Fundam. appl. Nematol., 1994, **17** (1), 17-27

Sperm competition in mated first generation hermaphrodite females of the HP 88 strain of *Heterorhabditis* (Nematoda : Heterorhabditidae) and progeny sex ratios in mated and unmated females

Ilona DIX *, Hinanit KOLTAI **, Itamar GLAZER **, and Ann M. BURNELL *

* *Department of Biology, St. Patrick's College, Maynooth, Co. Kildare, Ireland and*
** *Department of Nematology, A.R.O., The Volcani Centre, Bet Dagan 50-250, Israel.*

Accepted for publication 11 May 1993.

Summary – We have used a " dumpy " mutant to study the process of fertilisation in *Heterorhabditis*. We have found that in inseminated first generation hermaphrodite females, male sperm has a competitive advantage in fertilisation over the hermaphrodites' own sperm. Light microscope observations show that a spermatheca is absent in first generation hermaphrodite females and that inseminated sperm is stored in the proximal part of the ovotestis where it intermixes with hermaphrodite sperm. Inseminated sperm had well developed pseudopodia and appeared to be more active than hermaphrodite sperm. A spermatheca was observed in second generation amphimictic females and inseminated sperm was stored there and not in the proximal part of the ovary or in the uterus. The sex ratios of the outcross progeny of both hermaphrodites and amphimictic females ranged from 2.2 to 6.6 males per thousand progeny, a large deviation from the 1:1 ratio which would be expected among the outcross progeny of heterogametic males. A twenty to thirty fold higher proportion of male progeny was observed among the progeny of self fertilised hermaphrodites. Thus the source of the sperm, whether from males or hermaphrodites clearly influences progeny sex ratios in the HP 88 strain of *Heterorhabditis*.

Résumé – ***Compétition entre spermatozoïdes chez les femelles hermaphrodites de première génération de la souche HP 88 d'*Heterorhabditis *(Nematoda : Heterorhabditidae) et proportion des sexes dans la descendance de femelles fécondées et non fécondées –*** Nous avons utilisé un mutant « obèse » pour étudier le processus de la fécondation chez *Heterorhabditis*. Nous avons observé que, chez les femelles hermaphrodites de première génération inséminées, les spermatozoïdes provenant du mâle possèdent un avantage dans leur compétition avec les spermatozoïdes provenant de la femelle hermaphrodite elle-même. Les observations en microscopie optique ont montré qu'il n'y a pas de spermathèque chez les femelles hermaphrodites de première génération et que les spermatozoïdes introduits sont stockés dans la partie proximale de l'ovotestis où ils se mélangent avec les spermatozoïdes d'origine hermaphrodite. Les spermatozoïdes introduits ont des pseudopodes bien développés et paraissent plus actifs que les spermatozoïdes d'origine hermaphrodite. Les femelles amphimictiques de deuxième génération possèdent une spermathèque où sont stockés les spermatozoïdes introduits, et non dans la partie proximale de l'ovaire ou de l'utérus. La proportion des sexes dans la descendance croisée de femelles tant hermaphrodites qu'amphimictiques varie de 2,2 à 6,6 mâles pour mille descendants, chiffres très différents du rapport 1/1 qui aurait pu être attendu dans la descendance croisée de mâles hétérogamiques. Des mâles, en proportion 20 à 30 fois supérieure, ont été observés dans la descendance d'hermaphrodites autofécondés. Ainsi, l'origine des spermatozoïdes, suivant qu'ils proviennent de mâles ou d'individus hermaphrodites, influence nettement la proportion des sexes de la descendance chez la souche HP 88 d'*Heterorhabditis*.

Key-words : *Heterorhabditis,* fertilisation, sperm competition, sex ratio, dumpy mutant.

Insect parasitic nematodes of the genus *Heterorhabditis* were first described by Poinar (1975) with *H. bacteriophora,* which was isolated from a noctuid moth larva *(Heliothis punctigera)* in Brecon, South Australia, as the type species. Three further species of *Heterorhabditis* have since been described (Poinar *et al.,* 1987, 1992; Poinar, 1990) and this genus has been found to have a wide distribution in tropical and temperate regions of the world.

