Observations on the embryonic and post-embryonic development of *Diploscapter orientalis* (Nematoda : Rhabditida)

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SUMMARY

Diploscapter orientalis reproduces parthenogenetically. The eggs are elongate, measuring 45-55 × 20-30 µm with blunt spines on their surface. Most eggs are laid in the single cell stage. Hatching takes about 13-17 h from the time of oviposition. The newly hatched juveniles lack labial hooks which develop later in the second stage juveniles. The genital primordium in the early juvenile stage is oriented obliquely to the longitudinal axis. In late stages the germinal nuclei shift to the two ends while the somatic ones remain in the centre of primordium. The division of primordial nuclei is restricted to the periods of moulting but becomes continuous after the third moult. The life cycle from egg to adult is completed in 4-6 days.

RÉSUMÉ

Observations sur le développement embryonnaire et postembryonnaire de Diploscapter orientalis *(Nematoda : Rhabditida)*

Diploscapter orientalis se reproduit parthénogénétiquement. Les œufs, allongés, mesurent 45-55 × 20-30 µm et sont pourvus à leur surface d'épines émoussées. La plupart des œufs sont pondus au stade unicellulaire. L'éclosion a lieu environ 13 à 17 heures après la ponte. Les juvéniles qui viennent d'éclore sont dépourvus de crochets labiaux, lesquels se développeront plus tardivement, chez les juvéniles de deuxième stade. Chez les juvéniles de premier stade, le primordium génital est orienté obliquement par rapport au grand axe du corps. Chez les juvéniles des stades ultérieurs, les noyaux germinaux migrent vers les deux extrémités du primordium tandis que les noyaux somatiques demeurent au centre de celui-ci. La division des noyaux primordiaux est limitée aux périodes de mues, mais elle devient continue après la troisième mue. Le cycle d'œuf à adulte est accompli en 4 à 6 jours.

Nematodes of the order Rhabditida have since long been used in the study of developmental biology. Chuang (1962) and Hechler (1968) studied the embryonic and post-embryonic development of *Pelodera teres* and *Diploscapter coronata* respectively. More recently, the developmental biology of rhadbitids have been centred on *Caenorhabditis elegans* (Byerly, Cassada & Russell, 1976; von Ehrenstein & Schierenberg, 1980). Horvitz and Sternberg (1982) reviewed the cell lineage patterns of *C. elegans* and *Panagrellus redivivus*. Recently, the developmental biology of the rhabditid *Teratorhabditis andrassyi* was studied (Tahseen & Jairajpuri, 1988). In the present study an account of embryonic and post-embryonic development of *Diploscapter orientalis* is given.

Materials and methods

Soil samples containing *Diploscapter orientalis* were collected from Zoology Dept., AMU, Aligarh and processed by sieving, decantation and modified Baermann's funnel techniques. The nematodes so obtained were cultured in 1.5 % water agar (1.5 g of agar in 100 ml of water) in 5 cm diam. Petri dishes. 5 mg of milk powder (Lactogen) was spread over the agar surface to enhance the growth of bacteria, serving as diet of the nematodes. Embryonic development was studied in chambers similar to those designed by Ahmad and Jairajpuri (1979). Freshly laid eggs (n = 30) were observed continuously to determine the cleavage patterns. Terminology used by von Ehrenstein and Schierenberg (1980) was followed to denote stages during the embryonic development.

Post-embryonic development was studied by staining the juvenile stages in 2 % lacto-aceto-orcein for 2 h (2 g orcein stain + 33 parts lactic acid + 33 parts acetic acid + 34 parts distilled water). The juveniles were destained in 45 % lactic acid, if required. At least 40-50 juveniles of each stage were studied.

Results

The species *Diploscapter orientalis* was parthenogenetic with only females in the population.

