

Neoplastic Growths in Preparasitic Juveniles of *Meloidogyne incognita*¹

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Aberrant morphological forms have been reported for a number of free-living and plant-parasitic nematodes. In some cases aberrancy is genetic (4) and in others it is environmentally induced (5,6). More than 200 mutant genes known to control aberrant phenotypes of *Caenorhabditis elegans* (Maupas) Dougherty have been reported (4). Some of these mutants affect collagen synthesis and cuticle formation (4). Anal protrusions of *C. briggsae* (Dougherty & Nigon) Dougherty occurred when a cell-free extract of *Flavobacterium* was incorporated into the culture medium (6). Aberrant second-stage larvae of *Heterodera schachtii* (A. Schmidt) developed when this nematode was reared on insecticide-treated hosts (5).

In the current study, external growths were observed on freshly hatched juveniles (J2) of *Meloidogyne incognita* (Kofoid & White) Chitwood from a single egg-mass population that had been maintained in the greenhouse continuously for more than 15 years. Aberrant specimens were examined by light and electron microscopy to determine cytological details of the deformations.

Eggs of *M. incognita* (7) were collected (8) from greenhouse cultures of eggplant (*Solanum melongena* L. cv. Black Beauty) and hatched in distilled water on nylon cloth (TETKO, Inc., Elmsford, NY) with a pore size of 20 μm . Infective J2 that hatched and migrated through the nylon cloth were

collected every 24 hours and examined with a stereo microscope for gross deformations. Aberrant nematodes were mounted on glass microscope slides for light microscopy and photographed with interference contrast optics. For electron microscopy, hand-picked nematodes were fixed in 3% glutaraldehyde in 0.06 M phosphate buffer (pH 7.3) for 90 minutes at room temperature, rinsed three times with five volumes of 0.06 M phosphate buffer (pH 7.3), post-fixed with 1.5% osmium tetroxide in phosphate buffer, and dehydrated in an acetone series (10% increments). Dehydrated specimens were infiltrated with Spurr's 'B' in acetone (10% increments), and the blocks were cured at 60 C for 18-24 hours. Silver sections were cut and mounted on 74- μm (200-mesh) copper grids. Mounted sections were stained at room temperature for 20 minutes in 2% aqueous uranyl acetate, post-stained with Reynold's lead citrate for 10 minutes, and photographed on a Hitachi H-500 electron microscope at 75 kV.

Frequency of nematodes with deformations increased in proportion to the length of time required for hatching. One J2 per thousand after the first 24 hours and five J2 per thousand after 7 days were noticeably deformed. Aberrant growths affected the entire body of the J2 or were limited to local perturbations (Fig. 1). No organisms were detected within the tissues of the nematodes examined by electron microscopy. Symptoms were manifested only in the body wall (Fig. 2), especially in the cuticle. The striated basal layer, typical in preparasitic juveniles (1), was reduced, malformed, or lacking altogether (Fig. 2B, C). In areas outside the protuberance, the striations of the basal layer lacked unifor-

