

# Meiosis and mitotic chromosome numbers in certain species of the genus *Aphelenchoides*

Mohamed M. B'CHIR (\*) and Antoine DALMASSO

INRA, Station de Recherches sur les Nématodes, 123 boulevard du Cap, 06602 Antibes, France.

## SUMMARY

*Aphelenchoides besseyi* has six mitotic chromosomes at cleavage and a classical feature with three bivalents at diakinesis during the first meiotic division. In *A. fragariae* eight chromosomes have been observed during embryogenesis and two conspicuous cross-shaped tétravalents were seen at meiosis I. In *A. ritzemabosi* four diakinetid bivalents appeared from two diplotenic ellipsoidal masses. In *A. composticola* nuclei of the blastomeres,  $2n = 8$  chromosomes were present of which three were smaller. Changes in chiasmata during meiotic prophase would explain differences in previous countings at this stage.

*A. tuzeti* reproduces by obligate parthenogenesis. One bivalent and one univalent develop but meiosis is abortive; a single diploid polar body with three chromosomes is formed. Three mitotic chromosomes have been observed at early embryogenesis.

## RÉSUMÉ

### *Caryotypes méiotiques et mitotiques chez quelques nématodes du genre Aphelenchoides*

*Aphelenchoides besseyi* présente une évolution nucléaire classique avec six chromosomes mitotiques lors des divisions de segmentation et trois bivalents pendant la diacynèse. Chez *A. fragariae*, on a compté huit chromosomes au cours de la segmentation et la méiose se caractérise par deux tétravalents cruciformes. Chez *A. ritzemabosi*, quatre bivalents s'individualisent à la méiose à partir de deux masses chiasmatisques ellipsoïdales. Les noyaux des blastomères d'*A. composticola* possèdent un stock diploïde de huit chromosomes dont trois sont beaucoup plus petits. Des variations dans la formation des chiasmata pourraient être à l'origine des différences enregistrées chez cette espèce lors des comptages chromosomiques en méiose.

*A. tuzeti* se reproduit par parthénogenèse obligatoire. Un bivalent et un univalent sont élaborés à la méiose, mais celle-ci avorte; un unique globule polaire à trois chromosomes est évacué. Les figures nucléaires observées durant l'embryogenèse montrent que le nombre diploïde est  $2n = 3$ .

Little is known about the reproduction and karyotypes of species of *Aphelenchoides*. Only diplotene and diakinesis of the meiotic process have been studied in five species.

In *A. composticola* Franklin, 1957, Younès (1968) and Brun and Younès (1969) suggested

that irregularities occurring during meiosis render the interpretation of observations difficult. Dalmasso and Cayrol (1973) observed two large cross-shaped chromosomes in *A. fragariae* Christie, 1932 and two ellipsoidal chromatin masses in *A. ritzemabosi* (Schwartz, 1911) Goo-

(\*) Adresse actuelle : INAT, 43 avenue Charles Nicolle, Tunis, Tunisie.

dey, 1932. In the former species, the chiasmata occasionally formed circular figures, but more often crosses which persisted for a considerable time. In *A. ritzemabosi* four bivalents are formed at diakinesis from the two primitive masses. In fact, chiasmata seemed largely responsible for the variations of diakinetik chromosomes reported and may explain difficulties found in establishing reliable karyotypes in some species. The chiasmatic figures observed in an hybridization trial of *A. fragariae*, *A. ritzemabosi* and *A. besseyi* Christie, 1942 confirm this opinion (Dalmasso & Cayrol, 1973; Cayrol & Dalmasso, 1975). In this study, additional interpretations of these phenomena are described and the peculiar parthenogenetic reproduction of *A. luzeli* B'Chir, 1979 is discussed.

### Material and methods

Populations of five species of *Aphelenchoides* were obtained, each derived from one female, placed in a Petri dish containing either *Fusarium oxysporum* or *Allernaria citri* growing on malt agar (for *A. composticola*, *A. luzeli* and *A. besseyi*) or in glass tubes containing *Impatiens balsamina* growing on Skoog's medium (for *A. fragariae* and *A. ritzemabosi*).