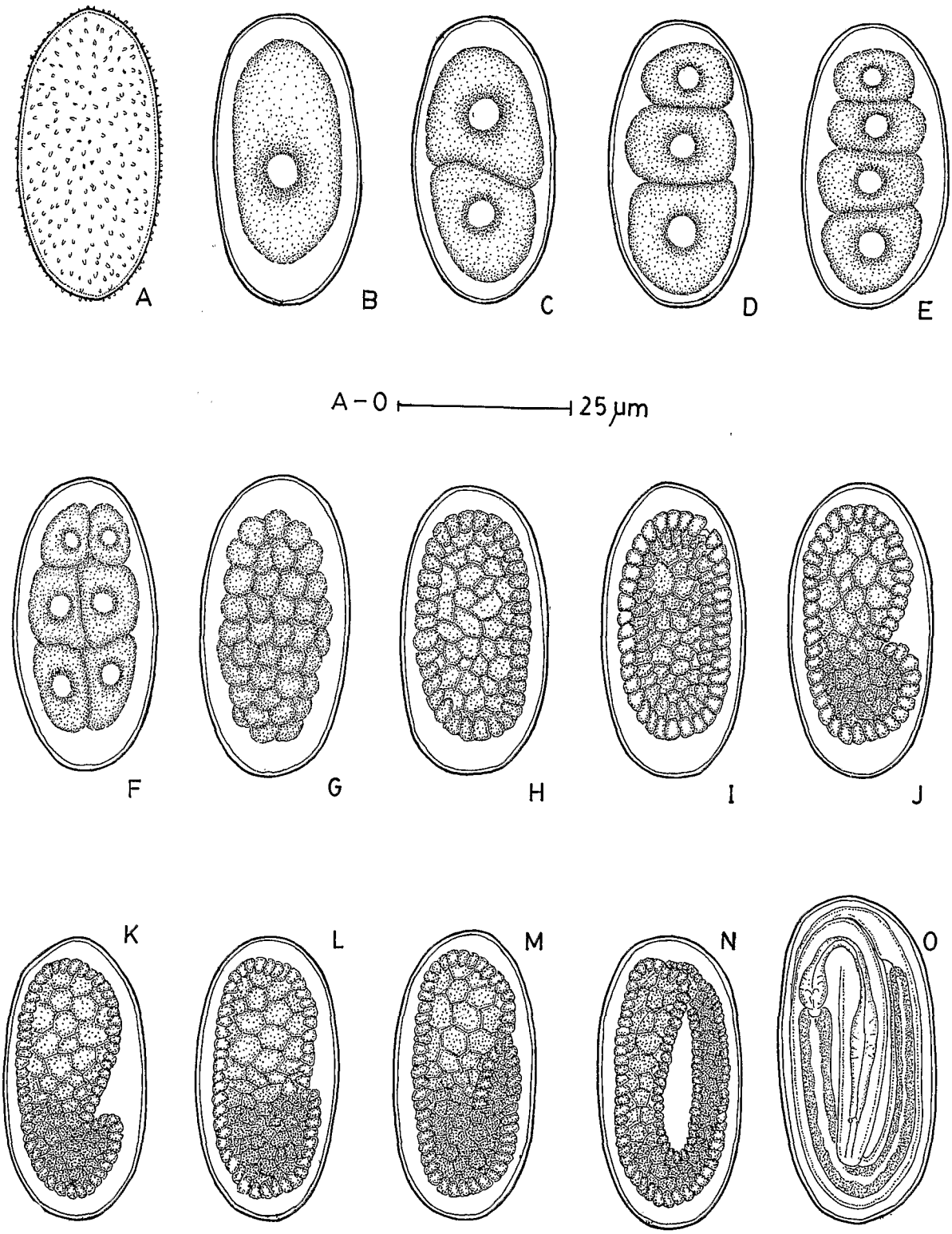

Fig. 1. *Diploscapter orientalis.* Embryonic development. A : Egg shell; B : Single-cell stage; C : Two-cell stage; D : Three-cell stage; E : Four-cell stage; F : Six-cell stage; G : Morula stage; H : Blastula stage; I : Gastrula stage; J : Lima bean stage; K : Comma stage; L : Tadpole stage; M : Plum stage; N : Loop stage; O : Pretzel stage.

EGG

The eggs were elongate measuring 38-48 × 20-30 (45 × 25 ± 0.02) µm with shells having blunt spines (Fig. 1A). No intra-uterine development occurred and the eggs laid were in single-cell condition (Fig. 1B).

