Cytogenetic status of *Meloidogyne kikuyensis* in relation to other root-knot nematodes (1)

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SUMMARY

This cytogenetic study has demonstrated that both males and females of one population of *Meloidogyne kikuyensis* from South Africa have seven chromosomes, *i.e.* the same number as *M. spartinae*, but different from that of all other *Meloidogyne* species which have n = 13-19. The chromosomes of *M. kikuyensis* are at least twice as large as those of other *Meloidogyne* species. Also, their behavior during gonial and maturation divisions is slightly different. An extra, small-size chromosome was detected in about 1/3 of the males and females studied. The oviduct-spermatotheca region of *M. kikuyensis* is almost typical of the genus *Meloidogyne*. Reproduction appears to be by amphimixis. The significance of the small number and the large size of the chromosomes of *M. kikuyensis* is discussed in relation to the evolution of root-knot nematodes.

RÉSUMÉ

Statut cytogénétique de Meloidogyne kikuyensis *en relation avec celui des autres espèces du genre*

Une étude cytogénétique a démontré qu'une population de *Meloidogyne kikuyensis* provenant d'Afrique du Sud possédait sept chromosomes, c'est-à-dire le même nombre que chez *M. spartinae*, nombre différent de celui des espèces de *Meloidogyne* les plus répandues chez lesquelles n = 13-19. Les chromosomes de *M. kikuyensis* sont au moins deux fois plus longs que ceux des autres espèces de *Meloidogyne*. D'autre part, leur comportement lors des divisions oogoniale et de maturation apparaît légèrement différent. Un petit chromosome supplémentaire a été observé chez environ un tiers des femelles et des mâles examinés. La zone de l'oviducte/spermathèque est chez *M. kikuyensis* presque typique des *Meloidogyne*. Il est conclu de cette étude que la présence de sept chromosomes chez *M. kikuyensis* correspond le mieux au nombre chromosomique de la forme ancestrale d'où ont évolué les espèces de *Meloidogyne* les plus répandues. Cependant, la grande taille de ces chromosomes peut indiquer que le caryotype de *M. kikuyensis* résulte d'une évolution secondaire, à partir probablement dans ce cas d'un caryotype semblable à celui de *M. spartinae*.

Presently, all 68 described species of root-knot nematodes have been assigned to a single genus, *Meloidogyne* (Luc, Maggenti & Fortuner, 1988). It is recognized, however, that some species exhibit morphological, behavioral or other characteristics that deviate to a certain degree from the typical features of the genus *Meloidogyne*. Whether such deviations are sufficient to justify splitting of the genus into a number of genera (*Hypsoperine*, etc.) is not clear. Recent cytogenetic examination of two such variant species [*M. spartinae* (Rau & Fassuliotis, 1965) Whitehead, 1968 and *M. nataliei* Golden, Rose & Bird, 1981] provided useful information regarding the cytogenetic relationships of these species to the rest of root-knot nematodes and other related genera (Triantaphyllou, 1985*a*, 1987). Similar information could elucidate the taxonomic status of other variant root-knot nematodes. The present study was conducted in an effort to clarify the cytogenetic status of the morphologically atypical species, *M. kikuyensis* De Grisse, 1960, in relation to other *Meloidogyne* species. A morphological evaluation of the same species will be presented in a separate article by J. D. Eisenback.

Materials and methods

The population of *M. kikuyensis* for this study was obtained in 1984 by J. D. Eisenback from a sugar cane field in South Africa. It has been propagated since then on sugar cane under normal greenhouse conditions. An abundance of males and egg-producing females were obtained from the very characteristic, leguminous-nodule-like galls of infected roots.

For the cytological study, young egg-laying females were smeared on microscope slides and the smears were processed and stained with propionic orcein according to established procedures (Triantaphyllou, 1985*b*). Young males found associated with the females in the same root

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galls were transferred with a fine needle to dry microscope slides and cut immediately by drawing the needle across the posterior part of their bodies. The reproductive system was thus spread on the slide and adhered to it as it dried within a few seconds. Slides were later processed for staining with propionic orcein in the same manner as slides with smears of the females.

