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Observations on the feeding behaviour of *Heterodera schachtii* throughout development, including events during moulting

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Summary — The feeding behaviour of *Heterodera schachtii* throughout development was observed and analysed with time-lapse video-enhanced contrast light microscopy, using observation chambers that allowed long term observations at 25 °C. Destructive intracellular migration of the second stage juveniles (J2) towards the permanent feeding site within the vascular cylinder was terminated by the selection of the initial syncytial cell (ISC). After careful wall perforation, the stylet-tip stayed protruded in the ISC for an average of about 7 hours. During this feeding preparation period the metacorpal bulb stayed motionless, and the number of granules in the ampullae and extensions of the two subventral secretory glands gradually decreased, whereas that of the dorsal gland increased. A few defecations preceeded the first pumping action of the metacorpus. J2 that had completed the preparation period were no longer capable of leaving the root. First visible changes in cytoplasmic streaming and density in the ISC, accompanied by an enlargement of the cell's nucleus, were noted a few hours after the J2 had started feeding. Troughout development feeding occurred in cycles, each consisting of three phases (I-III). Phase II (stylet retraction and reinsertion) and phase III (salivation, feeding tube formation) stayed rather constant, whereas phase I (continuous food withdrawal from the syncytium) increased with time, but decreased before the moult. In those juveniles that were not disturbed, the average numbers of feeding cycles varied between 32 and 69, depending on the developmental stage and sex of the juveniles. Anatomical features that distinguish the sexes in the J2 and J3 stage are described, as well as events during the moult, with emphasis on stylet dissolution and regeneration.

Résumé — ***Observations sur le comportement nutritionnel d'*Heterodera schachtii *pendant son développement, y compris les événements liés à la mue*** — Le comportement nutritionnel d'*Heterodera schachtii* a été observé et analysé grâce à un dispositif de vidéo-microscopie en contraste optique à temporisation, en utilisant des chambres d'observation permettant des observations de longue durée, à 25 °C. La migration intracellulaire destructrice des juvéniles de second stade (J2) vers le site nutritionnel permanent à l'intérieur du cylindre central s'achève par la sélection de la cellule initiale du syncitium (CIS). Après perforation prudente de la paroi cellulaire, l'extrémité du stylet fait saillie dans la CIS pendant 7 heures en moyenne. Durant cette période de préparation à la nutrition, le bulbe médian (metacorpus) demeure immobile et le nombre de granules contenus dans les ampoules et les extensions des deux glandes subventrales diminue graduellement tandis que celui des granules de la glande dorsale augmente. Quelques périodes de défécation précèdent l'action de pompage du metacorpus. Les J2 qui ont terminé la période de préparation ne sont plus capables de quitter la racine. Les premiers changements visibles dans le flux et la densité du cytoplasme de la CIS — accompagnés par un grossissement du noyau — sont notés quelques heures après que le J2 a commencé à se nourrir. Durant le développement, la prise de nourriture s'effectue par cycles comportant chacun trois phases (I à III). La phase II (rétraction et réinsertion du stylet) et la phase III (salivation, formation du tube nutritionnel) restent relativement constantes, tandis que la phase I (absorption continue de nourriture à partir du syncitium) croît avec le temps, mais diminue avant la mue. Chez ceux des juvéniles qui n'ont pas été troublés, le nombre moyen de cycles nutritionnels varie de 32 à 69 suivant les stades de développement et le sexe. Les caractères anatomiques différenciant les sexes chez les J2 et les J3 sont décrits, de même que les événements liés à la mue, en insistant sur la dissolution et la régénération du stylet.

Key-words : Behaviour, feeding, *Heterodera*.

Since Raski's fundamental contribution on the life history and morphology of the sugar-beet cyst nematode *Heterodera schachtii* (1950), our knowledge about the biology of this economically important parasite has steadily increased. A film of its life cycle has been published (Müller *et al.*, 1981 *b*), which served as a basis for the analysis of growth patterns and estimation of amounts of food consumed (Müller *et al.*, 1981 *a*). With the advance of video-enhanced contrast light microscopy, it became possible to observe nematodes inside roots. Using this technique, Wyss and Zunke (1986) described the feeding behaviour of second stage juveniles (J2), but information about the behaviour of all other developmental stages is still lacking. The present study was undertaken to fill these gaps and to provide additional information on cell responses to feeding and events occurring in the anterior feeding apparatus during the moults.

Material and methods

The feeding behaviour of *H. schachtii* throughout development was recorded with the aid of special chambers that allowed long-term observations without much loss in focus. These chambers, developed by Grundler (unpubl.), were prepared as follows : a 80 mm diam. coverslip (0.13 mm thick) was placed on the bottom of a 90 mm diam. plastic Petri dish. A few ml of Dropkin

and Boone (1966) nutrient agar were pipetted onto the centre of the coverslip and a young seedling of *Brassica rapa* var. *silvestris* f. *campestris* cv. Stielmus was placed into the thin agar layer. Previous surface sterilization and germination of the seeds was as described by Wyss and Zunke (1985). The Petri dishes were tightly sealed with parafilm and kept in the dark at 25 °C. After an extensive root system had developed, about 40 *H. schachtii* J2 from a monoxenic stock culture on *Sinapis alba* cv. Albatros were transferred singly with a fine needle close to well growing roots. The shoot of the seedling was cut off, the coverslip was carefully removed from the Petri dish under a clean bench and placed onto the brim (7 mm width) of a threaded 90 mm diam. lower brass ring. A 80 mm diam. autoclaved silicone ring of 1 mm thickness and 3 mm width was laid on the coverslip and covered by an identical 80 mm diam. coverslip. Then the upper threaded brass ring was twisted into the lower ring, until the " sandwich " between the silicone ring was tightly closed. Care was taken to prevent contact of the upper coverglas with the thin agar layer. Occasionally two silicone rings were necessary, with some loss in optical resolution. Oxygen supply in the observation chambers was sufficient to ensure development of invaded J2 to young adult females.

Over a period of three years the feeding behaviour of about 300 individuals was recorded with video time lapse, using a Panasonic recorder 6010 (maximal time lapse 24 fold) or 6720 (maximal time lapse 160 fold). Most of the observations under a Polyvar light microscope (Reichert/Vienna) were with the aid of video-enhanced contrast, differential interference contrast (AVEC-DIC) microscopy, using a Hamamatsu Chalnicon C 2400 camera with a DVS 3000 image processor for analogue contrast and digital processing (Hamamatsu Photonics Deutschland, Herrsching/FRG). The temperature of the microscope stage was kept constant at 25 ± 1 °C.

Results

Behaviour after the selection of an initial syncytial cell (preparation period)

Intracellular migration by *H. schachtii* J2 towards the permanent feeding site within the vascular cylinder was as described by Wyss and Zunke (1986). In the present study special emphasis was placed on the behaviour of the J2 after the selection of the initial syncytial cell (ISC). The period between ISC selection and the beginning of feeding is termed here " preparation period ". Seven J2 were recorded and evaluated from the moment the ISC had been penetrated. Sixteen J2 were recorded while already within in this period.

Prior to root invasion, the extensions (ducts) and ampullae of the two subventral secretory glands were packed with granules (Fig. 1 A), whereas the same parts of the dorsal gland contained fewer granules. Once the wall of the ISC had been penetrated by careful stylet thrusts, the stylet-tip stayed extended in the ISC (Fig. 1 B) for an average of 7 h, 15 min (6 h, 26 min - 8 h, 05 min) and the metacorpal bulb stayed motionless throughout. Only two visible changes occurred during this period. One concerned a distinct change in the number of granules in the ducts and ampullae of the three secretory gland cells. Initially the density of granules accumulating in the ampullae of the subventral glands was very high (Fig. 1 C) but it decreased gradually from about the fifth hour onwards, whereas the density of granules in the extension and ampulla of the dorsal gland increased (Fig. 1 D, E). The other change concerned one or two faint but sudden shrinkages of the body volume towards the end of the preparation period, when the J2 obviously defecated. In nearly all J2 a distinct defecation occurred just prior or after the first pumping action of the bulb associated with food ingestion. In addition to these changes, the presence of two to three larger " vacuoles " was often observed in the metacorpal bulb in front of the pump chamber. A few hours after the J2 had started feeding, these " vacuoles " were no longer visible. Although much attention was paid to possible changes in cytoplasmic streaming and in the size of the nucleus in the ISC during the preparation period, none could be detected. First changes became evident only about 3 h after the J2 had started feeding (see below).

Behaviour of the J2 after the beginning of feeding

Disturbed behaviour

All 23 J2 that had completed the preparation period were no longer capable of leaving the root. Frequently the ISC was destroyed at an early stage by other migrating J2. Whenever this occurred, the search for an alternative feeding site was restricted to the area where the anterior body of the affected J2 was located. Vigorous stylet thrusting was then resumed similar to that noted during migration inside the root. As the head and the anterior part of the body were still able to move in all directions, the J2 usually succeeded in establishing a feeding site after several hours. Because the preparation period had already been completed, the nematodes then usually started feeding about 1 h after a new ISC had been selected. Occasionally, however, 2 to 3 h elapsed before food ingestion was initiated. In other cases, J2 were not successful in establishing a new ISC, when all possible sites within range had been destroyed by competing migratory J2 or by the J2's own destructive stylet thrusting. These J2 continued stylet thrusting for many days within a cavity of necrotic cells; they used up their lipd reserves completely and finally starved to death.