Origins of the populations studied :

*A. composticola* : from cultivated mushroom, *Agaricus bisporus* (southern France).

*A. besseyi* (two populations) : from rice, *Oryza saliva* (Ivory Coast ; ) from rubber plant, *Ficus elastica* (Ivory Coast).

*A. luzeli* : from a pine forest of *Pinus halepensis* (southern France).

*A. fragariae* : from ornamental ferns, *Asplenium* sp. (southern France), from strawberry, *Fragaria vesca* (southern France).

*A. ritzemabosi* : from Chrysanthemum (southern France).

Dissected gonads and embryonated eggs were placed in Carnoy as fixative for 10 minutes and nuclei stained in Schiff reagent for 90 minutes.

Gonads were dissected in 9‰ NaCl. Embryo-

nated eggs were handpicked and five to ten eggs were treated on each side. An average of 500 eggs was examined for each species. As eggs of *A. besseyi* were too large and did not stick on the slide after fixation, glycerinated albumen from an hen's egg (Gabe, 1968) was used as an adhesive. After mounting, *Aphelenchoides* eggs were crushed gently in order to break the shell and to decrease stain retention in the cytoplasm. Observations were made on the following day.

### Results

Oogonial divisions in *Aphelenchoides* were rarely observed and located in germinal cells. Therefore mitotic counts can seldom be made at this stage and only on very young females. Early meiotic stages of oogenesis have been previously described. The variation in the arrangement of the chromatin filaments and the presence of loops during these stages made specific characterization of the chromosomes very difficult. Differences became apparent at the end of pachytene when chromosomes recondensed, thus making chromosome counts easier.

At this stage chromosome numbers recorded during meiosis could be compared to those of the somatic cells observed during embryogenesis.

#### MEIOTIC AND MITOTIC CHROMOSOME NUMBERS IN *A. besseyi*.

Prometaphase of cleavage divisions showed six chromosomes resembling sinuous threads (Fig. 1d), then as small club-shaped rods (Fig. 1e). Finally at metaphase six dense short spots were observed (Fig. 1f, g).

In meiosis of oogenesis, three compact chromatic rod-shaped structures contract progressively at diplotene and then condense to spherical metaphase chromosomes (Fig. 1a, b). Each maturation division forms two sets of three chromosomes (Fig. 1c, c').