The infective juveniles (IJ) of *Heterorhabditis* possess in their intestine a symbiotic bacterium (*Xenorhabdus* sp.) which is lethal for their insect host. The IJs actively seek insect larvae in the soil and having gained entry into the insect haemocoel, they release cells of their bacterial symbiont which multiply rapidly and usually kill the insect within 48 h. Bacterial growth in the haemolymph provides suitable conditions for nematode growth and reproduction. The IJ resumes development and gives rise to a hermaphrodite female. The first generation hermaphrodite females give rise to an amphimictic second generation which contains both males and females. Nematode reproduction continues until the nutrient sta-

ISSN 1164-5571/94/01/17 11 $ 4.00 / © Gauthier-Villars - ORSTOM

tus of the cadaver deteriorates, whereupon adult development is supressed and IJs are formed at the third larval moult. These non feeding stages emerge into the soil where they may survive for several months in the absence of a suitable host.

The nutrient status of the environment in which larval development occurs appears to play a major role in determining the adult fate of developing *Heterorhabditis* larvae. Poinar (1990) noted that second generation females are amphimictic. He suggested however, that it might be possible to maintain a continuous line of *Heterorhabditis* hermaphrodites but only when IJs are formed in each generation, as such IJs would be expected to give rise to hermaphrodite adults. Glazer *et al.* (1991) have found that when the HP 88 strain of *Heterorhabditis* was grown on dog food agar plates, it was possible to serially subculture hermaphrodites in the absence of males for fifteen generations. Dix *et al.* (1992), who studied three described species of *Heterorhabditis* and several undescribed *Heterorhabditis* strains, have shown that when development occurs *in vivo* in larvae of the wax moth *Galleria mellonella,* a second generation of males and non self-fertile amphimictic females occurs. A similar result was obtained by Poinar *et al.* (1992) for the newly described species *H. indicus*. When *H. indicus* was grown *in vivo* in *G. mellonella,* the second generation consisted of amphimictic males and females, however when this species was grown in small drops of *G. mellonella* haemolymph, the restricted amount of nutrients available resulted in the development of IJs, which subsequently developed into hermaphrodite females. A hermaphroditic line of *H. indicus* was serially subcultured in this manner for 4 generations.

Heterorhabditis has been successfully utilized in the biological control of a variety of soil dwelling insect pests (reviewed by Klein, 1990). The potential of *Heterorhabditis* in biological control could, however, be greatly improved if certain traits (e.g. UV and desiccation sensitivity, lack of infectivity at temperatures below 12 °C, improved host finding) which may be limiting for field application, could be improved by genetic manipulation. Glazer *et al.* (1991) have shown that considerable genotypic variation exists among IJs of *H. bacteriophora* for beneficial traits and that the heritability for some of these traits (viz. heat and UV tolerance, host finding) is very high. The feasibility of genetic selection for host finding has already been demonstrated by Gaugler and Campbell (1989) in the closely related insect parasitic nematode *Steinernema feltiae* and the basis for enhanced host finding was found to be the result of an enhanced chemosensitivity to carbon dioxide (Gaugler *et al.*, 1991). Strain improvement by mutagenesis is another approach which is currently being investigated in the authors' laboratories.

In order to carry out such genetic studies in *Heterorhabditis,* an understanding of the life cycle, mating system and mode of sex determination in this genus is highly desirable. Zioni *et al.* (1992 *a*) and Koltai *et al.* (unpubl.) have recently described the first morphological mutants in *Heterorhabditis*. These morphological mutants express a recessive " dumpy " *(dpy)* phenotype i.e. *dpy* individuals are shorter than wild-type nematodes but have a similar diameter, *dpy*-1 hermaphrodites, for example, are just 64 % the length of their wild type siblings (Zioni *et al.*, 1992 *a*). These *dpy* phenotypes have 100 % expressivity. The phenotype can be detected in larvae but is most pronounced in the adult stages, both male and female. We have used one of these *dpy* mutants to study the process of fertilisation in first generation hermaphrodite females and we confirm the observation of Zioni *et al.* (1992 *a*) that first generation hermaphrodite females can produce progeny by self fertilisation or by outcrossing with males. In this report we also demonstrate that male sperm has a competitive advantage over hermaphrodite sperm and we provide evidence that sex determination in the HP 88 strain of *Heterorhabditis* differs from the typical XY/XX or XO/XX mode.