Feeding J2, which had penetrated the root with their

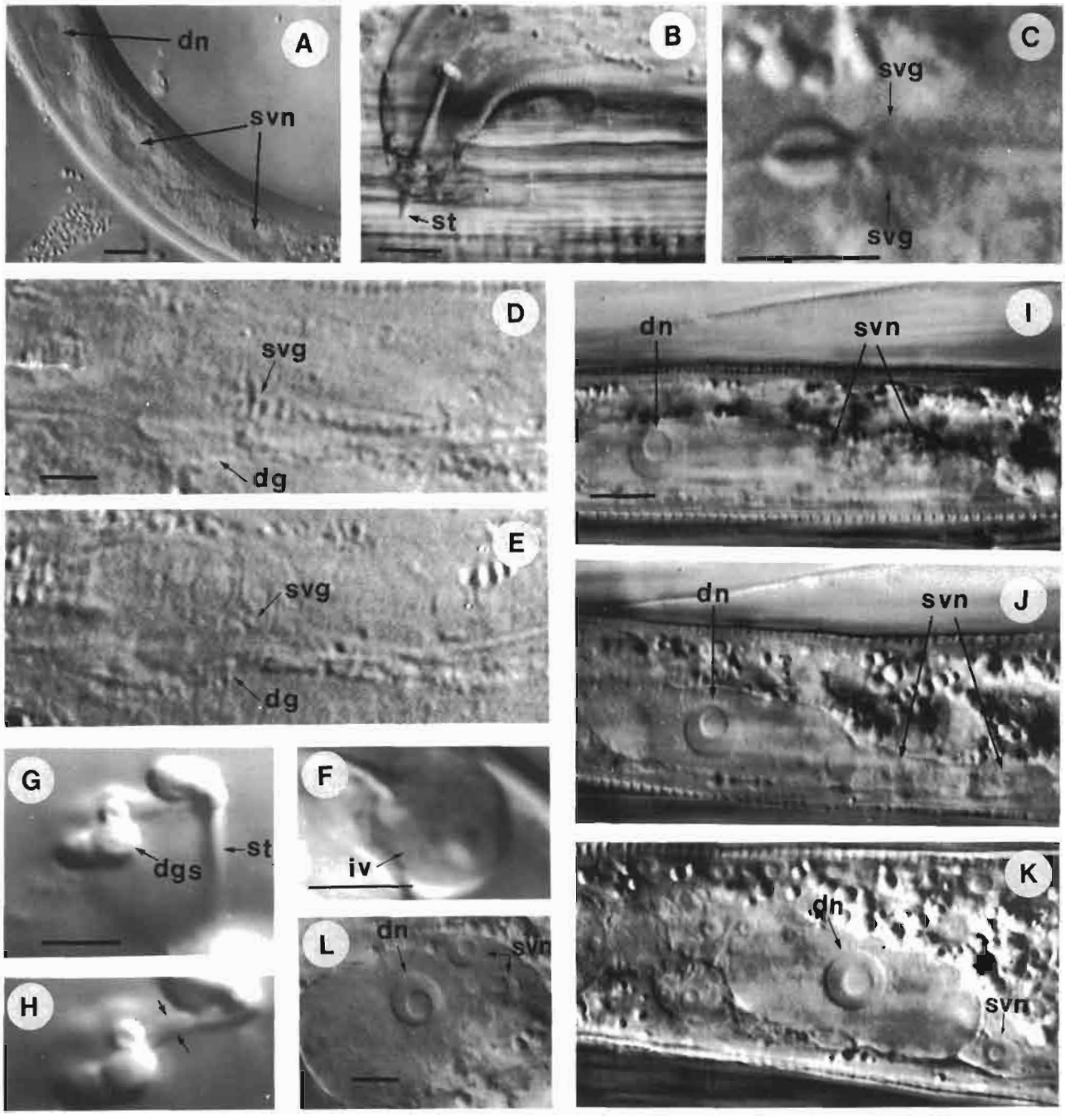

Fig. 1. A-K : J2 of *Heterodera schachtii* during different phases of parasitism in roots of *Brassica rapa* var. *silvestris* f. *campestris* — A : prior to root invasion, showing sizes of the three oesophageal secretory glands. dn, nucleus of dorsal gland; svn, nuclei of the two subventral glands; B-E : during preparation period (see text); B : stylet-tip (st) inserted into initial syncytial cell; C : granules of subventral glands (svg) accumulating in ampullae of their extensions behind pump chamber of metacorpal bulb, 1 h after stylet insertion; D : another J2, granules of subventral glands (svg) accumulating in ampullae, and granules of dorsal gland (dg) flowing forward through extension, 2 h after stylet insertion; E : 5 h after D, less svg still accumulating in ampullae, numerous dg flowing forward, 25 min before beginning of feeding; F-H : parts of a sedentary J2, having lost contact with root; F : oesophageal-intestinal valve (iv) with secretions from subventral glands; G : secretions from dorsal gland (dgs) emanating through orifice of stylet-tip (st); H : a few s later and at higher magnification, showing tube-like structure of secretions; I-K : changes in dimensions of the three oesophageal secretory glands of a J2 female, abbreviations as in A; I : 10 h after beginning of feeding; J : 24 after I; K : 32 h after J, just prior to moult; L : sizes of the glands in a J3 female, just prior to moult. *(Bars : A, B, I-K, L = 10 µm; C, D, E, F, G = 5 µm).*

anterior end only, occasionally lost contact with the root in their efforts to establish a new feeding site. Incapable of any further migration, they stayed outside the root and continued stylet thrusting. On one occasion a J2 was seen to have its stylet protruded for up to one hour, during which time secretions from the dorsal gland emanated continuously through the stylet-tip orifice, apparently in the form of a tube (Fig. 1 G-H). The two subventral glands, though already much reduced in size, were still very active, as revealed by the presence of numerous granules in the gland ducts and ampullae. Their secretions appeared to pass into the oesophago-intestinal valve (Fig. 1 F), which, due to the absence of body lipids, was clearly visible.

Undisturbed behaviour

Feeding by all stages, including adult females, was always in three phases. Phase I was characterized by a continuous rapid pumping action of the metacorpal bulb (5-8 muscle contractions per second), with the stylet-tip staying inserted in the ISC (Fig. 2 A). Phase II was characterized by stylet retraction (Fig. 2 B) and reinsertion, and phase III by a continuous forward movement of secretory granules, especially from the dorsal oesophageal gland, with the stylet-tip staying inserted in the ISC (Fig. 2 C). These three phases have been described in detail by Wyss and Zunke (1986) from selected observations of individual J2 only, with special emphasis on a J2 that was ideally situated inside the root. In contrast to this previous study, expansions of valve membranes and their depletion within the ampullae of the two subventral glands could only rarely be observed during phase II. The glands were still active, producing numerous secretory granules that accumulated in the ampullae, but, compared to the first few hours of the preparation period, the amount of granules accumulating was greatly reduced. Fig. 2 D-F present an overview of characteristic events during the three phases, especially with regard to the synthetic activity of the dorsal oesophageal gland. The formation of a " vacuole " or " vesicle " in the oesophageal tissue, a few µm in front of the metacorpal bulb during phase III and its rapid disappearance at the beginning of phase I, as described by Wyss and Zunke (1986), was observed in nearly all J2. In the J3 this phenomenon appeared occasionally in the anterior part of the metacorpal bulb.

CELLULAR RESPONSES IN THE FEEDINGS SITE

The earliest response, indicated by an increase in cytoplasmic streaming and density in the ISC, was recognizable about 3 h after the J2 had started feeding. A distinct hypertrophy of the ISC's nucleus was evident about 2 h later. Within 12 h after feeding had commenced, several cells in the vicinity of the ICS showed increased cytoplasmic activity and nuclear hypertrophy. A highly active syncytium, incorporating the ISC and numerous neighbouring cells, was established 24 h after the beginning of feeding. 12 h later it had reached dimensions as shown, for example, in Fig. 3 G for a male J2. The processes of partial cell wall dissolution were not studied, but cell wall fragments were numerous and distinct in about three days old syncytia (Fig. 3 H). In most cases syncytia expanded within the vascular cylinder in a direction away from the root-tip, i.e. in an acropetal direction. When the J2 started to moult, the syncytia of J2 females had reached dimensions of up to about twice the body length of the nematode. The syncytia of J2 males were less elongate and narrower in diameter.

In some time lapse studies special emphasis was placed on cytoplasmic changes in the immediate vicinity of the inserted stylet-tip. At an early stage of feeding (from about 2 h onwards), and occasionally towards the end of the preparation period, the stylet-tip was surrounded by a dome of accumulated cytoplasm (Fig. 3 A) which persisted during phase II when the stylet was retracted for a few minutes. Feeding tubes were never observed at an early stage but became evident 32-38 h after the beginning of feeding (Fig. 3 B) and subsequently they were always formed during phase III, throughout any stage of development. Secretory fluids, emanating from the stylet-tip orifice, progressively formed the tube (Fig. 3 E). Its formation was always accompanied by an expanding zone of modified cytoplasm that surrounded the tube. This zone persisted throughout phase I (food ingestion). Whenever the stytube was disconnected from the stylet-tip and remained for some time within or outside the modified zone (Fig. 3 F) before it was eventually carried away by streaming cytoplasm. Similarly, the feeding plug was not formed at the beginning of feeding. It first became clearly evident 12-15 h later (Fig. 3 C) and from then onwards it kept the lips firmly anchored in position during phase II and stylet reinsertion (Fig. 3 D). Prior to plug formation, slight lip rubbings were noticed during the gentle stylet thrustings within phase II.