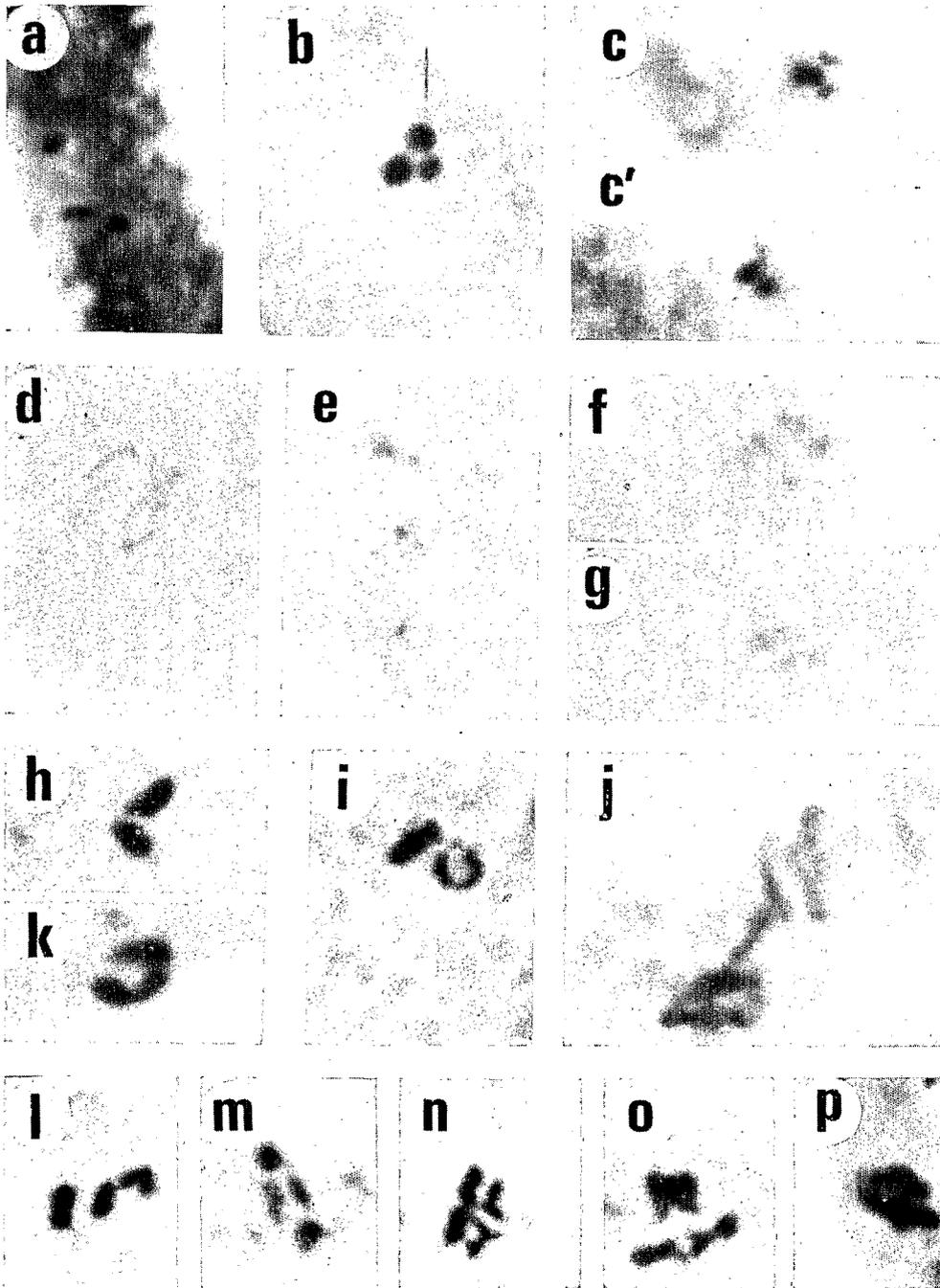


Fig. 1. Nuclear diakinetic evolution in an *A. besseyi* female ( $\times 5\,000$ ). a : Three bivalents ; b : meiotic I metaphasis showing three bivalents ; c, c' : anaphase I plates ; d, e, f, g : shapes of the six chromosomes during cleavage divisions. Nuclear evolution during meiosis I in *A. fragariae* ( $\times 5\,000$ ). h, i : diakinetic figures in oocytes ; j : one cross, the second is developing ; k, l, m, o, p : diakinetic evolution in spermatocytes. Progressive development of the two crosses.

MEIOTIC AND MITOTIC CHROMOSOME  
NUMBERS IN *A. fragariae* AND *A. ritzemabosi*.

In early stages of embryogenesis of *A. fragariae* eight very small chromosomes are present in metaphase (Fig. 2e, f) or proanaphase plates (Fig. 2g, g', h, i). However, chromosomes were difficult to count in the young morula because of their very small size and the irregular results using the squash technique.

The presence of eight chromosomes has been confirmed on oogonial divisions in freshly moulted females. In general, chromosome size seemed correlated with the number of cell divisions that occurred during embryogenesis. Cells at vegetative pole and more specifically the germinal cell, contained larger chromosomes than those of somatic lines (Fig. 2g').

During oogenesis of *A. fragariae*, diplotene chromosomes began to form two poorly defined chromatic masses (Fig. 1h). Two cross-shaped structures were formed (Fig. 1j); ring shaped chiasma described by Dalmaso and Cayrol (1973) were rarely observed (Fig. 1i). During spermatogenesis, the two primitive masses (Fig. 1k, l) produce four chromatic units (Fig. 1m) which pair forming two cross-shaped structures similar to those described in females (Fig. 1n, o, p). During spermatogenesis, as well as during oogenesis, two maturation divisions have been observed (Fig. 2a, b, c). Polar bodies were compounded of two chromatic masses which appeared to be composed of two parts in clear preparations (Fig. 2d, d').

