Morphological comparisons of females, males, and second-stage juveniles of cytological races A and B of *Meloidogyne hapla* Chitwood, 1949

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Summary - The morphology of females, males, and second-stage juveniles of eleven populations of *Meloidogyne hapla* representing various cytological forms of two cytological races (A and B) was compared by light and scanning electron microscopy (SEM). Head shape of females as revealed by SEM was similar in all the populations except population 42-Can (15). Perineal patterns were variable among specimens and were not useful for distinguishing cytological forms or populations. The morphology of the stylets of females was similar in all populations. The head shape and stylet morphology of males of some populations differed. Minor differences occurred in the shape of the head capsule, presence of lateral lips, the shape of the head annule, and the contour of the head annule with respect to the body annule. The head shape of males of population 42-Can (15) were extremely different from the other populations. The shape of the stylet of males from populations of race A gradually increased in width posteriorly, whereas the shaft was cylindrical in males of race B. The shape of the head of second-stage juveniles as revealed by SEM was similar for most populations. Small differences in the expression of the labial disc and medial and lateral lips occurred among populations. The head shape of juveniles of population 42-Can (15) was very different from all other populations of *M. hapla*. The shape of the tail of second-stage juveniles was variable and not considered useful for distinguishing the various populations.

Résumé - *Comparaison morphologique des femelles, mâles et juvéniles de deuxième stade des races cytologiques A et B de Meloidogyne hapla* Chitwood, 1949 - La morphologie des femelles, des mâles et des juvéniles de deuxième stade de onze populations de *Meloidogyne hapla*, représentant différents types cytologiques des deux races cytologiques (A et B), a été étudiée en microscopy optique et électronique à balayage. L'examen au microscope électronique à balayage montre que la forme de la tête des femelles est semblable chez toutes les populations à l'exception de la population 42-Can (15). Les dessins périnéaux varient suivant les spécimens et ne ont pas utilisables pour différencier les formes cytologiques. La forme de la tête ainsi que la morphologie du stylet des mâles sont différentes chez quelques populations. Des différences mineures ont été observées en ce qui concerne la forme de la capsule céphalique, la présence de lèvres latérales, la forme de l'anneau céphalique et le contour de ce dernier par rapport à l'annélation du corps. La forme de la tête des mâles de la population 42-Can (15) est très différente de celle des autres populations. La hampe du stylet des mâles des populations de la race A s'épaissit graduellement à la partie postérieure alors qu'elle est cylindrique chez les mâles de la race B. De petites différences entre populations sont également observées dans le type de disque labial et celui des lèvres médianes et latérales. La forme de la tête des juvéniles de la population 42-Can (15) est toutefois très différente de celle de toutes les autres populations de *M. hapla*. La forme de la queue des juvéniles de deuxième stade est variable et ne peut servir à distinguer les différentes populations.

Key-words : cytological races, *Meloidogyne hapla*, morphology, root-knot nematodes, SEM.
distribution; for example, the diploid line \((2n = 30-32)\) is widely distributed in South Korea, but is not known to occur on other continents.

The purpose of the present study is to compare, by light and scanning electron microscopy (SEM), the morphology of the various cytological forms of \(M.\) \(h\)apla. Initial investigations have been reported previously (Eisenback & Hirschmann, 1979, 1981, 1982; Eisenback et al., 1980). Detailed morphometric studies and the results of host range experiments will be presented in future publications.

**Materials and methods**

Eleven populations of \(M.\) \(h\)apla differing in chromosome number and mode of reproduction were selected from the Meloidogyne collection at North Carolina State University. Each population had been characterized according to the North Carolina host differential response (Sasser & Hartmann, 1984), perineal patterns, biochemical esterase phenotype, and cytological examination (Triantaphyllou, 1984). The populations were designated by the collection number, an abbreviation of their geographical origin, and the chromosome number in parenthesis as listed in Table 1. All populations were maintained on tomato (Lycopersicon esculentum Mill. cv. Rutgers) in a greenhouse kept at 22-28 °C. Second-stage juveniles, males, and females were prepared for light and scanning electron microscopy as previously described (Eisenback & Hirschmann, 1979, Eisenback et al., 1980). At least 100 specimens of each life stage from each population were examined by LM and SEM.

**Table 1.** The origin and cytological data of populations of Meloidogyne hapla compared morphologically by light and scanning electron microscopy.