Materials and methods

Source and maintenance of the nematode and bacterial isolates

The *Heterorhabditis* isolates used were the HP 88 strain from Utah, USA and a " dumpy " mutant *dpy*-2 which was isolated by mutagenesis from " 6 Dy ", an inbred strain of HP 88 (Zioni *et al.*, 1992 *a;* Koltai *et al.*, unpubl.). This recessive mutant allele has been designated *Hdpy*-2 by Koltai *et al.* to distinguish it from a similar allele in *Caenorhabditis elegans,* but hereunder it will be referred to as *dpy*-2. The HP 88 strain of *Heterorhabditis* is generally regarded as a species of *H. bacteriophora* (see e.g. Poinar and Georgis, 1990), however Dix *et al.* (1992) have provided evidence from crossbreeding studies that the HP 88 strain is not conspecific with the Brecon strain, the type species from which *H. bacteriophora* was originally described by Poinar (1975).

Nematodes were cultured *in vivo* in *G. mellonella* larvae as described in Woodring and Kaya (1988). Symbiotic bacteria *(Xenorhabdus luminescens)* were isolated from surface sterilized HP 88 IJs as described by Akhurst (1980). Primary forms of *X. luminescens* were maintained on NBTA agar plates (Akhurst, 1986). Lipid agar plates (5 cm diameter) were used for mating experiments and for *in vitro* cultivation of the resulting progeny. These plates were prepared as described by Dunphy and Webster (1989). The plates were inoculated with *X. luminescens* and incubated at 25 °C for two days before use, to allow for bacterial growth.

Outcrossing first generation hermaphrodites on lipid agar plates

G. mellonella larvae were infected with IJs of the *dpy*-2 strain and incubated at 25 °C. 68-72 h after infection,

the *G. mellonella* cadavers were dissected in sterile Ringer's solution (Oxoid BR 52). Developing hermaphrodites were collected by aspiration using a microcapillary pipette which was drawn out to give an external diameter of 150-250 μm. Thirty young hermaphrodites which had no fertilised eggs in their uteri and thirty hermaphrodites which had from 10-50 developing eggs in their uteri were selected. Each of these hermaphrodites was placed individually on a lipid agar plate. Three wild type HP 88 males were added to each of 20 plates from each group. These males had been collected by dissection from *G. mellonella* cadavers which had been infected with *Heterorhabditis* HP 88 IJs and incubated at 25 °C for 6-7 days. Males were not added to the plates containing the remaining 10 hermaphrodites from each group, who served as unmated controls.

The plates were incubated at 25 °C and 24 h later the hermaphrodites (but not the males) were transferred singly to a new plate. Every 22-24 h for the subsequent four days, the individual hermaphrodites were transferred to a fresh plate, so that the egg laying and sperm utilisation of each individual hermaphrodite could be monitored. By day five, larvae had begun to hatch within the uteri of the hermaphrodites therefore the hermaphrodites were left on the last plate for four to five days so that the phenotypes of the larvae which had developed *in utero* could be determined. All plates were incubated at 25 °C and four to five days after egg laying, the nematode progeny were washed off the plates and their sex and phenotype was recorded. The data presented in the results section are taken only from those females which continued to produce progeny for at least three days after the experiment was set up.

Outcrossing second generation nematodes on lipid agar plates

G. mellonella larvae were infected with either the wild type HP 88 or the *dpy*-2 strain of *Heterorhabditis,* and incubated at 25 °C. The resulting cadavers were dissected six to seven days postinfection in sterile Ringer's solution and second generation virgin females and mature males were collected by aspiration using a microcapillary pipette. One female and three to five males were placed on a 5 cm lipid agar plate (prepared as described above). One to two days after the cross was set up, the females (but not the males) were transferred to a fresh lipid-agar plate. The plates were incubated at 25 °C and six to seven days later the nematodes were washed off each plate and the sex and phenotype of the outcross progeny was recorded. For the unmated controls, 20 second generation females were placed on a lipid agar plate and incubated at 25 °C for seven days. As can be seen from Table 3, these unmated females did not produce progeny.