BEHAVIOUR OF J2 FEMALES AND MALES THROUGHOUT DEVELOPMENT

Fourteen J2 were continuously recorded with time lapse video microscopy throughout their development. Eight developed into female and six into male juveniles. The characteristic patterns of the feeding cycles, each composed of three feeding phases, are represented in Fig. 4 for a selected female and male J2. The patterns were generally similar for both sexes. After the beginning of feeding, phase III was initially prolonged (44-63 min) in all fourteen J2, and then gradually declined to a steady level. In the two J2 presented in Fig. 4 it averaged 15 min from the tenth cycle onwards. In those J2 that were not disturbed at some time during development, phase II was steady, averaging 5 min. The durations of continuous food ingestion (phase I) in-

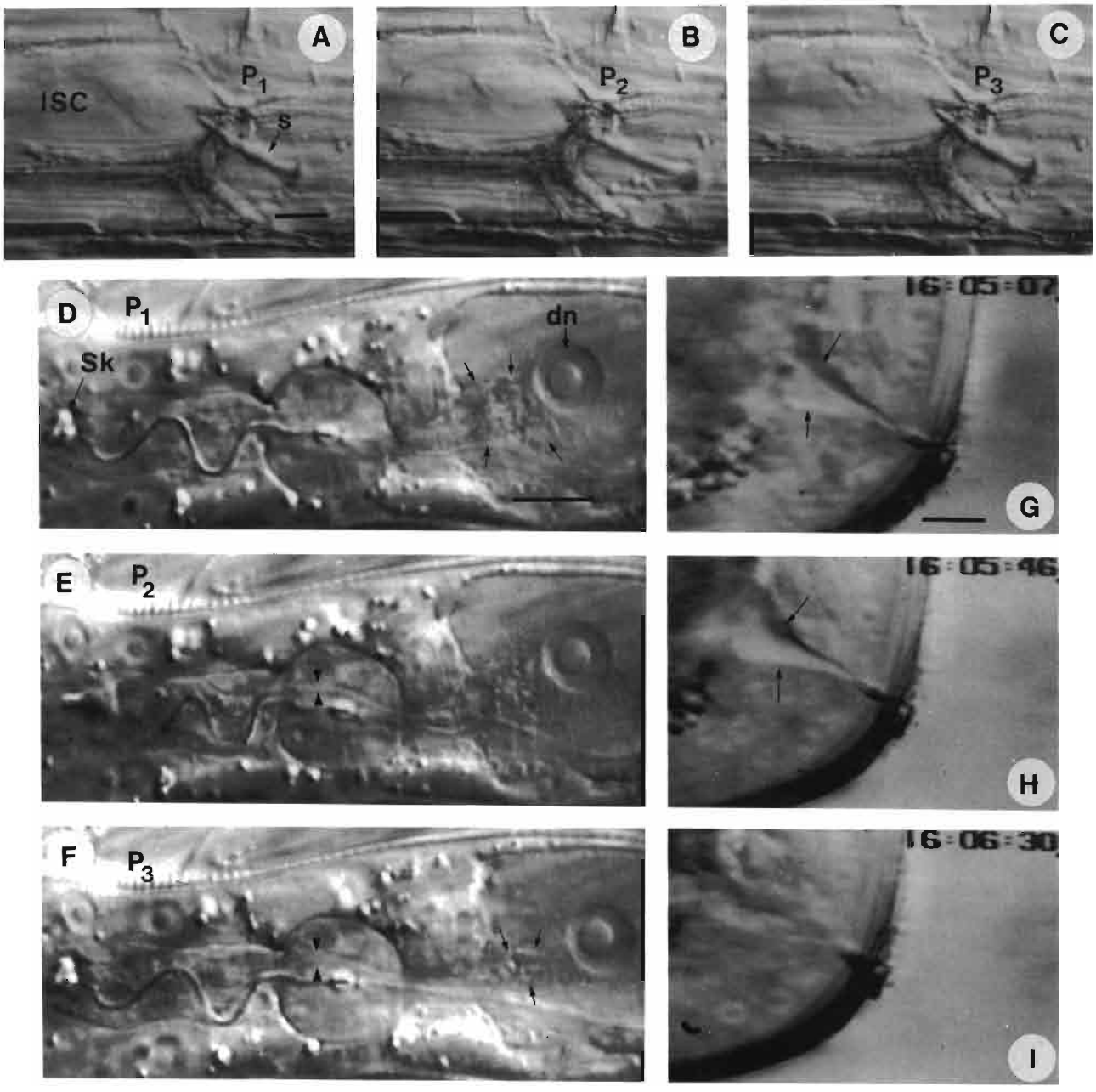

Fig. 2. A-F : J2 of *Heterodera schachtii* feeding in roots of *Brassica rapa* var. *silvestris* f. *campestris* — A: towards end of phase I (P1), stylet (s) still inserted in initial syncytial cell (ISC), 24 h after beginning of feeding; B : phase II (P2), stylet just retracted, 1 min after A; C : phase III (P3), stylet-tip just reinserted into ISC, 5 min after B; D : another J2 female during phase I (P1), 55 h after beginning of feeding. Stylet protracted (Sk = stylet knobs), note accumulation of secretory granules (arrows) in front of nucleus (dn) of dorsal gland; E : during phase II (P2), stylet retracted, secretory granules of dorsal gland flowing forward through extension in a single row (arrowheads) within metacorpal bulb, 8 min after D; F : towards end of phase III (P3), granules still flowing forward, in a triple row (arrowheads) through extension, 20 min after E. Note decrease in accumulation of granules (arrows) in front of gland nucleus. G-I : hind end of a J4 female at moment of defecation; G : intestino-rectal valve (arrows) and rectum expanded before expulsion of fluids from intestine; H : 39 s after G, fluids still being expelled, hind end shrunken (compare relative position of cuticle to time above); I : end of defecation, valve and rectum collapsed again. *(All bars = 10 µm).*