Results

Oogenesis

Most oogonia in the ovaries of young females are at interphase. A few oogonia that undergo mitotic divisions are distributed throughout the germinal zone and their divisions are not synchronized. At early prophase of oogonial divisions the chromatin forms long interconnected threads of about 0.5 µm in diameter (Fig. 1 A). At a slightly more advanced stage, it condenses and resolves into individual chromosomes which, however, remain interconnected and cannot be counted (Fig. 1 B). Finally, at late prophase, 14 rod-shaped chromosomes, varying in length from 2 to 4 µm, become clearly discernible inside the transparent nucleus (Fig. 1 C). The chromosomes contract even further as they arrange in a metaphase plate and become beaded along their length (Fig. 1 D). At this stage they vary in length from 1 to 3 µm. At anaphase and telophase the chromatids of each chromosome maintain a parallel orientation as they move apart from each other and they show no apparent bending that would indicate localized centromeric activity.

The germinal zone of the ovary is followed by a distinct zone of synapsis in which the chromatin of the young oocytes is highly condensed, forming a network that stains heavily with orcein (Fig. 1 E). Further development of the oocytes in the growth zone of the ovary is typical of other root-knot nematodes. Oocytes entering the spermatotheca are at late diakinesis or prometaphase-I. The seven bivalent chromosomes at this stage exhibit complex configurations, apparently due to incomplete terminalization of chiasmata (Fig. 1 F). One spermatozoon enters each oocyte as the oocytes pass through the spermatotheca (Fig. 1 G). Oocytes entering the uterus advance to metaphase-I. Most bivalents at metaphase-I are still complex (Fig. 1 H). Perfect tetrads, similar to those of other *Meloidogyne* species, are seen only in a few bivalents of some metaphase-I figures (Fig. 1 I). Even at advanced anaphase, many chromosomes do not form simple dyads, but they instead exhibit complex configurations and form chromosome bridges as they move toward the poles (Figs 1 J, 1 K). In telophase-I figures there are seven distinct chromosomes each consisting of two chromatids that lie parallel to each other (Fig. 1 L). They are regular dyads, but often appear as tetrads because of their distinct, pronounced heterochromatic ends. A second maturation division follows soon after the first. At metaphase-II each chromosome appears as a dyad with two chromatids parallel to each other (Fig. 1 M). Seven chromosomes, six long and a short one, are observed in many metaphase-II and telophase-II figures (Figs 1 N, 10). The sperm remains close to one end of the oocyte (the point of entrance), while the first and second maturation divisions are completed in the middle of the oocyte (Fig. 1 G).

Spermatogenesis

Spermatogonial divisions are observed along the entire germinal zone of the testis. At early prophase the chromosomes appear as long chromatin threads (Fig. 2 A). Later they condense considerably but remain interconnected and cannot be counted (Fig. 2 B), except in a few spermatogonial prometaphases.

The zone of synapsis in the testis is distinct, as in the ovaries. In spermatocytes at late pachytene, the chromosomes are elongated, with many chromomeres along their length and with thick, club-shaped heterochromatic ends (Fig. 2 C). Slightly more advanced spermatocytes enter a " diffuse " stage during which only the heterochromatic parts of the pachytene-diplotene chromosomes are visible (Fig. 2 D). A large nucleolus becomes distinct at this stage. Further posteriorly, spermatocytes at diakinesis have large chromosomes with very complex configurations (Fig. 2 E). At prometaphase the chromosomes contract considerably and appear as compact chromatin masses without forming distinct tetrads (Fig. 2 F). The second maturation division follows immediately after the first. Seven chromosomes (dyads) are clearly seen in metaphase-II figures (Fig. 2 G), and seven monads are seen in telophase-II figures and in young spermatids (Fig. 2 H).