EMBRYOGENESIS

The first cleavage occurred 15-20 min after egg laying and was transverse to the longitudinal axis (Fig. 1C). Of the resulting blastomeres, the anterior S_1 was larger than the posterior P_1. The anterior blastomere divided transversely into A and B blastomeres after 5-10 min (Fig. 1D). After 10-15 min. A divided horizontally into A_1 and A_2, the latter divided again in a vertical oblique plane into A'_2 and A''_2 resulting in a five-cell condition. The six-cell stage (Fig. 1F) was formed as a result of division of P_1 into S_2 and P_2, 30-40 min after first cleavage. The egg was in sixteen-cell stage 25-30 min after six-cell stage. The morula developed 40-50 min later or 2.5-3 h after first cleavage. The cleavage patterns from sixteen-cell to morula stage could not be traced because of heavy granulation and rapid proliferation and movement of blastomeres. About 5 h after the first cleavage the gastrula was formed. At this stage hyaline and granular regions were demarcated, the former occupying the larger half of the embryo. As an invagination developed in the granular region, the lima bean stage was formed. The deepening of invagination produced the comma stage first and tadpole stage later. The first movement in embryo occurred in the tadpole stage i.e. about 6-7 h after first cleavage. The plum stage, formed 20-25 min after tadpole stage, brought about a slight increase in the movement of embryo and also the development of a depression at the anterior end which represented the future stoma. Loop stage was characterized by a two egg-fold embryo and was later followed by early pretzel stage after 30-40 min or 7.5-10 h after first cleavage. This stage embryo had an elongate cavity in the anterior region representing the stoma and a long conoid tail posteriorly. The oesophago-intestinal junction, intestine and faint outline of oesophagus appeared 30-35 min after the embryo attained 2.5 egg-fold length. The stomatal rhabdions first appeared as refractory lines and became faintly visible. The early pretzel stage lasted for about 1-1.5 h and led to the late pretzel stage (Fig. 1O). During the latter stage the intestine, oesophagus and rectum were well formed and also the valvular plates of oesophagus. The tail narrowed attaining a somewhat filiform appearance. The rhabdions became well differentiated and labial region showed smooth cuticle without any hooks. The length of the juvenile prior to hatching was approximately 3.5-4 egg-folds. The fully formed juvenile within the eggshell showed continuous movement with short periods of quiescence. It probed the shell, which as a result stretched making its layers thinner. In the end a slit was formed for the exit of the juvenile. Retraction of the juvenile into the shell after an initial thrust forward, was observed. After a few minutes it left the shell and moved about in the medium. The time taken to complete embryogenesis was 13-17 h.

POST-EMBRYOGENESIS

In post-embryonic developmental study more attention was given to the development of the gonad. The length of the primordium increased with the increase in body length.

First stage juvenile (Fig. 2 A, F)

The first stage juvenile of *D. orientalis* measured 0.14-0.23 (0.22 ± 0.08) mm. In ventral view the primordium was oriented obliquely to the longitudinal axis of the body. In lateral view it gave an impression of two superimposed primordia. The primordium measured 3-7 µm and was positioned at 48-55 % of the body from the anterior end. There were two germinal nuclei in the centre flanked by two cap nuclei at tips. About 12-14 h after hatching, the juvenile stopped feeding and became inactive as moulting commenced. During this period somatic nuclei divided but no division occurred in the germinal nuclei.

Second stage juveniles (Fig. 2 B, G)

The body measured 0.20-0.27 (0.25 ± 0.07) mm. The spindle-shaped primordium, 9-12 µm long was located at 46-62 % from anterior end and was slightly oblique to the longitudinal axis. Within the primordium there were two germinal and two somatic nuclei along with two cap nuclei. The continuity of the ventral chord nuclei was disrupted by a slight gap appearing at the tip of the primordium. The second moult started after 8-12 h of completion of first moult and the juvenile possessed a primordium with three germinal, three to five somatic and two cap nuclei. The germinal nuclei were placed in the centre, flanked by somatic nuclei at the two ends. Moulting lasted for 3-5 h and after its completion the third stage female juveniles were produced.