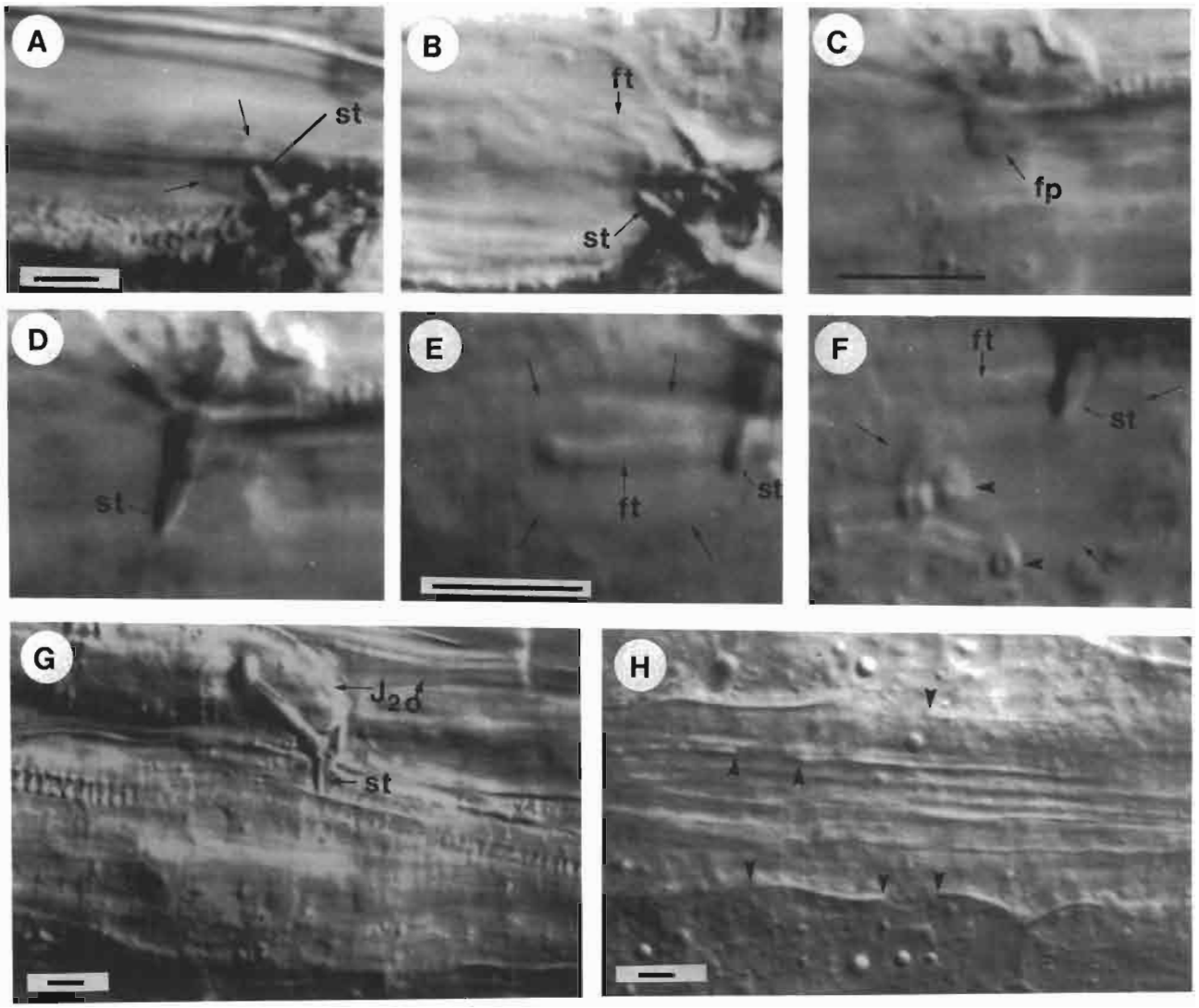

Fig. 3. A-H : J2 of *Heterodera schachtii* feeding in roots of *Brassica rapa* var. *silvestris* f. *campestris* — A : Dome of accumulated cytoplasm (arrows) surrounding stylet-tip (st), 2 h after beginning of feeding; B : 34 h after A, during phase I, feeding tube (ft) attached to stylet-tip (st); C-D : another J2, 14 h after beginning of feeding; C : stylet retracted during phase II; feeding plug (fp) clearly visible; D : 2 min after C, stylet-tip (st) just inserted through feeding plug; E : same J2, 21 h after D, size of feeding tube (ft), formed in 9 min after stylet insertion. Arrows outline zone of modified cytoplasm surrounding the tube; F : 6 h after E, size of feeding tube (ft), formed in 8 min after stylet insertion. Arrows outline zone of modified cytoplasm, arrowheads mark feeding tubes formed in two previous feeding cycles; G : syncytium of a J2 male, 36 h after beginning of feeding; H : part of a syncytium of a J2 female in the process of moulting, 74 h after beginning of feeding. Arrowheads point to partially dissolved cell walls. *(All bars = 5 µm).*

creased with time but decreased shortly before the moult.

Table 1 shows the total time of feeding activity (from the beginning of feeding after the preparation period until the last stylet retraction before the moult) for four J2 females and males, the total number of their feeding cycles and, derived from them, the average of the three feeding phases in percentages. Sudden shrinkages in body volume, as a result of defecation, usually occurred within one min after the nematodes had started feeding at the beginning of each feeding cycle. However, defecations were not always associated with the beginning of each cycle. Sometimes they occured at the beginning of every second, occasionally even at the beginning of a

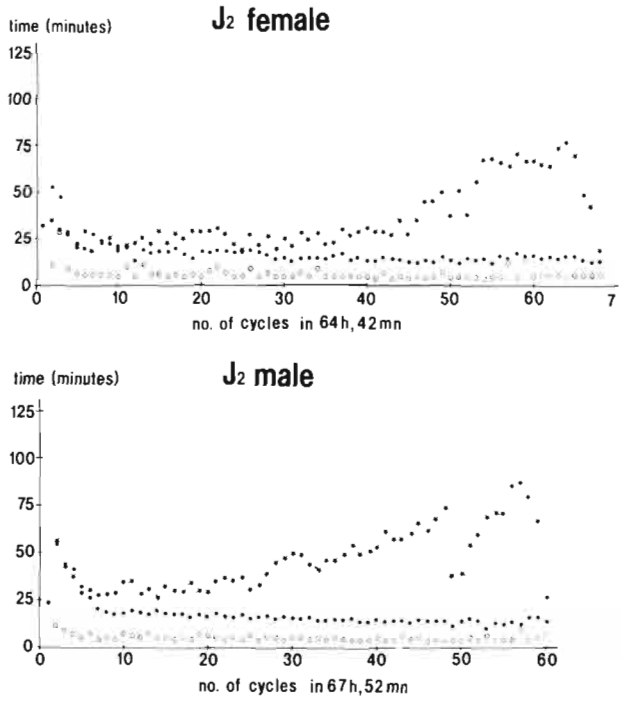

Fig. 4. Number of feeding cycles in a selected J2 female and male from beginning of feeding until last stylet retraction from feeding site before beginning of moult, and duration of the three feeding phases. *Asterisks = phase I* (continuous food ingestion; followed by *phase II* (time between stylet retraction and reinsertion into the feeding site), represented by *white spots;* followed by *phase III* (forward flow of secretory granules through the extensions of the oesophageal glands, especially of the dorsal gland), represented by black spots; followed again by phase I.

Table 1. Total time of feeding activity of *Heterodera schachtii* juveniles at different developmental stages, measured from the beginning of feeding until the last stylet retraction prior to the moult, with percentage averages of the three feeding phases (P1-P3), derived from the total number (n) of feeding cycles. Times for duration of moult measured from the last stylet retraction until the beginning of feeding of the following developmental stage. Asterisks mark those juveniles represented in Fig. 4, 6 and 7.

		P1 %	P2 %	P3 %	n	Moult
J2♂	62 h, 31 min	65.9	7.4	26.7	53	20h, 56min
*J2♂	67h, 52min	67.7	7.3	25.0	60	21h, 54min
J2♂	62h, 05min	59.7	14.3	26.0	50	23h, 14min
J2♂	65h, 14min	66.7	7.9	25.4	54	21h, 38min
J3♂	58h, 01min	81.9	4.1	14.0	35	
*J3♂	77h, 17 min	76.3	6.8	16.9	54	
J3♂	67h, 50min	77.6	8.6	13.8	44	
J3♂	64h, 55min	79.4	6.3	14.3	46	
*J2♀	64h, 42 min	62.0	9.4	28.6	68	22h, 23min
J2♀	67h, 15 min	60.1	7.9	32.0	69	19h, 55min
J2♀	61h, 30min	59.3	6.6	34.1	61	19h, 58min
J2♀	66h, 45min	60.5	7.1	32.4	67	20h, 28min
*J3♀	45h, 36 min	75.1	8.0	16.9	32	21h, 49min
J3♀	48h, 11 min	69.7	7.8	22.5	41	22h, 36min
J3♀	47h, 49min	70.2	7.6	22.2	40	20h, 27min
J3♀	39h, 47min	69.8	7.3	22.9	37	19h, 37min
J4♀	68h, 04min	68.6	10.3	21.1	47	23h, 12min
*J4♀	74h, 36 min	62.5	17.4	20.1	55	21h, 57min
J4♀	70h, 07min	68.8	7.2	24.0	57	22h, 16min
*J4♀	62h, 58min	77.1	5.1	17.8	44	22h, 15min

third cycle. The J2 female and male in Fig. 4, marked by an asterisk in Table 1, defecated for instance 46 and 42 times respectively during their total time of feeding activity. Defecation is shown in Fig. 2 G-I for a J4 female, which was chosen to illustrate the features of defecation more clearly.

A few J2 were disturbed once or several times during development. The factors responsible could not be determined, because optical limitations prevented resolution of details in the syncytia. The development of these J2 was accordingly prolonged. The longest time was recorded for a J2 male that started to moult as late as 154 h after it had started feeding. During most of the observations this juvenile was seen to thrust its stylet continuously, but sometimes it was also seen feeding.

A noticeable feature was an early reduction in the size of the two subventral oesophageal glands and an increase in size of the dorsal gland (Fig. 1 I-K). Towards the end of the J2 stage the dorsal gland was considerably larger in J2 females than males. The differences in size were even more pronounced in the early J3 stages just after the moult (Fig. 5 J-K). Fig. 1 L shows the dimensions of the three glands in a J3 female just before it started to moult.

Apart from the size of the dorsal gland, the sexes could also be distinguished by the form of the genital primordia, although, depending on the orientation of the body, a clear cut distinction was not always obvious. About half a day before moulting, the primordia of the J2 females were more rounded and less elongate than those of the males. In addition the pattern of cell divisions appeared different, at least in four cases (two J2 males, two J2 females) where cell division was studied. In the male primordium the anterior (cranial) and posterior (caudal) cap cells (Fig. 5 A) divided simultaneously about 10 h before the moult (Fig. 5 B). After one further division, one of the posterior cells stopped dividing, whereas the two anterior cells (Fig. 5 C) continued dividing (Fig. 5 D) during and after the moult. In