Supernumerary chromosomes

About 1/3 of both females and males examined had an extra, small-size chromosome in oocytes and spermatocytes at prometaphase and metaphase-I (Figs 3 A, 3 B, 3 C). During anaphase-I this univalent chromosome moved undivided toward one of the poles. Often it remained in the center until the two telophase plates were formed (Fig. 3 D), but was included eventually either in the egg nucleus or in the polar nucleus. The extra chromosome could be observed among the dyads of some metaphase-II figures. It is not clear whether it divided normally during the second maturation division, or it remained undivided and was included passively in either the egg nucleus or in the second polar nucleus. Several reduced oocytes and young spermatids were observed to have eight instead of seven chromosomes (Figs 3 E, 3 F).

Mode of reproduction

Eggs were deposited during, or shortly after, the

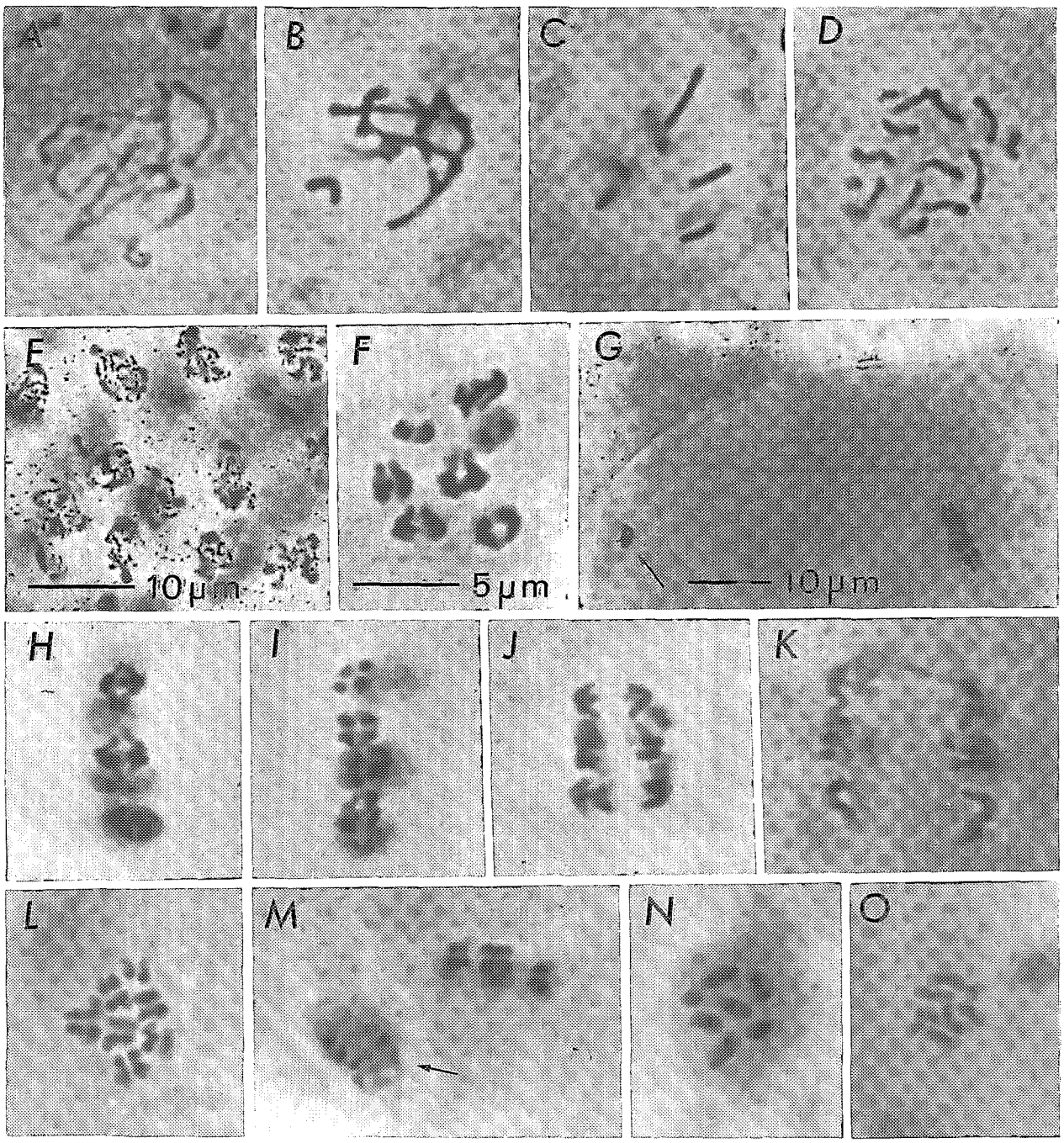

Fig. 1. The chromosomes of *Meloidogyne kikuyensis* during oogenesis : A : Early prophase of an oogonial division; B : Midprophase with condensed but interconnected chromosomes; C : Late prophase with distinct chromosomes; D : Early metaphase of an oogonial division; E : Zone of synapsis with oocytes at zygotene-early pachytene stage, typical of *Meloidogyne;* F : The seven diakinetic chromosomes of an oocyte shortly before it enters the oviduct-spermatotheca region; G : Oocyte at metaphase-I and a sperm nucleus (arrow); H : Typical metaphase-I figure in side view; I : Same as in Fig. 1 H but with two bivalents forming perfect tetrads, typical of *Meloidogyne;* J and K : Advanced anaphase-I figures with complex chromosomes (not perfect dyads) and some chromosomal bridges; L : Telophase-I in polar view; M : Metaphase-II in side view and the first polar body (arrow); N : Metaphase-II in polar view; O : Telophase-II in polar view, with six long and one short chromosome. (Scale for Figures 1 A-1 D and 1 H-10 as in Figure 1 F).