Mitotic chromosome number in *A. ritzemabosi* is probably  $2n = 8$ . But because the size of the egg, observations were difficult. One good figure obtained in the oogonial zone seemed to fit this hypothesis (Fig. 3g).

In oogenesis of *A. ritzemabosi* first described by Dalmaso and Cayrol (1973) chromosomes are formed from two ellipsoidal masses (Fig. 3a, b, c, d) but chiasmata do not produce crosses as in *A. fragariae* and four bivalent chromosomes may soon be distinguished. Later two normal polar bodies are formed each with four chromosomes. Spermatogenesis is similar. Some prediakinetik figures resemble those of *A. fragariae* (Fig. 3e) but typical diakinesis forms four bivalents (Fig. 3f).

MEIOTIC AND MITOTIC CHROMOSOME  
NUMBERS IN *A. composticola*.

Younès (1968) and Brun and Younès (1969) thought that the meiotic chromosome number in *A. composticola* was  $n = 3$  in females and  $n = 2$  and 3 in males. Because of anomalies resulting from desynapsis, the meiotic chromosome number was very difficult to establish. Present observations of the blastomeres showed that, in fact, the mitotic number is  $2n = 8$  (Fig. 3h). But in this case, as seen clearly in Figures 3i and j, there are obvious differences in the size of chromosomes in every set of the metaphase of segmentation. Three are very small, one is very elongate and the four others which are large and thick are grouped in pairs and seem to have special affinities. That may explain why, during diakinesis, only three large units were visible and some might be associated as tri or tetra-valents. Earlier figures reported by Brun and Younès (1969) support this hypothesis.

REPRODUCTION AND CARYOTYPE  
IN *A. tuzeti*.

No males have been observed in this species. Populations can develop from a single larva but spermatozoa have not been found in the genital tract nor in maturing oocytes. Therefore it has been concluded that reproduction is by parthenogenesis (B'Chir, 1979). As with other species of *Aphelenchoides*, gonial divisions were rarely observed and metaphase chromosomes were located very close to each other making counting difficult. Nevertheless, in some nuclei, three chromosomes have been observed (B'Chir, 1979). In young maturing oocytes, chromatin appeared as a single thread (Fig. 3k). Soon after, two units were visible of which one splits resulting in three units. Two of these paired again as a bivalent in a short time (Fig. 3l, m), but re-separated (Fig. 3n) at condensation (Fig. 4a). At metaphase, three chromosomes were present (Fig. 4b). A single polar body with three chromosomes has been observed (Fig. 4c, d).

Mitotic divisions during embryogenesis showed three metaphase chromosomes, thus the mitotic number is  $2n = 3$  (Fig. 4g).

The oocyte undergoes a single maturation...