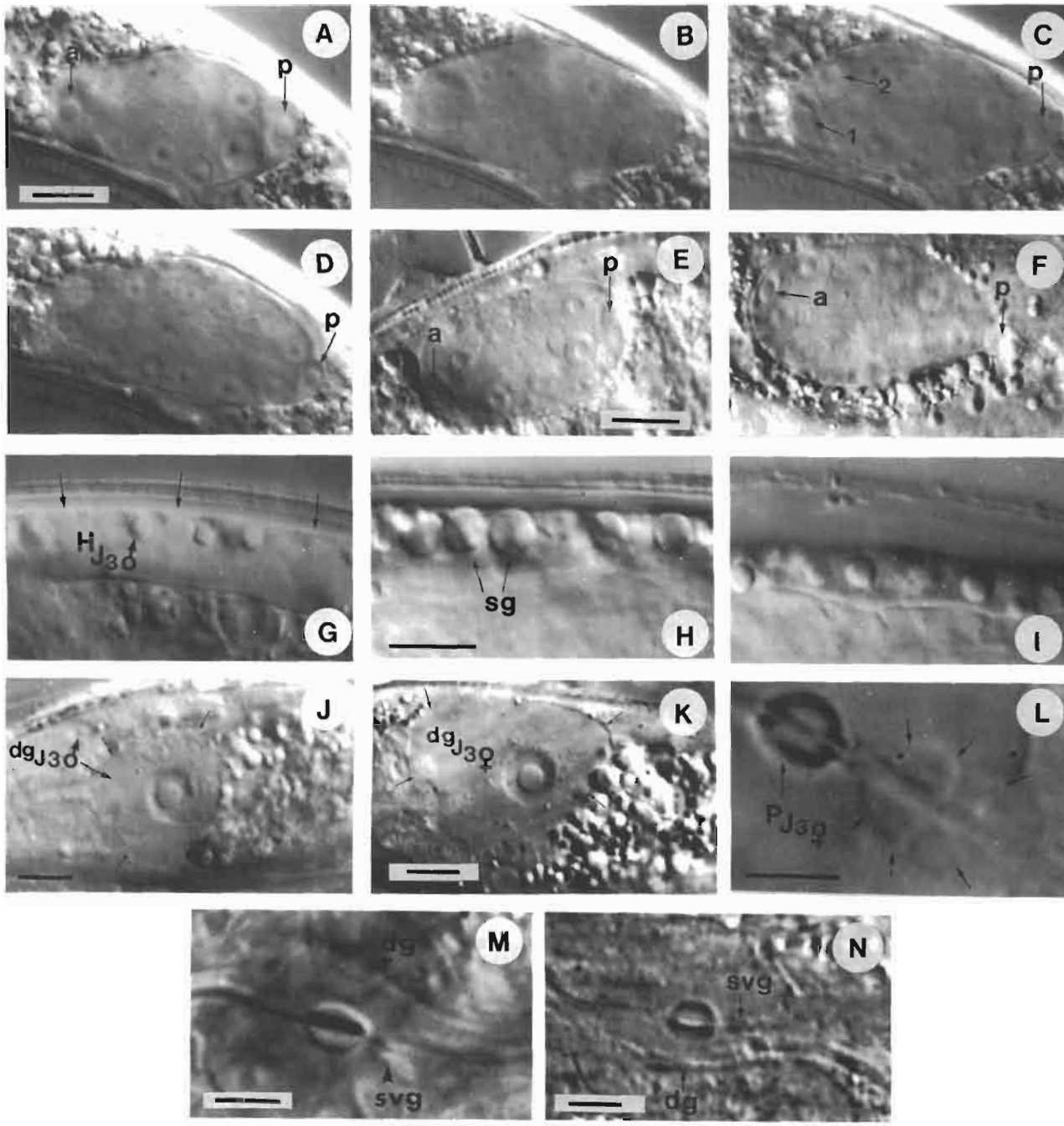

Fig. 5. A-D : genital primordium of a J2 male. A : 15 h before beginning of moult. a, anterior cap cell; p, posterior cap cell; B : 5 h after A, simultaneous division of the cap cells; C : 3 h afterB, the two (1, 2) cells from anterior cap cell will continue dividing, whereas posterior cell (p) will stay in position and no longer divide; D : 7 h after C, at beginning of moult and after cell 1 and 2 had divided once; E-F : genital primordium of a J2 female; E : 6 h before beginning of moult; F : 24 h after E, towards end of moult, anterior (a) and posterior (p) cap cells did not divide; G-I : J3 male before and during moult; G : Hypodermis (H) thickened and separated (arrows) from cuticle, 23 h before beginning of moult; H : hypodermis packed with large spherical globules (sg), just at beginning of moult; I : 7 h after H, spherical globules now much smaller and hypodermis clearly separated from cuticle, as body of moulting J3 becomes more elongate. J : dorsal gland (dg) of a J3 male, just after moult; K : dorsal gland (dg) of a J3 female, just after moult; L : pump chamber (P) of a J3 female, 20 min after beginning of moult (last stylet retraction); valve membranes (each outlined by two arrows) of subventral glands much expanded and formation of globules (each marked by a single arrow) that will be passed backwards; M-N : parts of metacorpal bulb of young J3 females, towards end of moult; M : distinct accumulation of granules subventral glands (svg) in ampullae behind pump chamber, numerous granules of dorsal gland (dg) flowing forward through extension, 1 h before beginning of feeding; N : another J3 female, again showing distinct accumulation of svg in ampullae and massive forward flow of dg, 1 h before beginning of feeding *(Bars : A-K = 10 µm; L-N = 5 µm).*

the primordia of the J2 female a division of the anterior and posterior cap cell was not recognizable prior to (Fig. 5 E) and during the moult (Fig. 5 F).

BEHAVIOUR OF J3 FEMALES AND MALES THROUGHOUT DEVELOPMENT

Most of the J2 observed throughout development were also continuously observed during and after the moult. The J3 female and male in Fig. 6 are the same nematodes shown in Fig. 4. In both, as well as all other J3, phase III was no longer prolonged at the beginning of feeding. When, after the moult, the newly formed stylet had been finally inserted into the syncytium, a feeding tube was immediately formed during this phase. The durations for phase III and II were as usual rather constant, whereas phase I increased in time and dropped again before the moult.

The feeding pattern of the J3 male in Fig. 6 was affected twice, as revealed by two prolonged phase II durations. In most of the juveniles examined (also including other stages), a sudden drop in food ingestion time (for the J3 male it occurred in the 24th cycle) was followed by a prolonged phase II. During this phase the stylet-tip stayed protracted several times for a few seconds and was then retracted again, indicating the presence of a temporary, unknown obstacle. As already

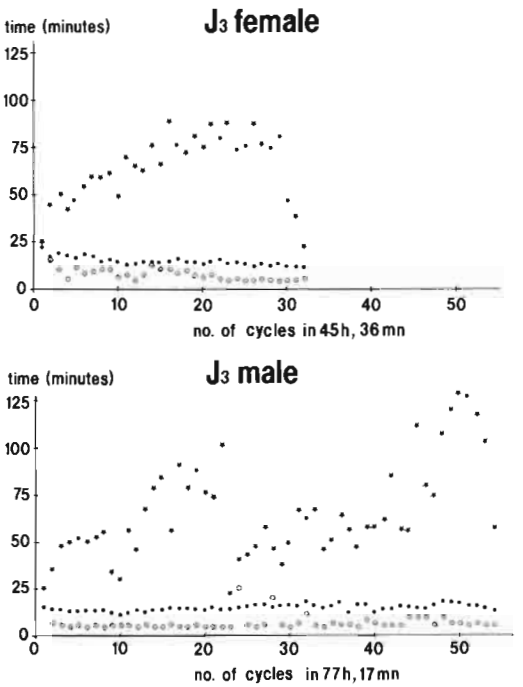

Fig. 6. Number of feeding cycles in a selected J3 female and male from beginning of feeding until last stylet retraction from feeding site before beginning of moult, and duration of the three feeding phases. Asterisks and spots as in Fig. 4.

described for the J2, defecations were not observed at the beginning of each feeding cycle. In J3 males, the durations of phase I towards the end of development were always considerably longer than in females. Values around 125 min were common, whereas in females they seldom exceeded 80 min. Consequently, and also due to a slightly higher number of feeding cycles (Table 1), the total time of feeding activity of the J3 males exceeded that of the J3 females by at least one third. Table 1 represents the values for four selected J3 females and males whose feeding behaviours were at most only temporarily affected, as for instance, the J3 male in Fig. 6, marked in Table 1 by an asterisk. The percentage of phase III was less in males than in females, indicating that less secretory fluids from the dorsal gland were injected by the males. J3 males could easily be distinguished from females about 24 h before the moult because of the thickened hypodermis of the J3 male that was clearly separated from the cuticle by a gap (Fig. 5 G). Subsequently, while the nematodes were still feeding, enlarged spherical globules developed in the hypodermis. At the beginning of the moult the width of these globules nearly equalled the thickness of the hypodermis (Fig. 5 H). A few hours after the J3 had started moulting, the sizes of the globules were much reduced and the hypodermis had more clearly separated itself from the cuticle (Fig. 5 I) as the body became more narrow and elongate. In contrast to males, the thickened hypodermis in J3 females was never separated from the cuticle by a distinct gap prior to the moult and spherical globules did not develop.

BEHAVIOUR OF J4 FEMALES THROUGHOUT DEVELOPMENT AND OF YOUNG ADULT FEMALES

Fig. 7 shows the patterns of the feeding phases of two selected J4 females, one without and the other with some disturbances during development, as revealed by prolonged phase II durations in the first third of development. Phase III was rather constant in all J4 examined as was phase II, except when feeding was temporarily disturbed. In all four J4 examined, the duration of phase I rarely exceeded 100 min, and then only towards the end of this developmental stage. Again defecations did not occur at the beginning of each feeding cycle, but they were now more numerous than in the J2 and J3 stages. In the first J4 represented in Fig. 7 they were, for example, only absent four times throughout development. The total time of feeding activity was similar to that of the J2 females, but the number of feeding cycles were less (Table 1).