Third stage juveniles (Fig. 2 C, H)

Third stage juvenile of *D. orientalis* measured 0.24-0.29 (0.26 ± 0.06) mm in length. The primordium was at 44-58 % from the anterior end and measured 14-28 µm. It consisted of four germinal and eight to ten somatic nuclei including two cap nuclei. Four specialized ventral chord nuclei located opposite the primordium were observed. During moulting the multiplication of nuclei occurred and the primordium elongated anteriorly and posteriorly. The number of germinal and somatic nuclei increased to five to seven and eleven to twenty three respectively. Some of the germinal nuclei migrated into the elongating arms. The moulting lasted

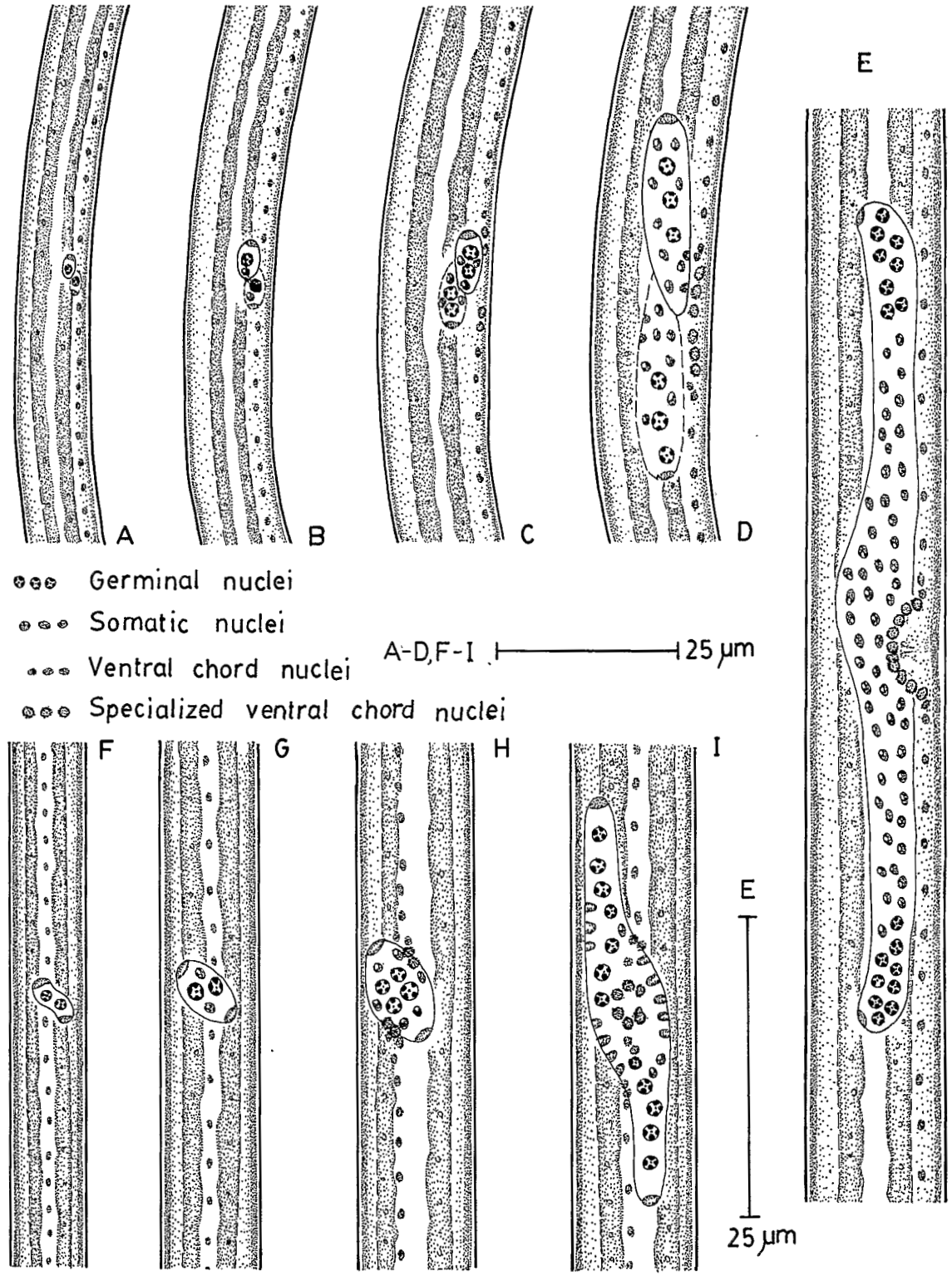

Fig. 2. *Diploscapter orientalis.* Post-embryonic development — A-E : Developing primordia (lateral view); F-I : Developing primordia (ventral view) — A, F : First stage juvenile; B, G : Second stage juvenile; C, H : Third stage juvenile; D, I : Early fourth stage juvenile; E : Late fourth stage juvenile.

for 4-5 h and finally gave rise to a juvenile of fourth stage with much developed primordium.

Fourth stage juvenile (Fig. 2 D, E, I)

The fourth stage female juveniles measured 0.27-0.35 (0.32 ± 0.09) mm. The length of the primordium varied from 30-112 µm in the beginning and it was situated at 38-54 % from the anterior end of body. It showed an obliquely placed central part with one extension directed anteriorly and one posteriorly. The extensions represented the two gonads while the central part represented the location of future uteri and vagina. The somatic nuclei 12-26 in number, were exclusively present in the central area. Those close to the boundary were long flattened and light stained in contrast to other somatic nuclei which were dark stained. At the terminal stage the number of germinal nuclei increased to eight to ten in each arm and somatic nuclei also proliferated repeatedly, making their count difficult. The specialized ventral chord nuclei were six to ten in number and formed a circle ventrally to demarcate the position of vagina. In moulting stage the developing anterior and posterior ovaries reflexed over. All germinal nuclei were contained in the flexures which also elongated because of multiplication of the nuclei. The specialized ventral chord nuclei, arranged in a circular manner, became compact and formed the vagina which ultimately connected to the exterior through a transverse vulva. The total post-embryonation time was 3-5 days.