<table>
<thead>
<tr>
<th>Population name</th>
<th>Country of origin</th>
<th>Chromosome number</th>
<th>Cytological race</th>
</tr>
</thead>
<tbody>
<tr>
<td>E284-Hol (14)</td>
<td>Nederland</td>
<td>14</td>
<td>A</td>
</tr>
<tr>
<td>42-Can (15)</td>
<td>Canada</td>
<td>15</td>
<td>A</td>
</tr>
<tr>
<td>6-NC (16)</td>
<td>North Carolina, USA</td>
<td>16</td>
<td>A</td>
</tr>
<tr>
<td>86-Va (17)</td>
<td>Virginia, USA</td>
<td>17</td>
<td>A</td>
</tr>
<tr>
<td>E470-Kor (30)</td>
<td>South Korea</td>
<td>30</td>
<td>B</td>
</tr>
<tr>
<td>E471-Kor (31)</td>
<td>South Korea</td>
<td>31</td>
<td>B</td>
</tr>
<tr>
<td>465-Fra (43)</td>
<td>France</td>
<td>43</td>
<td>B</td>
</tr>
<tr>
<td>E15-Kor (45)</td>
<td>South Korea</td>
<td>45</td>
<td>B</td>
</tr>
<tr>
<td>48-NC (45)</td>
<td>North Carolina, USA</td>
<td>45</td>
<td>B</td>
</tr>
<tr>
<td>66-Md (45)</td>
<td>Maryland, USA</td>
<td>45</td>
<td>B</td>
</tr>
<tr>
<td>230-Chile (48)</td>
<td>Chile</td>
<td>48</td>
<td>B</td>
</tr>
</tbody>
</table>

**Results**

**Females**

*Head shape* (Fig. 1): As revealed by SEM, the heads of females of Meloidogyne hapla of all populations examined are very similar in morphology (Fig. 1 A, B), except for population 42-Can (15) (Fig. 1 C, D). In general, the slit-like stoma is centrally located on the labial disc within the oval shaped prestoma. Six small pore-like openings of the inner labial sensilla surround the stoma. Often the submedial sensilla open into the prestoma, whereas the lateral sensilla open onto the labial disc. Six lips surround the labial disc. The subdorsal and sub-ventral lip pairs combine to form one dorsal and one ventral lip. These lips also fuse with the labial disc and make up one smooth, continuous structure. Sometimes one or both of the medial lips may be indented medially. The small, triangular lateral lips usually merge with the ventral lip pair, but are separated from the dorsal lip by a shallow groove. Elongate, ovate amphidial apertures occur between the labial disc and lateral lips. One large smooth head annule, lying posterior to the lips, may be marked by transverse folds. The head annule may be difficult to distinguish from regular body annules, but often is wider than the first body annule or demarcated by a deep groove.

The head morphology of females from population 42-Can (15) (Fig. 1 C, D) is quite different from that typical for the species (Fig. 1 A, B). The medial and lateral lips are triangular and posteriorly extend further onto the head annule. The small, triangular lateral lips fuse almost totally with the ventral lip and a deep groove separates them from the dorsal lip. The head annule is similar to that of other \(M.\) \(h\)apla populations examined. The differences are not obvious with the light microscope (LM).

*Stylet morphology* (Figs 2, 3): The stylets of females of \(M.\) \(h\)apla populations are similar in overall morphology in both LM and SEM. The sharply pointed cone gradually increases in width along its entire length posteriorly and curves dorsally slightly. The cone is cylindrical to slightly tapered posteriorly, and the knobs are small, rounded, and set-off from the shaft. Differences among the populations of \(M.\) \(h\)apla are mainly in overall length and the distance of the DGO to the base of the stylet. These morphometric differences will be described and evaluated in a future manuscript.

*Perineal patterns* (Fig. 4): The shape of the perineal pattern of females of \(M.\) \(h\)apla is usually characteristic for the species; however, the patterns of some individuals are variable in overall shape and other structural details. In general the patterns appear as rounded hexagons (Fig. 4 A, B) to flattened ovals (Fig. 4 C-I). The striae are usually fine (Fig. 4 A-C) to moderately coarse (Fig. 4 D-G), and smooth to slightly wavy. The dorsal arch is usually flat and rounded (Fig. 4 A-C), but may be high and squarish in some specimens (Fig. 4 D-H). The lateral lines may be marked by slight irregularities in the striae (Fig. 4 B, G, H) or by dorsal and ventral striae that meet at an angle (Fig. 4 A, C-F). Often the dorsal and ventral sectors of the pattern are divided by a wide,
deep groove (Fig. 4 A, B, D, F, G) which is most obvious in SEM. In other specimens the lateral lines may be more inconspicuous (Fig. 4 I). Sometimes ventral striae may extend laterally to form one or two wings (Fig. 4 D-F). Subcuticular punctuations occur in the tail terminal area of all patterns, although they may not be readily apparent in poorly preserved specimens.

