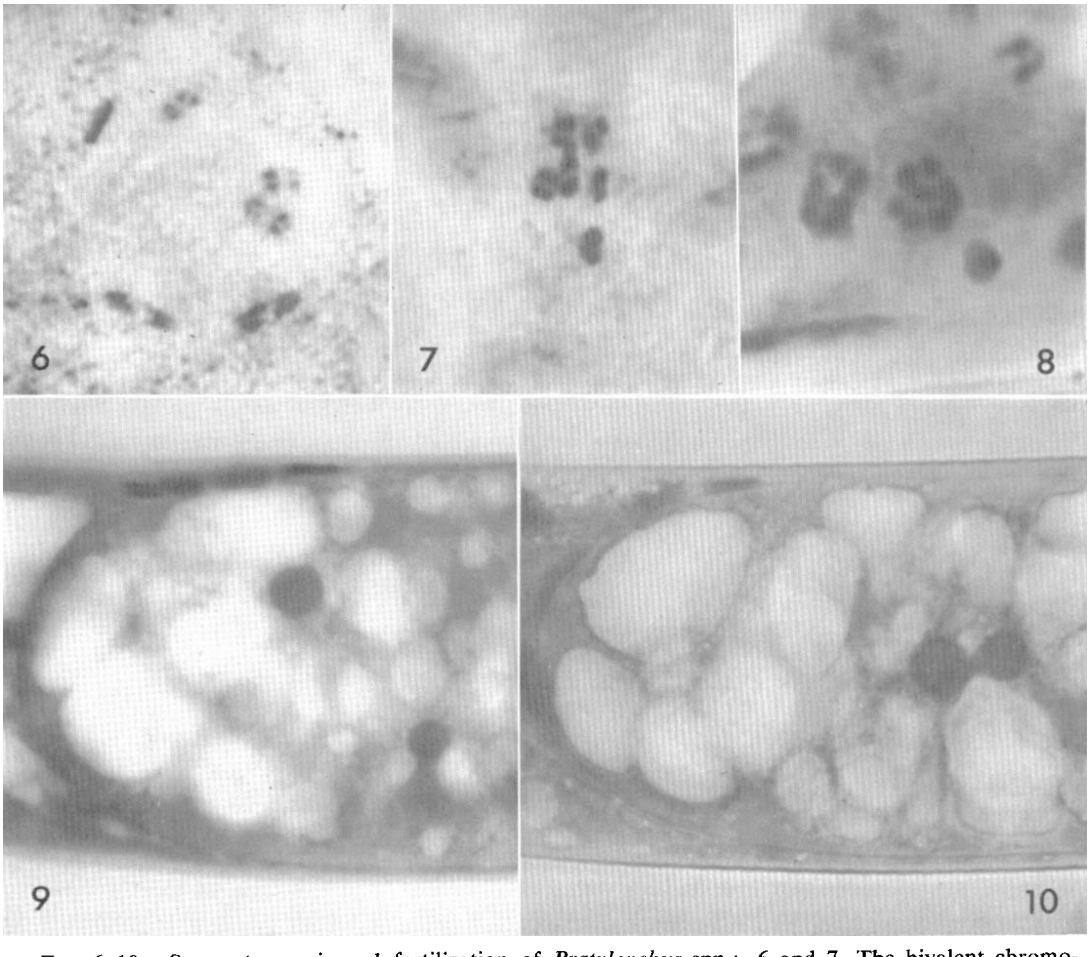

FIG. 6–10. Spermatogenesis and fertilization of *Pratylenchus* spp.: 6 and 7. The bivalent chromosomes of *P. vulnus* at late diakinesis and prometaphase I, respectively, × 3600; 8. Metaphase I during spermatogenesis of *P. coffeae* with 7 bivalent chromosomes, × 3600; 9–10. Progressive stages toward fusion of the sperm and egg pronuclei illustrating the amphimictic nature of *P. penetrans*, × 1800.

tion of the chromosome number. However, from late telophase I figures, an estimate of the chromosome number (2n = 25–26) of the future polar nucleus was possible. No second maturation division takes place, and the eggs cleave shortly after the completion of the first division. Reproduction, therefore, is by mitotic parthenogenesis.