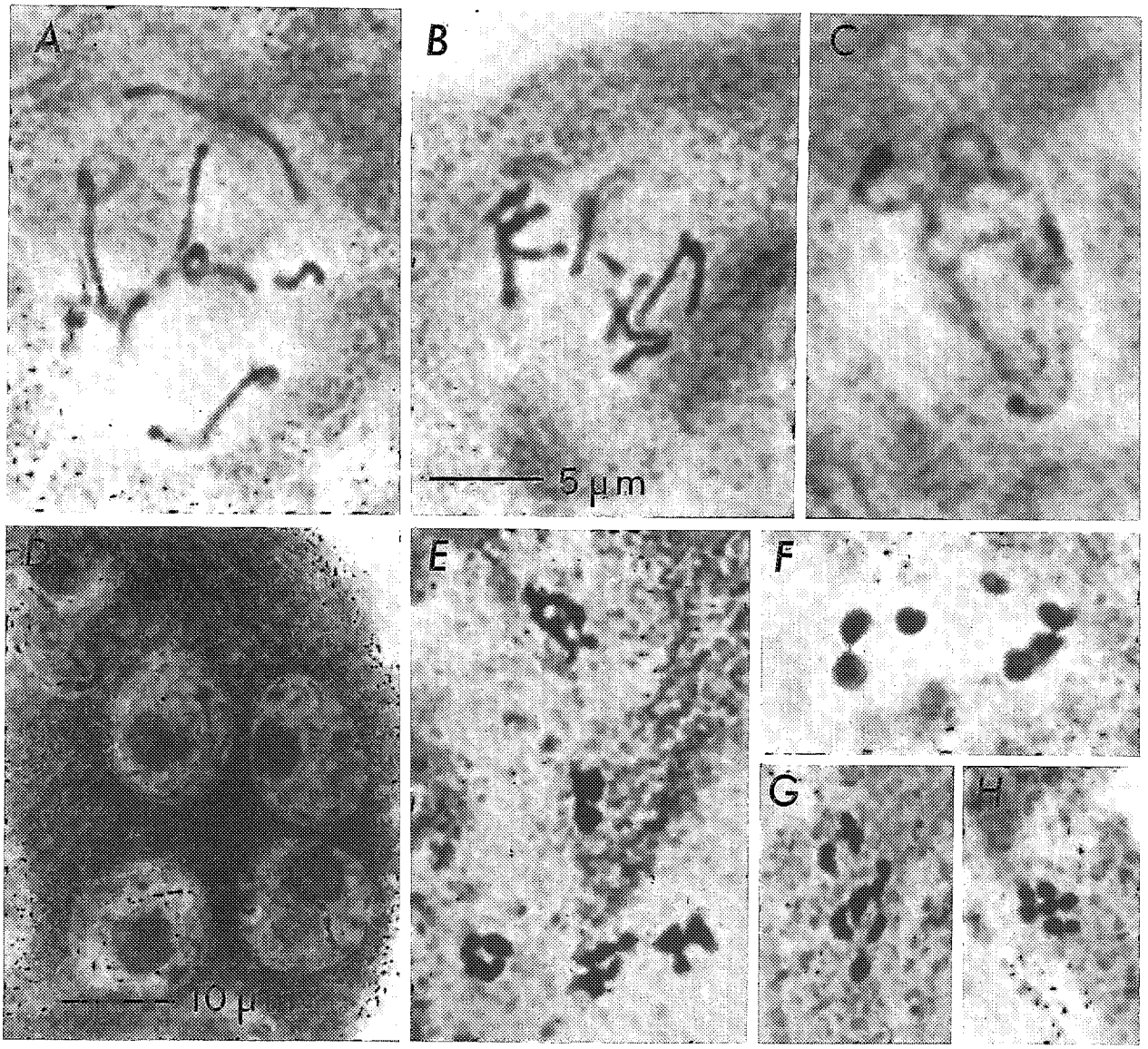

Fig. 2. Spermatogenesis in *Meloidogyne kikuyensis* : A : Early prophase chromosomes in a spermatogonium; B : Mid-prophase with condensed, interconnected chromosomes; C : Late pachytene chromosomes with pronounced heterochromatic ends; D : Spermatocytes at pachytene-early diplotene stage, with a large nucleolus inside the distinct nucleus; E : Five of the seven diakinetic chromosomes of an advanced spermatocyte; F : The seven metaphase-I chromosomes of a spermatocyte; G : Metaphase-II with seven dyads; H : The seven chromosomes of a spermatid. (Scale for Figures 2 A, 2 C and 2 E - 2 H as in Figure 2 B).