Males

Head shape (Figs 5-7): The shape of the head of males of most of the cytological populations of M. hapla is similar in gross morphology; however, small differences are evident as revealed by SEM (Figs 5, 6) and light microscopy (Fig. 7). The oral disc and medial lips are fused to form a medially elongate and rounded rectangle in most of the populations of the northern root-knot nematode. The head cap is relatively high and it does not extend posteriorly onto the head annule as in the other most common species (Eisenback et al., 1981). The head cap is rounded in lateral view. Lateral lips may be present in some populations or in some specimens, particularly in populations of race A. Lateral lips are common in males of population 86 (Figs 5 C, 6 C); they sometimes occur in population 6, but are infrequent in all other populations examined. The shape of the head cap of population E284-Hol (14) (Figs 5 A, 6 A) and 42-Can (15) (Figs 5 B, 6 B) is different from the other populations. In E284-Hol (14), the head cap is narrow and the lateral disc and dorsal lip pair are demarcated by shallow indentations on the lateral edges of the head cap. The head cap of population 42-Can (15) is narrower than that of population E284-Hol (14) and both medial lips are demarcated from the labial disc. Usually the indentations between the labial disc and the dorsal lip pair are more pronounced than those between the ventral lip. The shape of the head cap and the head annule of males of population 42-Can (15) is different from most other populations of M. hapla (Figs 5 B, 6 B), but the shape of the head cap is similar to population E284-Hol (14) (Figs 5 A, 6 A).

As viewed with the light microscope, the head shape of all the populations except populations 42-Can (15) (Fig. 7 B) and 465-Fra (43) (Fig. 7 E), are similar in shape. The head cap is rounded and its diameter is much narrower than that of the head annule. The head annule, on the other hand, is larger in diameter than the first body annule and appears set off. In population 42-Can (15) the head annule is nearly the same diameter as the first body annule and in the same contour (Fig. 7 B). The head region of population 465-Fra (43) is much smaller in diameter than the first body annule (Fig. 7 E). As a result, the head region and the larger body annules are not in the same contour.

Stylet morphology (Figs 7, 8): The stylet of males of M. hapla is small and thin, relative to the other common species (Eisenback et al., 1981). The cone gradually increases in width posteriorly, but the shaft is variable in shape. In most populations of cytological race A the shaft gradually increases in width along its entire length (Fig. 8 B, C), or it may narrow at the junction with the knobs (Fig. 8 A). The shaft is cylindrical in most populations of cytological race B (Fig. 8 D-F). In some specimens there is a slight protuberance posterior to the opening of the stylet lumen. The knobs are small, rounded, and set off from the shaft. The knobs of population E284-Hol (14) (Figs 7 A, 8 A) are smaller and do not project as far from the shaft and is similar to population 42 Can (15) (Figs 7 B, 8 B). The knobs of the race B populations (Figs 7 D-F, 8 D-F) often project further from the shaft and also slope posteriorly more than in populations of race A (Fig. 8 E, F). The distance of the DGO to the base of the stylet is moderately long, but it is quite variable depending on the population. In some populations the DGO is short (Fig. 7 B-E), but in others it is long (Fig. 7 A, F). Measurements of the stylet and distance of the DGO to the base of the stylet will be reported in a future manuscript.

Second-stage juveniles

Head shape (Figs 9, 10): The head shape of second-stage juveniles of different populations of M. hapla is similar, except for population 42-Can (15). In general the labial disc and medial and lateral lips are fused into a head cap. The labial disc may be slightly raised above the medial lips in some populations (Fig. 9 A, E, F), or in the same contour (Fig. 9 B-D). Lateral lips are present in all the populations except population 42-Can (15) (Figs 9 B, 10 B). The posterior edges of the medial lips of most of the populations are rounded to slightly indented medially (Figs 9 A, C-F; 10 A, C-F); however in population 42-Can (15) they are pointed medially. The head annules of all populations are large and smooth, without additional annulations.

Stylet morphology: The stylet of juveniles of M. hapla is delicate compared to the four most common species (Eisenback et al., 1981). The knobs are rounded and appear set off from the shaft.