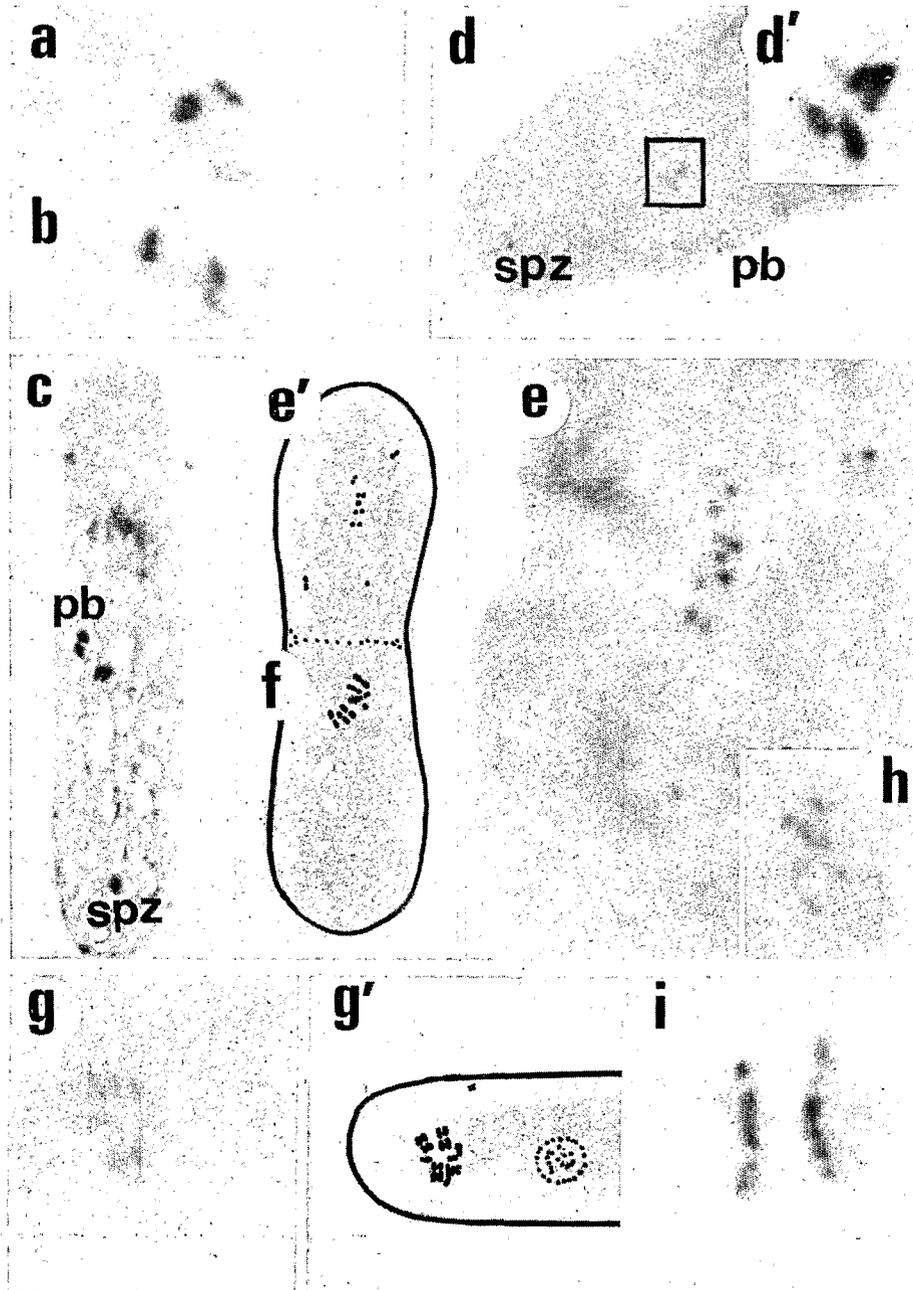


Fig. 2. Nuclear evolution in *A. fragariae*. a, b : oogenesis telophase of the first maturation division ( $\times 5\ 000$ ); c : first polar body (p.b.), spermatozoid (spz.) in oocyte undergoing first maturation division ( $\times 1\ 700$ ); d : second female maturation division ( $\times 1\ 700$ ); d' : detail of female meiotic II anaphase showing the two discrete couples of chromosomes ( $\times 5\ 000$ ); e, h, g : proanaphase of different cleavage divisions (chromosomes have been scattered by the squash) ( $\times 5\ 000$ ); e', g' : schema of related views; f : schema of cleavage metaphase ( $\times 1\ 700$ ); i : anaphase of cleavage division; lateral view.