(ii) *P. zeae, P. neglectus and P. brachyurus*: No synapsis of homologous chromosomes takes place in these species. The somatic chromosome number (Table 1, Fig. 5) is maintained throughout maturation which consists of a single mitotic division. Some males were observed only in the North Carolina population of *P. brachyurus* from both, greenhouse and callus cultures, but their role in reproduction was not ascertained. The absence of sperm in the spermatotheca or in the oocytes indicates that males are not functional. Reproduction in these three species is by mitotic parthenogenesis.

SPERMATOGENESIS: Limited observations

of spermatogenesis were made in males from the bisexual species *P. penetrans*, *P. vulnus* and *P. coffeae*. Spermatogenesis apparently follows the normal pattern for amphimictic nematodes: two maturation divisions that result in spermatids with the haploid chromosome complement.

Six bivalent chromosomes were observed at prometaphase or metaphase I in all three populations of *P. vulnus* (Figs. 6, 7). The Florida and one of the Puerto Rican populations of *P. coffeae* had 7 bivalent chromosomes (Fig. 8). This suggests that the chromosome number is the same in males and females of these two species. No sex chromosomes were recognized. The chromosome number for males of *P. penetrans* was not determined.

DISCUSSION

These studies demonstrate that gametogenesis in the bisexual species *P. penetrans*, *P. vulnus* and *P. coffeae* follows the classical pattern of other diploid amphimictic nematodes. No fusion of sperm and egg pronuclei was observed in *P. vulnus*, but this may be attributed to the limited amount of material available for study. It is believed that all three bisexual species studied reproduce by cross-fertilization.

The observed variation in chromosome numbers ($n = 5$ in *P. penetrans*, $n = 6$ in *P. vulnus* and $n = 7$ in *P. coffeae*) indicates that extensive chromosomal changes have occurred during the evolution of the genus. It is difficult at present to identify the basic chromosome number in the genus, and to understand how the existing variation has been derived. The chromosomes are small, indistinguishable from each other, and no morphological comparison can be made between the chromosomes of the various species. Detailed cytological analysis of additional members of the genus supplemented by critical evaluation of important morphological, and physiological characters, will probably result in elucidation of the true phylogenetic relationships and the evolutionary pathways within this genus.

Among the monosexual species, *P. scribneri* appears to be in a state of active evolution with regard to gametogenesis and mode of reproduction. The Florida population of this species undergoes regular meiosis during maturation of the oocytes which later develop parthenogenetically. In the California population, however, maturation consists of a single mitotic division and reproduction is by mitotic parthenogenesis. It is likely that mitotic parthenogenetic populations of this species have evolved from meiotic parthenogenetic populations, or that both have evolved from an amphimictic relative independently. The situation is similar to that of *Meloidogyne hapla* Chitwood, in which populations, reproducing by meiotic, and others reproducing by mitotic, parthenogenesis have been encountered (4). The high chromosome number ($2n = 25$–26) of the mitotic parthenogenetic population of *P. scribneri* indicates that this population is probably a tetraploid form. Consistent with the previous assumption, this form must have derived from a meiotic parthenogenetic, or an amphimictic relative with a haploid number of $n = 6$ chromosomes. All other monosexual species, *i.e.*, *P. zeae*, *P. neglectus* and *P. brachyurus* reproduce by mitotic parthenogenesis and have a high chromosome number also indicating polyploidy. *P. zeae*, *P. neglectus* and *P. brachyurus* must have evolved from related diploid amphimictic species, but it is difficult to identify those at present.

The observed variation in chromosome numbers and mode of reproduction among members of this genus may explain the difficulties taxonomists have encountered in properly characterizing individual members of this group. Further cytogenetic studies

will help clarify the phyletic relationships of the various members and establish the foundation for a meaningful taxonomic treatment of the genus.

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