After the moult, young adult females initially showed a feeding pattern similar to the J4 females before the moult. As usual, phase III stayed rather constant but was now longer than in the previous stages, averaging 22 min. Phase II, if undisturbed, averaged 5 min as in the previous stages. From three days beyond the moult, phase I became much prolonged and often lasted about

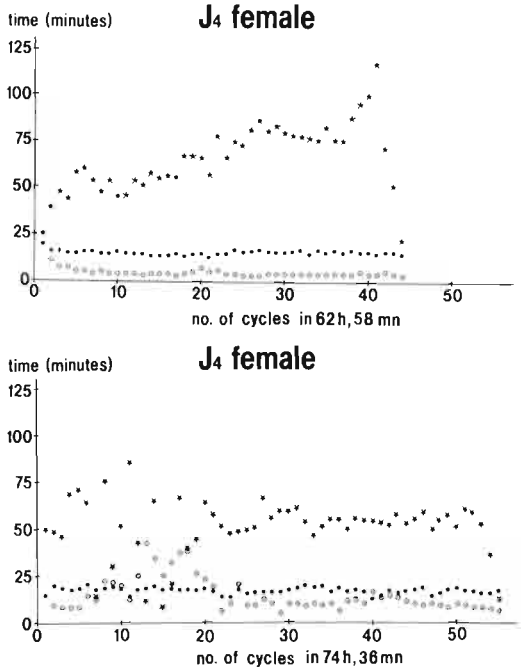

Fig. 7. Number of feeding cycles in two selected J4 females from beginning of feeding until last stylet retraction from feeding site before beginning of moult, and duration of the three feeding phases. Asterisks and spots as in Fig. 4.

2 h. After the moult, the young females started to expel fluids through the vulva which gradually formed a drop of gelatinous material that steadily increased in size. The fluids were expelled by muscle contractions at regular intervals, occurring every seventh to eighth minute.

The present study concentrated primarily on the feeding behaviour of the nematodes throughout their development and on events occurring during the moult, but with little attention to changes in the body size. However, measurements of the size of the metacorpal bulb in four female juveniles demonstrated that the bulb, but not the pump chamber, increased in size during the feeding activities of the three developmental stages. In contrast, no increase in the size of the bulb was noticed in three J3 males where it was measured. Length and width of the pump chambers in the metacorpal bulbs (Fig. 8 N-P) averaged 3.1/2.3 µm in the J2, 4.4/3.6 µm in the J3, 6.3/5.2 µm in the J4 and 8.9/7.8 µm in young adult females (n = 7).

Events in the anterior feeding apparatus during the moults

Table 1 shows that the durations of the moults, measured from the last stylet retraction of a feeding juvenile until the beginning of feeding (phase I) by the following developmental stage, was uniform for all stages, lasting from between about 20 to 23 h.

The first visible change in the anterior feeding apparatus was the expansion of the valve membranes within the ampullae of the two subventral oesophageal glands that occur behind the pump chamber in the metacorpal bulb. These valves were difficult to observe in some specimens. Fig. 5 L shows an expanded valve in a J3 female, about 20 min after the last stylet retraction. When recognizable, these valve expansions occurred in all developmental stages. The membranes finally appeared to burst, as rather large globules, possibly consisting of fused secretory granules, appeared to flow backwards, sometimes as late as 90 min after the beginning of the moult (last stylet retraction).

The most striking feature in the early phase of moulting was the rapid dissolution of the stylet knobs and stylet shaft and the retention of the stylet cone (Fig. 8 A-D). First signs of stylet knob disintegration were visible about 70-90 min after the last stylet retraction. When the process of stylet knob disintegration was observed at high magnification, the factors involved in the disintegration could not be determined; the knobs just faded away (Fig. 8 H, I). At the time the knobs had disappeared, parts of the stylet shaft were already degraded. Its dissolution progressed from back to front. In two cases, out of a total of 28 observations, the initiation of stylet degradation was delayed by 4 to 6 h, but once it had started, it progressed rapidly.

About 6 h after moulting had been initiated, the head of the nematode started to retract from the original stylet cone and slowly moved around in all directions within the old cuticle as the new stylet cone was developing (Fig. 8 E). The formation of the cone progressed from front to back. At the time the cone was fully developed (Fig. 8 J), the new pump chamber in the metacorpal bulb was also formed. This was about 9-10 h after the beginning of the moult. Head movements of the juvenile were still sporadic during stylet shaft formation (Fig. 8 K), which, similar to stylet cone development, progressed from front to back. After completion of the stylet shaft, the stylet knobs were regenerated (Fig. 8 F, L), during a process that lasted about 2 h. There was pronounced activity of granules around the developing knobs while the head remained virtually motionless.

When the stylet knobs were fully formed (Fig. 8 G, M), the extension of the dorsal oesophageal gland and its ampulla behind to knobs were partially filled with secretory granules, even as early as 6 to 7 h before the termination of the moult. About 2 h before feeding, the expanding head of the juvenile had contacted the previous feeding site and made coordinated head movements with gentle exploratory stylet movements. At this time the duct and the ampulla of the dorsal gland were packed with granules, and also the ampullae of the two subventral glands contained numerous granules (Fig. 5 M, N), at least in all six J3 studied. Granule activities in the ampullae of the subventral glands prior to feeding were not examined in such detail in later developmental

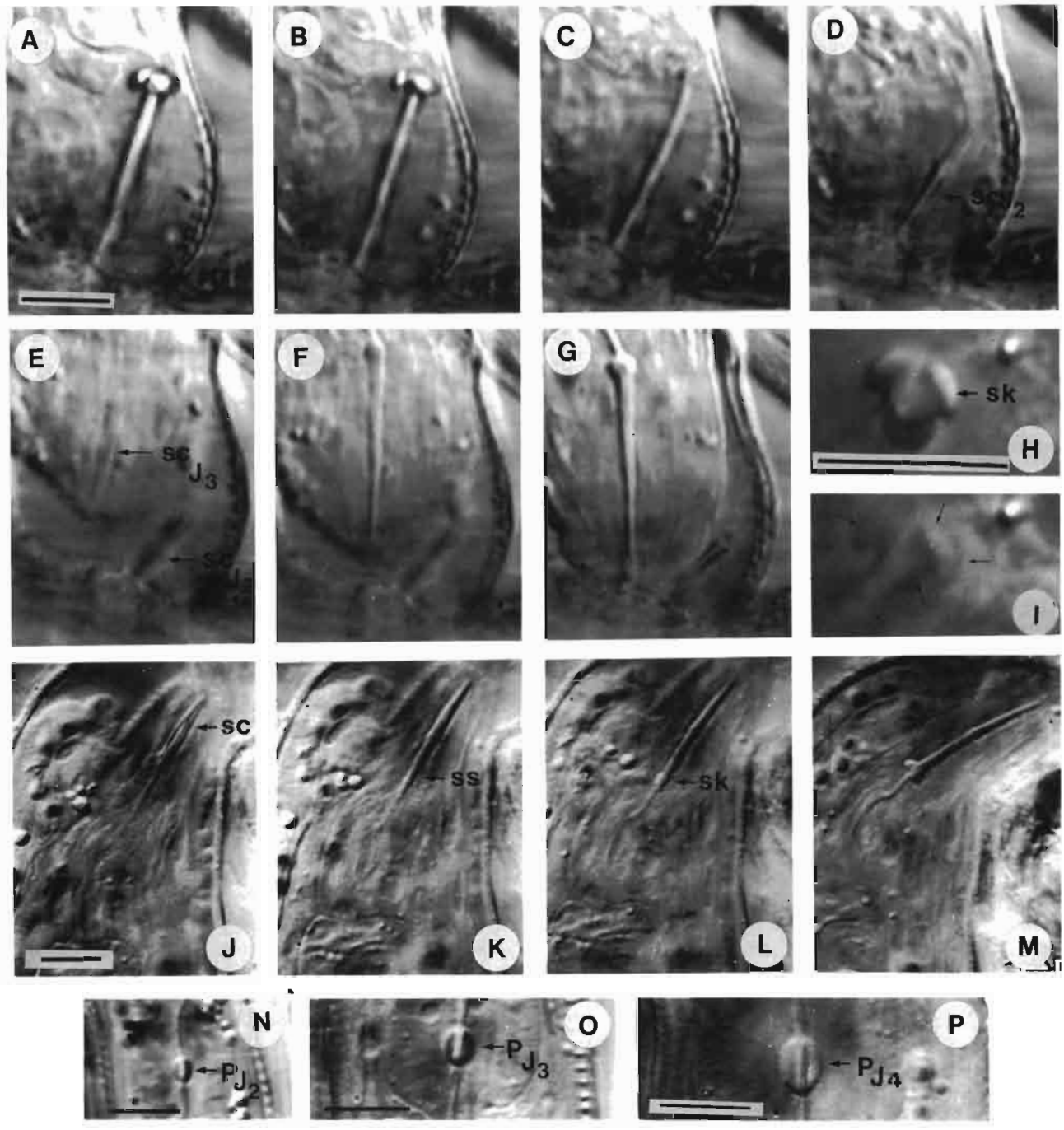

Fig. 8. A-G : head of a J2 male, showing stylet dissolution and regeneration during moult — A : Beginning of moult, stylet just retracted after last feeding cycle; B : 1 h 35 min after A; C : 22 min after B, stylet knobs partially dissolved; D : 20 min after C, stylet knobs and most of stylet shaft dissolved; E : head of early J3 retracted, showing undissolved stylet cone (sc) of J2 and newly forming sc of J3, 7 h 04 min after D; F : stylet shaft and parts of stylet knobs of J3 stylet formed, 3 h 38 min after E; G : new stylet formed, 4 h 48 min after F and 2 h before first feeding cycle — H-I : dissoluton of stylet knobs of a J2 female; H : stylet knobs (sk), 1 h 15 min after last stylet retraction; I : 33 min after H, outlines of fading knobs marked by arrows — J-M : formation of new stylet in J4 female; J : stylet cone (sc) formed, 9 h after beginning of moult; K : stylet shaft (ss) partially formed, 1 h 50 min after J; L : stylet knobs (sk) partially formed, 58 min after K; M : stylet formed, 3 h after L and 4 h before first feeding cycle — N-P : size of pump chamber (P) of same female juvenile in three developmental stages; N : J2, 10 h after beginning of feeding; O : J3, 1 h after beginning of feeding; P : J4, 2 h after beginning of feeding *(Bars = 10 µm).*

stages, i.e. in J4 and adult females. Up to 1 h elapsed before the stylet was finally reinserted into the syncytium after vigorous stylet thrustings. During this time the lips moved in all directions until a suitable spot was located, usually close to the old stylet cone. Immediately after stylet insertion, a feeding tube was formed. About 15-20 min later the freshly moulted nematodes started to withdraw food for the first time in a new series of feeding cycles.