Light microscope observations

All observations and photographs were made using an inverted Nikon Diaphot microscope equipped for Nomarski differential interference observation. The following categories of female were observed : unmated and mated first generation immature hermaphrodites (i.e. with no fertilised eggs in their uteri); unmated and mated mature hermaphrodites (i.e. with 10-50 fertilised eggs in their uteri) and unmated and mated second generation females. First generation hermaphrodites were obtained by dissection from *G. mellonella* cadavers which had been infected with *Heterorhabditis* HP 88 IJs and incubated at 25 °C for two to three days. The hermaphrodites were placed on lipid agar plates with males overnight to obtain freshly mated females. Mated second generation females with 10-50 developing eggs in their uteri were obtained by dissection from *G. mellonella* cadavers eight to nine days post infection. Unmated second generation females were obtained by dissection from *G. mellonella* cadavers six to seven days post infection. They were transferred to lipid agar plates and aged for one to two days before dissection and observation.

The nematodes were transferred to a drop of sterile *Caenorhabditis elegans* egg buffer (Edgar & McGhee, 1986) and the reproductive tract was dissected using a pair of fine needles. The dissected, but intact, reproductive systems remained viable for several hours so that continuous observations of sperm and egg movements along the female reproductive tract could be made. For photographs, glass coverslips were sometimes gently placed over the dissected reproductive systems.

Results

Progeny of unmated *dpy*-2 first generation hermaphrodites

The mean numbers of dumpy progeny produced per hermaphrodite per day are presented in Table 1. Young hermaphrodites which had no eggs in their uteri at the start of the experiment produced an average of 240 progeny each. Of these, 5.8 % were males and 76.8 % of all male progeny from this cohort of hermaphrodites was produced on day 2, their first day of egg laying (Fig. 1). The mature hermaphrodites (which already had some 10-50 fertilised eggs in their uteri at the start of the experiment) produced an average of 254 progeny each, of which 4.1 % were males. Over half (62.6 %) of these males were produced on day 1, their first day of egg laying (Fig. 1).

Progeny from *dpy*-2 first generation hermaphrodites mated with wild type males

Since *dpy*-2 is a recessive allele, any wild type progeny result from the utilization of male wild type sperm by the hermaphrodite, whereas progeny displaying a " dumpy " phenotype are the result of self-fertilisation by the hermaphrodite.

Table 1. *Mean number of progeny produced per unmated first generation* dpy-*2 hermaphrodite per day.*

	day 1	day 2	day 3	day 4	day 5
Young hermaphrodites *	0	82.2 ± 16	96.2 ± 18.2	43.1 ± 15.0	14.2 ± 5.3
Mature hermaphrodites **	92.3 ± 12.0	72.0 ± 9.6	64.9 ± 13.4	10.9 ± 5.2	13.0 ± 6.1

1. * Young hermaphrodites which had no eggs in their uteri at the start of the experiment (n = 9).
2. ** Mature hermaphrodites which had 10-50 fertilised eggs in their uteri at the start of the experiment (n = 8).

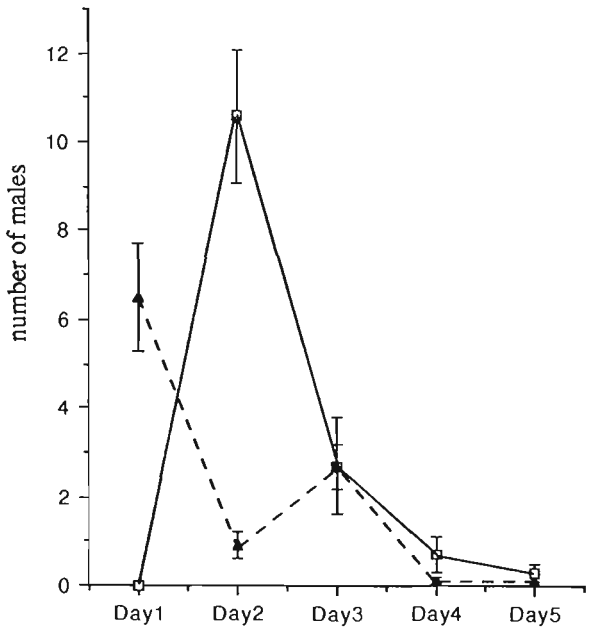

Fig. 1. *Mean number of male progeny produced per unmated first generation* dpy-*2 hermaphrodite per day (○—○: young, n = 9; ▲ - - - : mature, n = 8).*