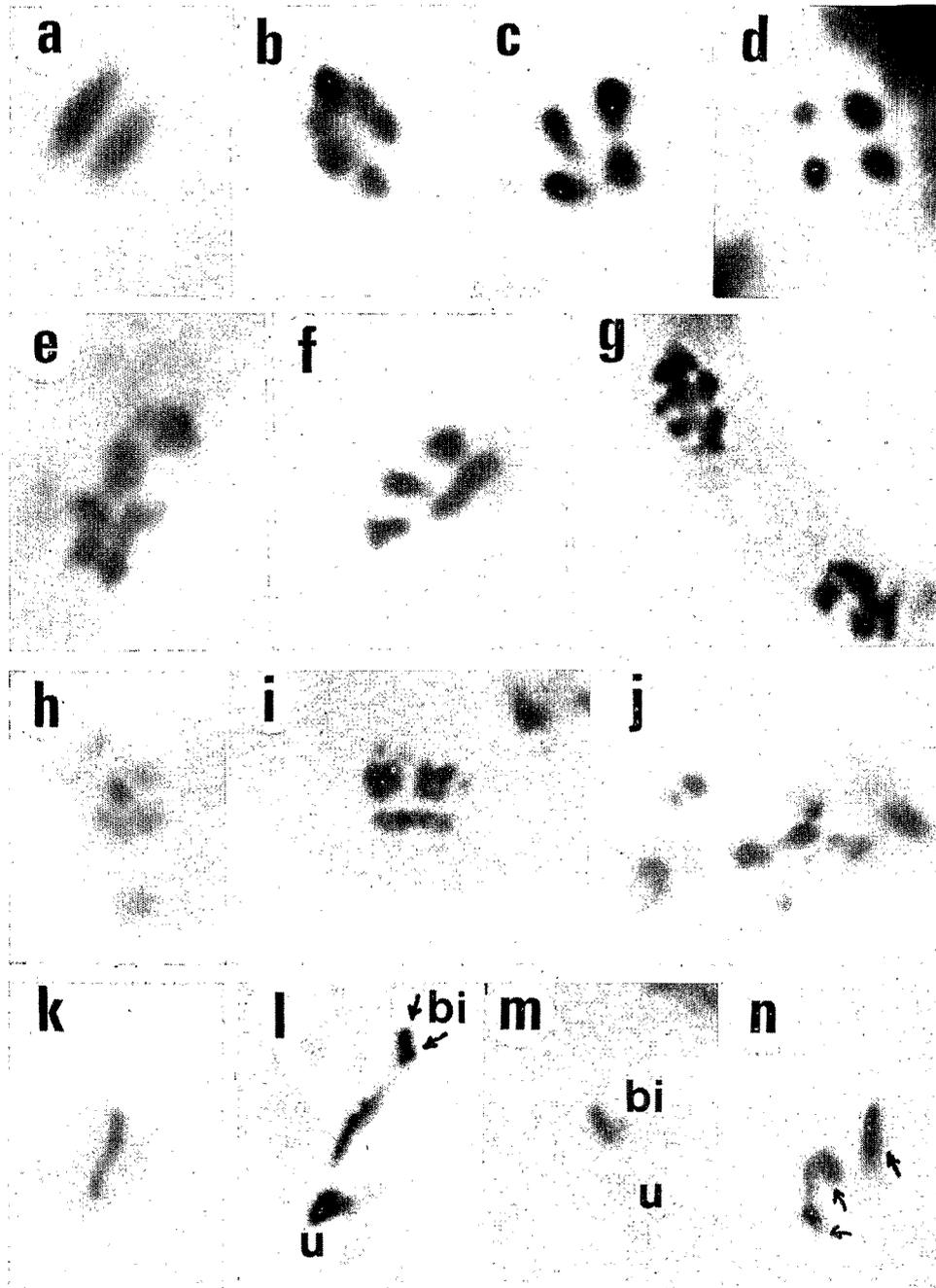


Fig. 3. Nuclear evolution in *A. ritzemabosi* ( $\times 5\ 000$ ). a, b, c, d : diakinetic evolution in oocytes until formation of four bivalents ; e, f : some diakinetic evolution in spermatocytes ; g : metaphase of oogonial division showing approximatively eight units. Nuclear evolution in *A. compositicola* ( $\times 5\ 000$ ). h : eight prometaphasic chromosomes during the third cleavage division ; i : metaphase during fifth cleavage ; j : proanaphase during a cleavage division (some chromosomes are duplicating). Nuclear evolution in *A. tuzeti* ( $\times 5\ 000$ ). k, l, m, n : pseudomeiosis in oocytes, bivalents (bi), univalent (u).