Reentry of the stylet-tip into the syncytium proved to be a critical phase for a number of juveniles. Some of them finally gained access only after considerable effort, during which the stylet was vigorously thrust towards the syncytial wall for many hours. A few did not succeed at all and two were seen to be so desperate and violent in their efforts to reinsert the stylet that they injured the syncytium. In these two cases contents of the syncytium suddenly flushed towards the juveniles and coagulated, forming an insurmountable barrier. All juveniles that did not succeed in reentering the syncytium continued to make stylet thrusts for many days and finally starved to death.

Discussion

Before root invasion, the J2 of cyst nematodes respond to the root diffusates of their host plants. Apart from stimulating the dorsal oesophageal gland prior to hatching (Perry *et al.*, 1989), the diffusates initiate stylet thrusting, as recently shown for *H. schachtii* (Grundler *et al.*, 1991). Inside the root, the J2 are programmed to migrate intracellularly towards the vascular cylinder, where they select a living cell that becomes transformed into the initial syncytial cell (ISC). The mechanisms of recognition are not yet known but the destructive stylet thrustings, used for intracellular migration, are replaced by subtle stylet thrusts, usually aimed at one point to perforate the wall of the ISC. After wall perforation, the stylet-tip is gently inserted and then stays protruded for many hours, without any pumping action of the metacorpal pump. This long rest period before the beginning of feeding (bulb pulsations) was intuitively believed to occur in J2 of *Globodera rostochiensis* after ISC penetration (Hammond-Kosack *et al.*, 1990) but evidence was lacking.

At the time of writing this discussion, the author obtained an excellent contribution by Steinbach (1973) that has not been cited in pertinent articles. With the aid of simple observation chambers, Steinbach observed the feeding behaviour of *G. rostochiensis* J2 and J3 in tomato roots. He was the first to report a rest period after a J2 had perforated a parenchymatous cortical cell, selected as the ISC. No activities in the feeding apparatus could be visualized during this rest period. Steinbach's optical equipment was not as advanced as that used in the present study, and hence no data on gland activities and other features were given.

The rest period in *H. schachtii* lasts longer than in *G. rostochiensis*. In the present study it is termed preparation period, as it is thought to " transform " the migratory J2 into a sedentary J2. This supposition is supported by several observations : no changes in the protoplast of the ISC could be recognized during this period, which had an average duration of 7 h. The gradual decrease in the number of secretory granules in the ampullae and extensions of the two subventral glands indicates that the secretions of these granules may be used to mobilize lipid reserves, while the intestine is transformed into an absorptive organ, as already suggested by Atkinson and Harris (1989). Forward flow of these subventral gland secretions from the pump chamber (into which they are discharged) into the oesophageal lumen towards the ISC is most unlikely, for reasons given by Wyss and Zunke (1986). The few defecations towards the end of the preparation period strongly support the presumption that the secretions are passed into the intestine. No signs of passive ingestion, described, for instance, for *Ditylenchus dipsaci* (Doncaster, 1976), were evident. It seems that atrophy of the locomotory muscles is initiated during the preparation period, as the J2 were no longer able to leave the root after completion of this period. In emigrant J2 of *G. rostochiensis*, Forrest *et al.* (1988) recorded differences in lectin-binding to the cuticle as well as changes in the amphidial exudate between preparasitic and emigrated J2. They supposed that these changes were the result of an early interaction between host and parasite.

In their first description of the feeding behaviour of *H. schachtii* J2, Wyss and Zunke (1986) did not record a preparation period after ISC selection in the two J2 observed. Obviously these two J2 had already started feeding, but were disturbed and hence had to search for another feeding site close to their heads, where short forward and backward body movements were still possible. After completion of the preparation period, feeding starts sooner in a newly selected ISC. The function of vacuoles in the anterior part of the metacorpal bulb, often observed during the preparation period, is as yet difficult to explain. Perhaps the dorsal gland extension in this region may have been affected. In preparasitic J2 of *H. glycines*, the gland extension is bordered by a neurosecretory cell and a membrane complex (Endo, 1984). This complex also occurs in the same region in preparasitic *Meloidogyne incognita* J2, and Endo and Wergin (1988) supposed that the membranes function as supporting elements of the metacorpus muscles. Electromicroscopic examinations of *H. schachtii* J2 during the preparation period should reveal whether the membrane complex may also be involved in the formation of these vacuoles, which disappear a few hours after commencement of feeding.

When the nematodes were disturbed by yet unknown factors during feeding, the stylet appeared to perform a gustatory function, as during thrusting it was intermittently protracted for a few seconds and then retracted again. Probably this supposition cannot be substantiated, as a " gustatory neuron " associated with a network of fine channels in the stylet shaft of an aphelenchid nematode (Shepherd *et al.*, 1980) has not been observed in the J2 stylet of either *H. glycines* (Endo, 1983) or *Meloidogyne incognita* (Endo & Wergin, 1988).

The three feeding phases, described for *H. schachtii* J2 (Wyss & Zunke, 1986) occurred throughout development, including young adult females. Exactly the same phases and a similar pattern were also observed by Steinbach (1973) in feeding J2 and J3 of *G. rostochiensis.* Due to lack of optical resolution, phase III (salivation) could not be interpreted by him, but he too observed a prolonged duration of this phase just after the initial rest period (Ruheperiode R) which gradually decreased as feeding continued. Phase II (Ruheperiode R_1) and phase III (Ruheperiode R_2) then stayed rather constant while phase I (Saugperiode) increased.

The similarity of the feeding pattern of *H. schachtii* and *Globodera rostochiensis* suggests that it may be characteristic of all cyst nematodes. It differs considerably from that in *Meloidogyne incognita* J2 (Wyss *et al.*, 1992). The functional significance of stylet retraction from the syncytium by cyst nematodes at regular intervals and subsequent reinsertion cannot yet be explained satisfactorily. Possibly a suitable " condition " of the cytoplasm surrounding the stylet-tip has to be continuously maintained for an efficient withdrawal of nutrients from within a zone of modified cytoplasm. During phase III, secretions from the dorsal oesophageal gland are beyond doubt injected into the ISC. About one and a half days after the beginning of feeding, these secretions form visible feeding tubes, probably due to an interaction of the secretions with a change in nature of the cytoplasm. However, it cannot be excluded that the properties of the dorsal gland secretions may change during parasitism. Although secretory fluids from the dorsal oesophageal gland appeared to emanate through the stylet orifice in the form of a tube (Fig. 1 H) in a parasitic J2 displaced from the root about 2 days after the beginning of feeding, tubes are initially not formed in the cytoplasm of the ISC. In the early stage of *H. glycines* parasitism, the secretions were not separated by a definite membrane from the adjacent ER (Endo, 1991). One day later, distinct membrane-bound feeding tubes were formed in the modified cytoplasm around the stylet-tip. Rumpenhorst (1984) proposed that food is withdrawn by sedentary nematodes via an extensible ampulla, connected to the feeding tubes. This assumption can no longer be maintained, as in the present study feeding tubes were repeatedly formed in all developmental stages during phase III and remained connected to the stylet-tip orifice until stylet retraction at the beginning of phase II.

The marked and rapid decrease in the size of the two subventral secretory glands, accompanied by an enlargement of the dorsal gland during the early stage of parasitism, conforms with observations in the J2 of *H. glycines* (Atkinson & Harris, 1989) and *M. incognita* (Hussey & Mims, 1990). In both species considerable changes were also recorded in the ultrastructural morphology of the secretory granules in the dorsal gland and the two subventral glands (Endo, 1987; Hussey & Mims, 1990). In the parasitic J2 stage the granules of the subventral glands appeared degenerate, at least they were smaller and more electron dense. In the present light microscopic observation the density of the granules in the extensions and ampullae of the ventral glands was much decreased after completion of the preparation period, but numerous granules were still present in these parts of the ducts throughout feeding, including the J3 stages. In the J4 stages they were, however, difficult to recognize even at very high magnification, using digital AVEC-DIC image processing in real time. The function of the subventral secretory granules in parasitic stages is not yet resolved. Their increased rate of synthesis before completion of the moult, at least in the early J3 stages, indicates that they may still be necessary for digestive purposes.