Tail shape: The tail of second-stage juveniles of M. hapla is long and slender with a narrow, tapering terminus that may bear several distinct annulations. The hyaline tail terminus is not clearly demarcated and the tail tip is finely rounded to pointed. Morphological differences among the populations were not detected and differences in size will be reported in a future manuscript.

Discussion

When Chitwood (1949) originally described M. hapla, he presented several varieties based on differences in the shape of the head cap, stylet length, and distance of the dorsal esophageal gland orifice (DGO) to the base of the stylet in the male, and stylet length and DGO
Fig. 1. Scanning electron micrographs of heads of females of *Meloidogyne hapla*. A, B: Head shape typical for most populations of *M. hapla* examined; C, D: Head shape typical for population 42-Can (15). (All photographs are at the same scale.)
Fig. 2. Light micrographs of stylets of females of five populations of Meloidogyne hapla. A: Population E284-Hol (14); B: Population 86-Va (17); C: Population E470-Kor (30); D: Population 465-Fra (43); E: Population 230-Chile (48). (All photographs are at the same scale.)

Fig. 3. Scanning electron micrographs of excised stylets of females of five populations of Meloidogyne hapla. A: Population E284-Hol (14); B: Population 86-Va (17); C: Population E470-Kor (30); D: Population 465-Fra (43); E: Population 230-Chile (48). (All photographs are at the same scale.)
Fig. 4. Scanning electron micrographs of perineal patterns of females of *Meloidogyne hapla* showing variation within and among populations. (All photographs are at the same scale.)
in the female. He suggested that the morphological variations were controlled by genetic factors as is true for all species.

Some morphological characters of populations of *M. hapla* vary and others are quite stable. The head morphology of females of all populations studied was very similar except for one population, 42-Can (15). Scanning electron microscopy of the head is necessary to observe these differences in head morphology of the females because they are very small and easily obscured by the preparation of specimens for observation with the light microscope. The stylet shape of females was similar for all populations and remains a stable and useful character for the identification of *M. hapla*.

The stylet morphology of all the populations was very similar but not identical, and differences occurred within individuals of the same population. Minor differences occurred in the shape of the shaft and knobs. The major differences noted were in the distance of the DGO to the base of the stylet. These measurements will be reported in a separate paper.

Perineal patterns are widely used as the primary means of identifying species of root-knot nematodes. The morphology of the perineal patterns was extremely variable and many differences were observed. Variability was great between specimens of the same population and no characters could be related to cytological race or chromosomal form.

The basic shape of the perineal pattern of all the *M. hapla* populations was hexagonal to rounded; the striae were fine, and most of the details were variable. The most stable character of the species was subcuticular punctations in the tail terminal area. They were visible in all the specimens examined, although other species also have been reported to have these punctations; namely, *M. deomincki* Elmilig, 1968 and *M. lusitana* Abrantes & Santos, 1991.
A deep trough was observed separating the dorsal and ventral arches of the pattern in almost all the specimens examined. This depression of the lateral field may not be unique to *M. hapla*, but when combined with overall shape and the occurrence of punctations in the tail terminal area, it contributes to the identification of this species and the usefulness of the perineal pattern for identification.

The head shape and stylet morphology of males have been shown to be useful characters for the identification of species of *Meloidogyne* (Eisenback & Hirschmann, 1980). In this study these characters were quite variable. The major differences were in the shape of the head cap and the diameter of the head annule in comparison to the first body annule. Minor differences resulted from the occurrence of lateral lips. Although differences in head shape occurred in comparisons of males of *M. hapla* populations from around the world, the variation among populations in a more limited geographical region is likely to be smaller.

The typical head shape occurred in all the populations except two, 42-Can (15) and 465-Fra (43); however, stylet morphology was more variable. The stylets varied in the shape of the shaft and knobs. For many populations, combining the characters of the head shape and stylet morphology made them unique. Individual populations could be identified as such. Stylet morphology was correlated with cytological race: the shaft widened posteriorly in all populations of race A, whereas it was cylindrical in all populations of race B.

The head shape and stylet morphology of second-stage juveniles has been shown to be a stable and useful character for species identification. The typical head shape as revealed by light microscopy occurred in all the populations. One population, 42-Can (15), had unique head morphology as revealed by SEM. The head cap of population 86 was also slightly different from the typical head morphology. Unfortunately, their small size makes the head and stylet of second-stage juveniles unsuitable...
Fig. 7. Light micrographs of the head of males of six populations of *Meloidogyne hapla*, in lateral view. A: Population E284-Hol (14); B: Population 42-Can (15); C: Population 86-Va (17); D: Population E470-Kor (30); E: Population 465-Fra (43); F: Population 230-Chile (48). (*All photographs are at the same scale. Figs B, C, F from Eisenback and Hirschmann, 1981.*)
for observation in the light microscope for routine identifications.