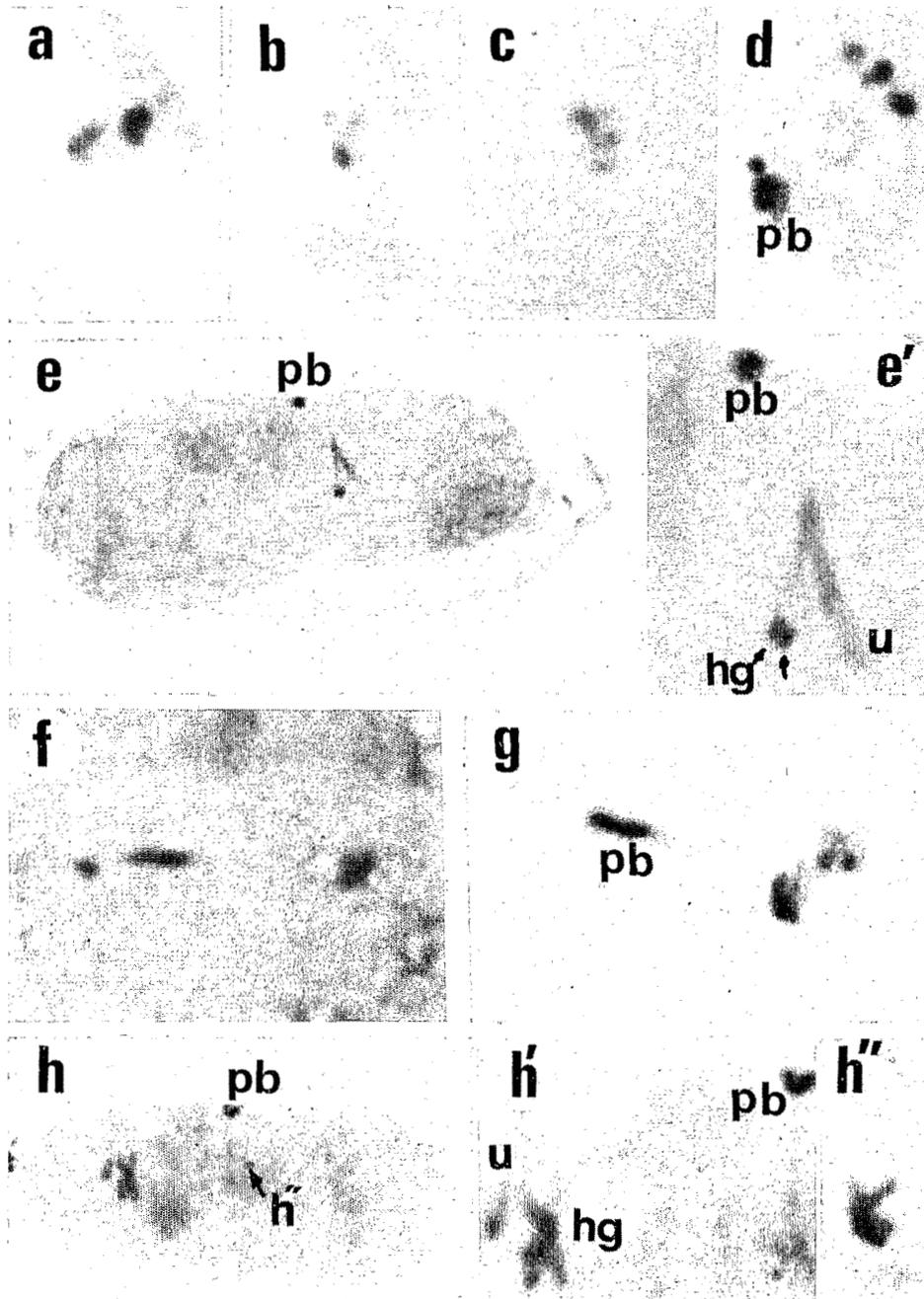


Fig. 4. Nuclear evolution in *A. tuzeti*. a, b : metaphase of the single maturation division ( $\times 5\ 000$ ); c, d : two plates each with three chromosomes during anaphase and telophase of maturation division ( $\times 5\ 000$ ); e, e' : prometaphases of the first cleavage ( $\times 1\ 700$  and  $\times 5\ 000$ , respectively), polar body (p.b.), homologous (hg), single chromosome (u) showing two daughter strands; f : 3 metaphasic chromosomes during a cleavage ( $\times 5\ 000$ ); g : anaphase of first cleavage, sets with three chromosomes and the single polar body; h : second cleavage egg ( $\times 1\ 700$ ); h' : detail of prometaphase plate showing a pair of homologous (hg), a single chromosome (u) and the polar body (p.b.) ( $\times 5\ 000$ ); h'' : other metaphase chromosomes belonging to the same egg, but at another focal plane ( $\times 5\ 000$ ).