Apart from forming feeding tubes, the secretions of the dorsal gland are required to maintain the syncytium. In *H. schachtii* J3 males, the dorsal gland is much smaller than in J3 females and from the values given in Table 1, it can be inferred that J3 males inject less secretions from the dorsal gland in spite of their much longer ingestion periods. Possibly the food quality in syncytia of males may be different to that present in syncytia of J3 females. Nematode development is not affected by the total amount of amino acids and proteins in the syncytia (Grundler *et al.* 1991 *a*), but by changes in the concentration of some specific amino acids (Betka *et al.*, 1991).

Sex determination in *H. schachtii* is most probably controlled by trophic factors (Grundler, 1989) that occur in an early stage of parasitism. Related anatomical features, expressed in the size of the dorsal gland and genital primordia with different division patterns, are distinct before the moult of the J2. The development of large spherical globules in the hypodermis of the J3 males about one day prior to the moult may be preparatory to rapid changes in body volume once moulting has started. Wright and Perry (1991) have shown that in moulting *Aphelenchoides hamatus* a decrease in the volume of the nematode at the beginning of the moults is due to a loss of water. They presented one of the first *in vivo* observations of the dynamics of stylet dissolution and regeneration. The present results with *H. schachtii* are basically in agreement with those of Wright and

Perry (1991). Endo (1985) provided a detailed account of ultrastructural changes in stylet formation in J3 of *H. glycines*. No attempt is made here to discuss the dynamic events with those of the static ultrastructural observations. However, it cannot be emphasized enough that for a better understanding of nematode-plant interactions and the interpretation of the functional dynamics, *in vivo* observations with video-enhanced light microscopy should be combined with an analysis of observations at the ultrastructural level. Specific questions can be answered when specimens are immediately fixed after *in vivo* observation and processed for electron microscopic examination. A first step in this direction was taken to clarify the origin and transfer of exudates through the cuticle of parasitic *H. schachtii* J2 (Endo & Wyss, 1991).

The approximate amount of food consumed by *H. schachtii* during growth has been calculated from body volume changes and the determination of continuous ingestion periods (Müller *et al.*, 1981). According to the present study, defecations involved with volume changes, do not occur regularly at the beginning of each feeding cycle. Hence this estimation is not very reliable and should be improved by calculating the quantity of food withdrawn from volume changes in the pump chamber during metacorpal bulb pulsation and the frequencies of bulb pulsations (e.g. Seymour, 1983). In agreement with Günther (1967), the sizes of the heavily cuticularized pump chambers stay constant during each developmental stage. As generally the rate of bulb pulsations did not differ between the stages, J4 females with about three times larger pump chambers than J2 females, will remove much more food, in spite of similar developmental and feeding values (Table 1) for both stages.

Acknowledgment and dedication

I thank Howard Atkinson, Paul Burrows, Burt Endo and Rolo Perry for critically reading the manuscript and for their helpful comments. This work is dedicated to my friend Eli Cohn, a wonderful man and most stimulating pioneer in phytonematology, who passed away from us much too early.

References

ATKINSON, H. J. & HARRIS, P. D. (1989). Changes in nematode antigens recognized by monoclonal antibodies during early infections of soya beans with the cyst nematode *Heterodera glycines*. *Parasitology*, 98 : 479-487.

BETKA, M., GRUNDLER, F. & WYSS, U. (1991). Influence of changes in the nurse cell system (syncytium) on the development of the cyst nematode *Heterodera schachtii*. Single amino acids. *Phytopathology*, 81 : 75-79.

DONCASTER, C. C. (1976). Feeding of the stem nematode, *Ditylenchus dipsaci* on leaf tissue of field bean, *Vicia faba*. *J. Zool.*, 180 : 139-153.

DROPKIN, V. H. & BOONE, W. R. (1966). Analysis of host-parasite relationships of root-knot nematodes by single-larva inoculations of excised tomato roots. *Nematologica*, 12 : 225-236.

ENDO, B. Y. (1983). Ultrastructure of the stomatal region of the juvenile stage of the soybean cyst nematode, *Heterodera glycines*. *Proc. helminth. Soc. Wash.*, 50 : 43-61.

ENDO, B. Y. (1985). Ultrastructure of the head region of molting second-stage juveniles of *Heterodera glycines* with emphasis on stylet formation. *J. Nematol.*, 17 : 112-123.

ENDO, B. Y. (1987). Ultrastructure of oesophageal gland secretory granules in juveniles of *Heterodera glycines*. *J. Nematol.*, 19 : 469-483.

ENDO, B. Y. (1991). Ultrastructure of initial responses of susceptible and resistant soybean roots to infection by *Heterodera glycines*. *Revue Nématol.*, 14 : 73-94.

ENDO, B. Y. & WERGIN, W. P. (1988). Ultrastructure of the second-stage juvenile of the root-knot nematode, *Meloidogyne incognita*. *Proc. helminth. Soc. Wash.*, 55 : 286-316.

ENDO, B. Y. & WYSS, U. (1991). Ultrastructure of cuticular exudations of *Heterodera schachtii*, as related to cuticle development and morphology. *Protoplasma* (in press).

FORREST, J. M. S., ROBERTSON, W. M. & MILNE, E. W. (1988). Changes in the structure of amphidial exudate and the nature of lectin labelling on freshly hatched, invaded and emigrant second stage juveniles of *Globodera rostochiensis*. *Nematologica*, 34 : 422-431.

GRUNDLER, F. (1989). *Untersuchungen zur Geschlechtsdetermination des Rübenzystennematoden* Heterodera schachtii. Ph. D. Thesis, University of Kiel, 114 p.

GRUNDLER, F., BETKA, M. & WYSS, U. (1991 *a*). Influence of changes in the nurse cell system (syncytium) on sex determination and development of the cyst nematode *Heterodera schachtii*. Total amounts of proteins and amino acids. *Phytopathology*, 81 : 70-74.

GRUNDLER, F., SCHNIBBE, L. & WYSS, U. (1991 *b*). *In vitro* studies on the behaviour of second stage juveniles of *Heterodera schachtii* (Nematoda : Heteroderidae) in response to host plant root exudates. *Parasitology*, 103 : 149-155).

GÜNTHER, B. (1967). *Beiträge zur Morphologie und Histologie von* Heterodera schachtii *(Schmidt)*. Ph. D. Thesis, University of Greifswald, 291 p.

HAMMOND-KOSACK, K. E., ATKINSON, H. J. & BOWLES, D. J. (1990). Changes in abundance of translatable mRNA species in potato roots and leaves following root invasion by cyst nematode *G. rostochiensis* pathotypes. *Physiol. mol. Plant Path.*, 37: 339-354.

HUSSEY, R. S. & MIMS, C. W. (1990). Ultrastructure of esophageal glands and their secretory granules in the root-knot nematode *Meloidogyne incognita*. *Protoplasma*, 156 : 9-18.

MÜLLER, J. REHBOCK, K. & WYSS, U. (1981 *a*). Growth of *Heterodera schachtii* with remarks on amounts of food consumed. *Revue Nématol.*, 4 : 227-234.

MÜLLER, J. WYSS, U. & Inst. Wiss. Film (1981 *b*). Entwicklung des Zystennematoden *Heterodera schachtii*. Film C 1387 des IWF, Göttingen. *Publikation Wissenschaftlicher Film, Sektion Biologie*, Serie 14, 20 pp.

PERRY, R. N., ZUNKE, U. & WYSS, U. (1989). Observations on the response of the dorsal and subventral oesophageal glands of *Globodera rostochiensis* to hatching stimulation. *Revue Nématol.*, 12 : 91-96.

RASKI, D. J. (1950). The life history and morphology of the sugar-beet nematode, *Heterodera schachtii* Schmidt. *Phytopathology*, 40 : 135-152.

RUMPENHORST, H. J. (1984). Intracellular feeding tubes associated with sedentary plant parasitic nematodes. *Nematologica*, 30 : 77-85.

SEYMOUR, M. (1983). The feeding pump of *Ditylenchus dipsaci* (Nematoda : Tylenchida). *Nematologica*, 29 : 171-189.

SHEPHERD, A. M., CLARK, S. A. & HOOPER, D. J. (1980). Structure of the anterior alimentary tract of *Aphelenchoides blastophthorus* (Nematoda : Tylenchida, Aphelenchina). *Nematologica*, 26 : 313-357.

STEINBACH, P. (1973). Untersuchungen über das Verhalten von Larven des Kartoffelzystenälchens (*Heterodera rostochiensis* Wollenweber, 1923) an und in Würzeln der Wirtspflanze *Lycopersicon esculentum* Mill. *Biol. Zbl.*, 92 : 563-582.

WRIGHT, K. A. & PERRY, R. N. (1991). Moulting of *Aphelenchoides hamatus*, with especial reference to formation of the stomatostyle. *Revue Nématol.*, 14 : 497-504.

WYSS, U., GRUNDLER, F. M. W. & MÜNCH, A. (1992). The parasitic behaviour of second-stage juveniles of *Meloidogyne incognita* in roots of *Arabidopsis thaliana*. *Nematologica*, 38 (in press).

WYSS, U. & ZUNKE, U. (1985). *In vitro* feeding of root parasitic nematodes. *In* : Zuckerman, B. M., Mai, W. F. & Harrison, M. B. (Eds). *Plant Nematology Laboratory Manual*. The University of Massachusetts. Agr. Exp. Sta. Amherst, Massachusetts : 91-96.

WYSS, U. & ZUNKE, U. (1986). Observations on the behaviour of second stage juveniles of *Heterodera schachtii* inside host roots. *Revue Nématol.*, 9 : 153-165.