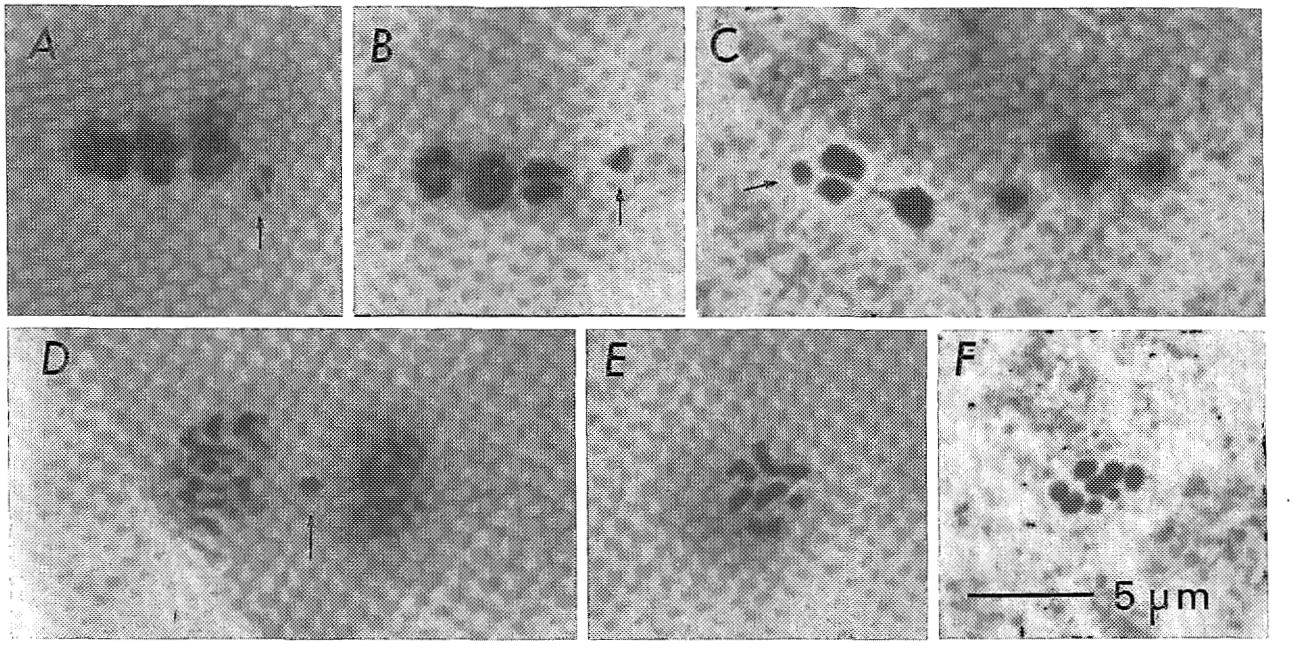

Fig. 3. Supernumerary chromosomes in gametocytes of *Meloidogyne kikuyensis* : A : Metaphase-I of an oocyte with an extra, univalent chromosome (arrow); B and C : Metaphase-I figures of spermatocytes with an extra chromosome (arrow); D : Telophase-I in an oocyte with the extra chromosome still remaining in the equatorial plane (arrow); eventually it will be included either in the egg nucleus or in the polar nucleus; E : A reduced oocyte with an extra chromosome; F : A spermatid with eight chromosomes — its regular set of seven and an extra one. (Scale for Figures 3 A-3 E as in Figure 3 F).

second maturation division was completed. Fusion of egg and sperm pronuclei, *i.e.*, actual fertilization, occurred after the eggs had been deposited in the egg sac. A few females (less than 1 %) secreted a large gelatinous mass that contained no eggs. Such females had no sperm in their spermatothecae and no oocytes in their uteri. These observations suggest that reproduction in *M. kikuyensis* is by obligatory amphimixis (cross fertilization).

Discussion

The present study has shown that *M. kikuyensis* is a unique root-knot nematode species with the following peculiar cytogenetic characteristics :

1. It has only seven chromosomes (haploid number) compared to 16-18 of most other *Meloidogyne* species.
2. Its chromosomes, both in oogonia and oocytes, are considerably larger than those of any other *Meloidogyne* species (Triantaphyllou, 1985*c*).
3. In oogonial prophases, its chromosomes are not as discernible as in other *Meloidogyne* species. This feature, however, may not represent a true deviation in mitotic behavior, but may be due to the larger size of the *M. kikuyensis* chromosomes that inevitably leads to interconnections between chromosomes.
4. Maturation divisions follow the same pattern as observed in other *Meloidogyne* species, but the prometaphase chromosomes rarely form the characteristic tetrads that are so common in other *Meloidogyne* species. Instead, they exhibit complex configurations probably as a consequence of their large size and the difficulty in undergoing complete and timely terminalization of chiasmata.

Besides *M. kikuyensis*, the only known root-knot nematode species with seven chromosomes is *M. (Hypsoperine) spartinae* whose generic status has been a controversial subject among nematode taxonomists. Cytogenetic studies have suggested that, in spite of its small chromosome number, this latter species should be regarded as a true root-knot nematode (Triantaphyllou, 1987). The anatomy of its oviduct-spermatotheca region, which is identical with that of females of most *Meloidogyne* species, provided strong supporting evidence that *M. spartinae* indeed belongs to the genus *Meloidogyne*. This view was formally adopted in a recent review of the genus *Meloidogyne* (Luc, Maggenti & Fortuner, 1988).