Young hermaphrodites

Nineteen of the 20 young *dpy* hermaphrodites were fertilised by wild type males and 17 of these continued to lay eggs for at least three days. Mating plugs were observed on many of the females 12-20 h after setting up the crosses, however the presence of a mating plug did not prevent egg laying. No egg laying occurred in the first 24 h after fertilisation. Thus egg laying was initiated at the same stage in both the mated and unmated young hermaphrodites. The mean number of progeny produced per hermaphrodite was 220, and 41.3 % of the progeny had a wild type phenotype. Fig. 2 A illustrates the utilization of male sperm by the young hermaphrodites on successive days following fertilisation. From this figure it can be seen that male sperm was utilized to fertilise 69.5 % of the eggs on the first day of egg laying. In subsequent days the proportion of zygotes resulting from fertilisation with male sperm declined steadily and by five days after mating 91.5 % of the progeny resulted from self fertilisation. These data indicate that the male sperm is more successful in fertilisation than the hermaphrodites' own sperm and that male sperm is rapidly utilised following copulation.

Mature hermaphrodites

All 20 hermaphrodites were fertilised by the wild type males and 16 of these continued to lay eggs for at least three days. These mature hermaphrodites, which already had fertilised eggs in their uteri when mated, started to lay eggs within 24 h of the cross being set up. The mean number of progeny produced per hermaphrodite was 310 and 45.9 % of the progeny had a wild type phenotype. Fig. 2 B illustrates the utilization of male sperm by the mature hermaphrodites on successive days following fertilisation. Despite the fact that these hermaphrodites already had from 10-50 fertilised eggs in their uteri when outcrossed, it can be seen from this figure that 50.3 % of the progeny which developed from eggs laid on the first day had a wild type phenotype. The proportion of zygotes which gave rise to wild type progeny had increased to 69.3 % on day two and in subsequent days the proportion of wild type progeny declined.

SEX RATIOS AMONG THE PROGENY OF FIRST GENERATION MATED AND UNMATED *DPY*-2 HERMAPHRODITES

If sex determination in *Heterorhabditis* were of the typical XX/XO or XX/XY type, with the male as the heterogametic sex, then half of the progeny resulting from male fertilisation should be males. From Table 2 it can be seen, however, that the proportion of males resulting from outcrossing in the inseminated hermaphrodites ranged from 2.2 to 3.8 males per thousand progeny. When the same groups of hermaphrodites utilised hermaphrodite sperm in fertilisation, a twenty to thirty fold higher proportion of male progeny was observed. That the fertilisation of the eggs by the male sperm is a true fertilisation and not pseudogamy is evident from the wild type phenotype of the progeny. Thus the source of the sperm, whether from males or hermaphrodites clearly influences progeny sex ratios and the sex ratios of the outcross progeny which we observed deviate greatly from the 1:1 ratio expected among the outcross progeny of heterogametic males.