division which initially is a normal meiosis but which fails at diakinesis. Supposedly diploidy is established by retention of the first polar body. Thus, the single polar body rejected is diploid. At the first segmentation division, it is still possible to observe the single polar body and the central diploid nucleus (Fig. 4e, e'). This nucleus contains three chromosomes at metaphase (Fig. 4f), but two of them exhibit special affinity perhaps due to a translocation. This affinity persists during the second cleavage (Fig. 4h, h', h''). This confirmed the low karyotype number observed during pseudomeiosis and the special relationship between two of the chromosome sets which are considered homologous. The third could be a univalent without a homologue.

## Discussion

As a general remark squashes might occasionally break the chromosomes rendering the interpretation difficult. On the other hand, since separate observations were made on various gonads or eggs, ordered sequences from early diakinesis to amphimixis were often difficult to establish.

Three different karyotypes have been found in *Aphelenchoides*:  $2n = 3$  (*A. tuzeti*);  $2n = 6$  (*A. besseyi*);  $2n = 8$  (*A. composticola*, *A. fragariae*, *A. ritzemabosi*). Such variability within a genus is similar to that reported in *Rhabditis* (Belar, 1923) and in *Pratylenchus* (Roman & Triantaphyllou, 1969). In addition, differences exist in the size of the chromosomes in *A. ritzemabosi* or in *A. tuzeti* karyotypes for example.

The two chromatic cross-shaped configurations observed in *A. fragariae* must represent four chromosomes (Fig. 1j, m, n, o), and both crosses are considered as tetravalent chromosomes. Their sizes agree with this hypothesis.

The four bivalents observed in *A. ritzemabosi* also arise from two ellipsoidal diplotene chiasmata (each grouping two bivalents; Fig. 3a, b, c, d). Thus we thought that we might be dealing in both species with some close phenomena

affecting the respective chromatin sets. The possibility of hybridization, previously reported (Cayrol & Dalmasso, 1975), even if it gives rise to sterile forms, would account for that.

In *A. tuzeti*, the univalent may indicate a loss of one chromosome rather than a supranumerary one as indicated by the perfect affinity of the two homologues.

Karyotype of *A. composticola* was difficult to establish in meiotic figures. The chromosome number visible during mitotic divisions was probably higher than the five or six chromosomes which had been previously reported by Brun and Younès (1969) according to observations during meiosis. It is possible that this species could possess five large and three small chromosomes. During meiosis some could be paired as bivalents, but others as trivalents thus accounting for the lower number of chromatin masses observed during metaphase I.

The low chromosome number recorded for *A. tuzeti* is not exceptional; Hechler (1968) reported that the Rhabditida, *Diploscapter coronata* which reproduces by mitotic parthenogenesis, has only  $2n = 2$  chromosomes. At present *A. tuzeti* is the only *Aphelenchoides* species known to reproduce by obligate parthenogenesis. So this process is not really associated with high chromosome number.

To explain the reduction in chromosome size observed in nuclei of cells located towards the animal pole during the segmentation process, two hypothesis can be forwarded: it would result either from an increase in chromatin condensation while the cells divide during embryogenesis or by chromatin decrease ("diminution") (not observed for technical reasons) as recorded in *Ascaris* (Boveri, 1909). In the case of the species of *Aphelenchoides* studied here, no chromatin rejection was noted. In other respects there is no difference in chromosome number between germinal and somatic cells that could arise from fragmentation related to polycentric phenomena generally associated in *Ascaris* with the chromatin decrease.

Several fundamental questions as to the basic chromosome number in *Aphelenchoides* or changes in chromosome size during embryogenesis remain unresolved and further investigations on the genetics and cytology within *Aphelenchoides* are needed.

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