The oviduct-spermatotheca region of *M. kikuyensis* also appears to be similar to that of *Meloidogyne*. The oviduct, consisting of two rows of four thick epithelial cells, is definitely typical of the genus *Meloidogyne*. The

spherical spermatotheca is also typical in anatomy and general shape, but it deviates slightly in that its thick epithelial cells are relatively flat, with a less pronounced " lobe-like " shape than spermatothecal cells of most *Meloidogyne* species (McClure & Bird, 1976). Nevertheless, it can be accepted that *M. kikuyensis* has the same, characteristic oviduct-spermatotheca region as other *Meloidogyne* species.

The fact that both *M. kikuyensis* and *M. spartinae* have seven chromosomes suggests that they may have followed the same or a similar pathway of cytological evolution, different from that of other *Meloidogyne* species which have haploid chromosome numbers of 13-19. As discussed earlier (Triantaphyllou, 1987), the smaller chromosome number may more accurately represent the ancestral chromosomal form from which the rest of the root-knot nematodes have evolved. The obligatorily amphimictic mode of reproduction of *M. kikuyensis* and *M. spartinae* compared to the predominantly parthenogenetic reproduction of most other *Meloidogyne* species further supports the contention that these two species represent relatively ancestral forms of *Meloidogyne*. Obligatory amphimixis would indeed be expected in slowly evolving members of a biological group such as the genus *Meloidogyne*.

The chromosomes of *M. kikuyensis* are at least twice the size, of those of other *Meloidogyne* species. This size difference, which is difficult to interpret, leads to contradictory conclusions when evaluating the evolutionary relationship of the karyotypes of *M. kikuyensis* and the rest of the *Meloidogyne* species. Earlier it was assumed that chromosomal forms with n = 13-19 have evolved through polyploidization from ancestral forms with one-half as many chromosomes (Triantaphyllou, 1984). If a chromosomal form similar to *M. kikuyensis* is now considered as the true progenitor of root-knot nematodes, species with 13-19 chromosomes would have to be viewed as advanced forms that have evolved through fragmentation of the ancestral chromosomal complement. This process would give rise to forms with higher numbers of smaller chromosomes. Still, this alternative interpretation does not explain how forms like *M. spartinae*, with seven, small-size chromosomes, may be related to *M. kikuyensis*.

The extra, small-size chromosome observed in about 1/3 of both males and females of *M. kikuyensis* may represent a remnant of a regular-size chromosome that was partially translocated to another chromosome. It probably includes the centromere and a small heterochromatic portion of the original chromosomes. The observation that all primary gametocytes of an individual have this extra chromosome suggests that the extra chromosome divides normally during gonial divisions. It does not divide during the first maturation division and, most likely, even during the second maturation division. If this is the case, only 1/4 of the mature gametocytes of such individuals will carry the extra chromosome. At such a low frequency of transmission to the gametes this chromosome should be expected ultimately to be eliminated from the population, unless it confers a competitive advantage to the individuals that carry it. Its persistence in high frequency may also indicate that there is a cytological mechanism that favors its maintenance. It is not known whether this extra chromosome is unique to the single population of this study or is commonly present in other natural populations of the species.

The presence of an extra chromosome in *M. kikuyensis* may also suggest that the ancestral form of this species had eight chromosomes. A form with eight or, preferably, with nine chromosomes would fit better as the progenitor of the common, present-day root-knot nematodes, most of which have haploid numbers of 16, 17 or 18 and are presumed to be tetraploid. Further search may reveal other forms with eight or nine chromosomes that could qualify as more direct ancestors of root-knot nematodes.

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