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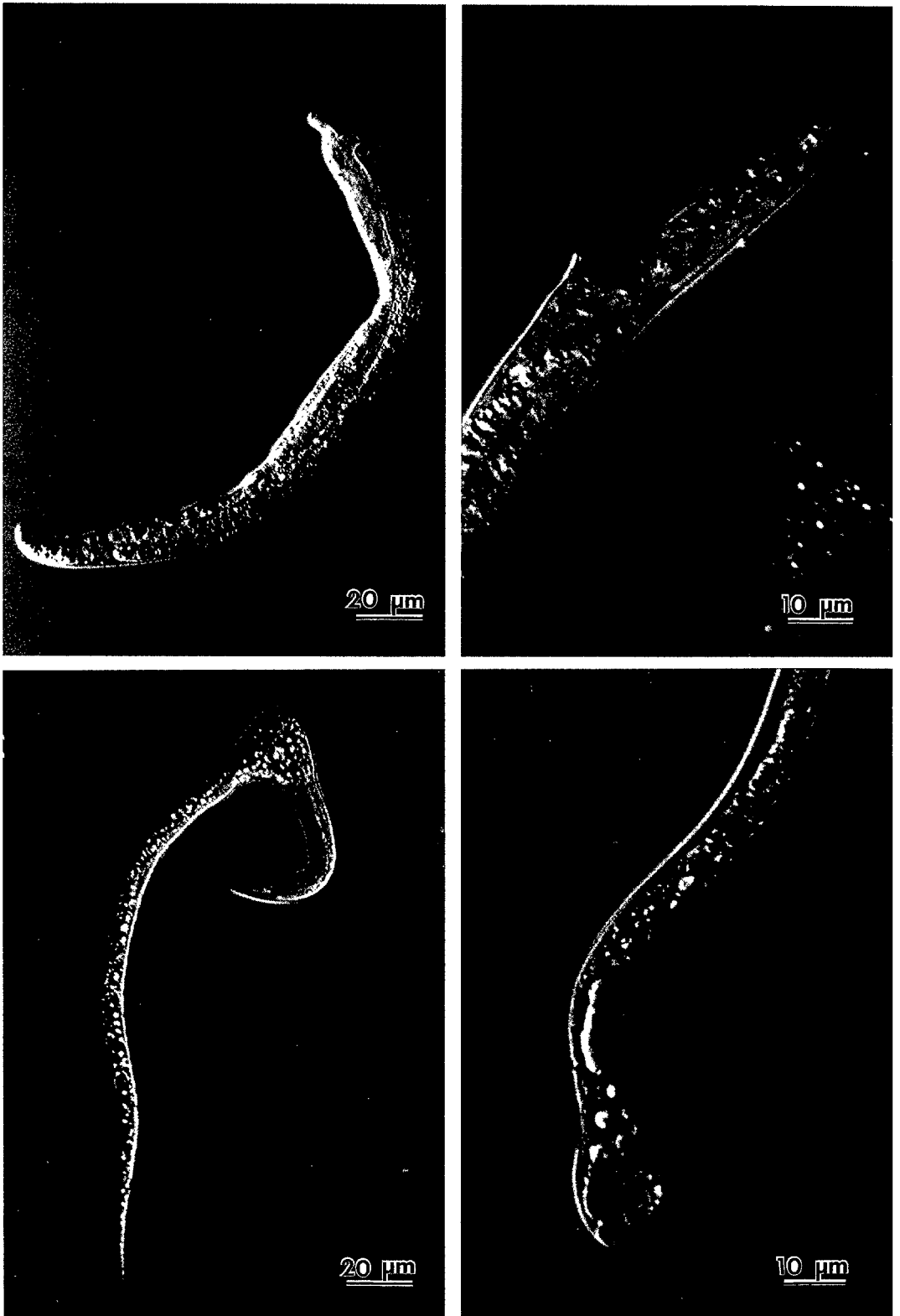


FIG. 1. Neoplastic growths on preparasitic juveniles of *Meloidogyne incognita*.