Discussion

The eggs laid in single-cell condition indicated the possibility of a late oogenesis in *D. orientalis*. The ornamentation on the egg shell was similar to *Acrobeles complexus* (Thomas, 1965) and *Chromadorita tenuis* (Jensen, 1983).

The cleavage pattern apparently did not resemble the rhabditids as much as it did to the tylenchids. Except for the first division which is transverse in all nematodes, the preceeding two cleavages, also being transverse, were similar to *Ditylenchus triformis* (Hirschmann, 1962) and *Scutellomena cavenessi* (Demeure, Netscher & Quénéhervé, 1980) and were in sharp contrast to longitudinal oblique divisions seen in *C. elegans* (von Ehrenstein & Schierenberg, 1980) and *T. andrassyi* (Tahseen & Jairajpuri, 1988). Thus in the former cases the resulting division produced four cells arranged in tandem and in the latter cases in a rhomboid manner. But conformity of the initial divisions of *D. orientalis* to the tylenchs did not seem to be universal and a difference was seen in *Ditylenchus destructor* where the third division was longitudinal (Anderson & Darling, 1964).

Juveniles of nematodes generally resemble the adult, differing only in their size and development of the reproductive system. However absence of certain morphological features on the lip region have been reported in those species where elaborate ornamentation of the lips are found. The absence of labial hooks in first stage juveniles of *D. orientalis* corroborated the observations of Hechler (1968) on *D. coronata*, where absence of labial hooks in first stage juveniles was also reported. Thomas (1965) also observed lesser columns of labial probolae in the first stage juveniles of *Acrobeles complexus*. The number of germinal nuclei in the first stage juvenile was consistent with observations made on other didelphic species such as *Helicotylenchus vulgaris* (Yuen, 1965), *H. dihystera* (Hirschmann & Triantaphyllou, 1967) and *C. elegans* (von Ehrenstein & Schierenberg, 1980). The development of gonad followed the pattern seen in *D. coronata* (Hechler, 1968). Like *D. coronata* the multiplication of cells was restricted to moulting periods only till third stage, thereafter becoming continuous. The arrangement and dispositioning of the specialized ventral chord nuclei was similar to that seen in *H. dihystera* (Hirschmann & Triantaphyllou, 1967); the vaginal initial cell, however, was not observed.

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