*Meloidogyne hapla* is one of the four most common species of the genus (Sasser & Carter, 1985). Based on differences in the morphology of the head and stylet morphology, several variants of *M. hapla* exist. Morphological variations are controlled by genetic factors and may be related to the method of reproduction.

Variants of *M. hapla* have been detected in relation to DNA restriction fragment length polymorphisms. The two cytological races, A and B, appear to have different EcoRI restriction fragment length polymorphisms (Curran et al., 1986). These differences were not conclusively shown to be correlated with cytological race, however, because only one population of each race was examined.

Populations of *M. hapla* have very similar phenotypes for several enzymes (Esbenshade & Triantaphyllou, 1985). Ninety-four percent of the 34 populations of both race A and B examined by Esbenshade and Triantaphyllou could be identified on the basis of esterase phenotype alone. The two populations that were variant in esterase phenotype patterns were both from race A. The superoxide dismutase (Sod), malate dehydrogenase (Mdh), and glutamate-oxaloacetate transaminase (Got) phenotypes of *M. hapla* were the same for all populations examined, but they were different from that of *M. arenaria* (Neal, 1889) Chitwood, 1949; *M. incognita* (Kofoid & White, 1919) Chitwood, 1949; and *M. javanica* (Treub, 1885) Chitwood, 1949. The Sod, Mdh, and Got phenotypes of *M. hapla* were similar in some aspects to that of *M. carolinensis* Eisenback, 1982; *M. chitwoodi* Golden, O'Bannon, Santo & Finley, 1980; *M. graminicola* Golden & Birchfield, 1965; *M. microtyla* Mulvey, Townshend & Potter, 1975; *M. naasi* Franklin, 1965; *M. oryzae* Maas, Sanders & Dede, 1978; *M. platani* Hirschmann, 1982; and *M. querciana* Golden, 1979.
The host response to *M. hapla* populations varies little (Sasser, 1979; Riggs, 1991). At least one population has been reported parasitizing marigolds (*Tagetes erecta* L.) (Eisenback, 1987), *M. hapla*-resistant tomato (*Lycopersicon esculentum* Mill.) (Stoyanov, 1979), Charleston Grey watermelon (*Citrullus vulgaris* Schrad.) (di Vito & Greco, 1982), and *M. hapla*-resistant peanut (*Arachis hypogaea* L.) introduction lines (Miller, 1971). Selection for virulence to cucumber (*Cucumis sativus* L.) was demonstrated in one population of *M. hapla* (Stephen, 1982) which revealed a mixture of genotypes. Host range tests have also indicated the possibility of host races of *M. hapla* (Brzeski & Basik, 1981); however, reproduction of *M. hapla* on barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) may be evidence of a mixture of species or the occurrence of a new species. In the Pacific Northwest of the United States, populations of *M. hapla* were thought to be host races that reproduced on barley and maize (Ogbuji & Jensen, 1972). These populations were probably *M. chitwoodi* that was described in 1980 (Golden et al., 1980).

In conclusion, these studies show that the morphology of cytological races A and B of *M. hapla* is similar for some characters and different for others. The stylet morphology of the females is considered to be the most stable character examined; whereas the stylet morphology of males is correlated with cytological race. The perineal patterns show considerable intraspecific variation but are useful for identifying this species from others. The male head shape and stylet morphology also show considerable intraspecific variation, but are useful for species identification. The head shape and stylet morphology of second-stage juveniles are stable characters with little intraspecific variation, but they may be too small to be of practical value. Population 42-Can (15) is quite unique in the morphology of the head of females, males, and second-stage juveniles, but other characters such as perineal pattern, female stylet morphology, host response, and esterase phenotype support its retention as *M. hapla*.
Fig. 10. Scanning electron micrographs of heads of second-stage juveniles of six populations of *Meloidogyne hapla*, in lateral view. A: Population E284-Hol (14); B: Population 42-Can (15); C: Population 86-Va (17); D: Population E470-Kor (30); E: Population 465-Fra (43); F: Population 230-Chile (48). (All photographs are at the same scale; Figs B and C from Eisenback and Hirschmann, 1979.)

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