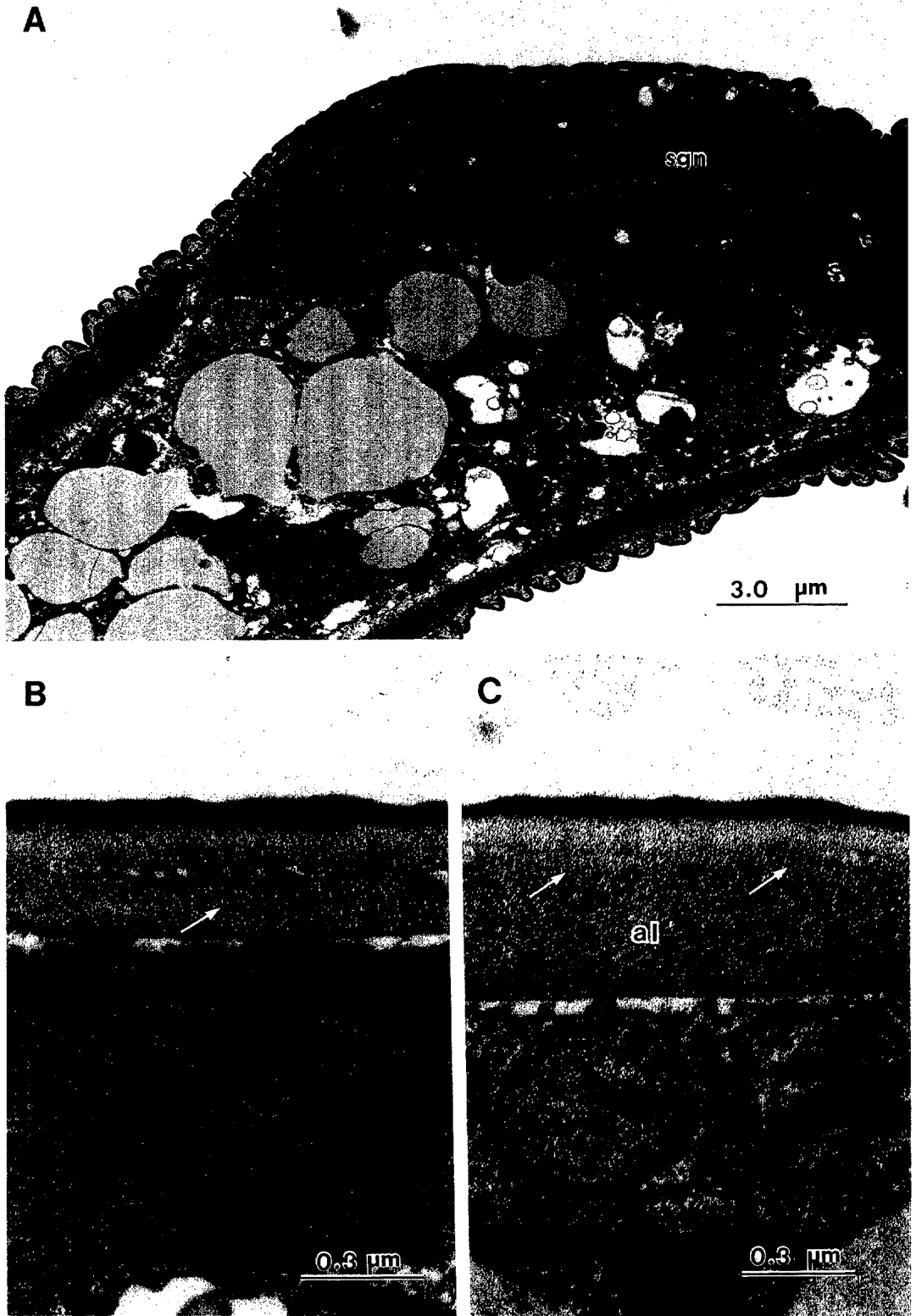


FIG. 2. Parasagittal sections of preparasitic juveniles of *Meloidogyne incognita*. A) Section through a neoplasm in the region of the esophageal glands. sgn = subventral gland nucleus. B) Section through an area adjacent to a neoplasm. Basal striated layer of cuticle (arrow) is present, but malformed. C) Section through the neoplasm shown in Figure 2A. Basal striated layer of cuticle (arrows) is reduced or lacking. It is subtended by a thick amorphous layer (al).

mity in width and orientation (Fig. 2B). The intensity of perturbation decreased in sections cut further from the protuberances. The region normally occupied by the striated basal layer contained, instead, a thickened amorphous layer which sometimes contained a sublayer of extremely fine fibrils (not shown), parallel and adjacent to the basement membrane. Body wall muscles were lacking in the affected area (Fig. 2B). It was apparent that the swellings and distortions could be attributed to these defects in the body wall, since the basal striated layer is thought to maintain cuticular integrity. Aberrant nematodes moved more slowly than their healthy counterparts and movements were jerky and uncoordinated. Most were capable of stylet thrusting activity and produced amphidial secretions when placed in Coomassie brilliant blue stain (9). Of 50 aberrant specimens placed on the roots of tomato (*Lycopersicon esculentum* Mill. cv. Bonnie Best), however, none penetrated. Twenty-six J2 without deformations penetrated and developed past the second molt in 12 days.

Since new tissues are formed, the protuberances and other defects, such as forked tails, may properly be designated neoplasms. The cause of neoplastic growths in J2 of *M. incognita* is unknown. Elimination of microbial contamination from hatching eggs is not practical, and the number of affected J2 increases proportionate to length of incubation. Therefore, microbial constituents or metabolites may be responsible, as they are in *C. briggsae*. However, the neoplasms obviously occur during formation of the J2 cuticle when the egg is impermeable to substances with molecules as small as osmium tetroxide (2). The effects of exogenous substances during this stage probably are minimal. Another explanation for the relationship between incubation time and frequency of emerging J2 with neoplasms is that nematodes with neoplasms require longer to emerge from the egg, a supposition supported by their inhibited movements.

A striking resemblance was noted be-

tween the neoplasms reported here and certain genetic defects recorded for *C. elegans*. Some dumpy mutants of *C. elegans* not only are similar in appearance to the specimen in Figure 1, but produce individuals with lumps and protuberances as well (4). Many of these show aberrant cuticular structure (3). If the neoplasms of *Meloidogyne* J2 have a genetic basis, the mutations may arise spontaneously or be induced by cultural conditions. Applications of the insecticides Malathion (o,o-dimethylphosphorodithioate of dithiolmercaptosuccinate) and Isotox (1,2,3,4,5,6-hexachlorocyclohexane) increased the number of aberrant *H. schachtii*, but aberrancy was not inherited (5). Malathion and other insecticides have been applied over the past 20 years to the host plants sustaining the population of *M. incognita* used in this study, and the effect of these treatments will be investigated. Studies on heredity, however, must await the establishment of reproductive populations of aberrant forms.

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