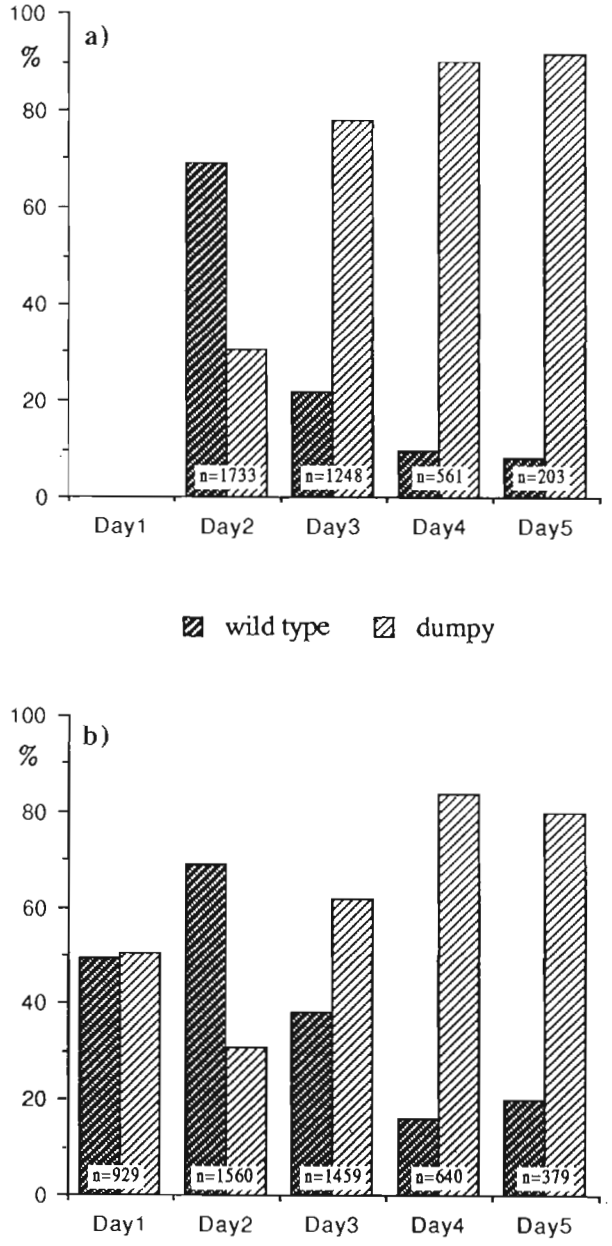

Fig. 2. *The utilisation of wild type* Heterorhabditis *HP 88 male sperm by mated* dpy-*2 hermaphrodites on successive days following fertilisation. A : young hermaphrodites; B : mature hermaphrodites.*

Sex ratios among the progeny of mated second generation amphimictic females

The male sex ratios obtained among the progeny of mated second generation amphimictic females are similar to those obtained among the outcross progeny of mated first generation hermaphrodite females (Table 3). Just eleven males (0.66 %) were observed among the 1952 progeny obtained from the HP 88 second generation females mated with HP 88 males, while no males were observed among the 333 progeny obtained from mating *dpy*-2 second generation females with HP 88 males.

Table 2. *Sex ratios among the progeny of unmated* dpy-*2 hermaphrodites and of* dpy-*2 hermaphrodites inseminated by wild type HP 88 males.*

Parents	N	% male progeny	Total progeny (N)
Unmated hermaphrodites *	9	5.77	2165
Unmated hermaphrodites **	8	4.09	2028
Mated hermaphrodites *	17		
dpy-2 progeny (self fertilisation)		7.46	2197
wild type progeny (male fertilisation)		0.38	1548
Mated hermaphrodites **	16		
dpy-2 progeny (self fertilisation)		7.07	2686
wild type progeny (male fertilisation)		0.22	2281

1. * Young hermaphrodites which had no eggs in their uteri at the start of the experiment.
2. ** Mature hermaphrodites which had 10-50 fertilised eggs in their uteri at the start of the experiment.

Table 3. *Sex ratios among the progeny of mated second generation amphimictic females.*

Parents	N	% male progeny	Total progeny (N)
HP 88 ♀ × HP 88 ♂♂	17	0.66	1652
Unmated HP 88 ♀ control	20	–	0
dpy-2 ♀ × HP 88 ♂♂	20	0	333
Unmated *dpy*-2 ♀ control	20	–	0

Light microscope observations

Unmated hermaphrodites

The tubular ovotestis is thin walled and is connected to a thick walled, constricted oviduct which leads to a large thin walled uterus. A spermatheca was not observed in either mated or unmated hermaphrodites. Hess and Poinar (1986) have described the structure of the genital ducts in hermaphrodite females of the Brecon strain of *H. bacteriophora* and they also noted that a spermatheca was absent in these females. Fig. 3 shows an egg passing through the oviduct of an unmated hermaphrodite and in this figure, hermaphrodite sperm can also be clearly seen in the proximal part of the ovotestis. In some cases, sperm were also seen as far up as the loop

of the ovotestis and occasionally a single row of 4-6 sperm was observed above the zone of mature oocytes, an arrangement which was suggestive of intermittent spermatogenesis in the mature hermaphrodites (Fig. 4). Sperm was not observed in the uteri of these unmated hermaphrodites. When the proximal part of the ovotestis was dissected to release hermaphrodite sperm, very few of the spermatozoa had pseudopods and some cells were larger and seemed more like spermatids, or possibly even secondary spermatocytes.

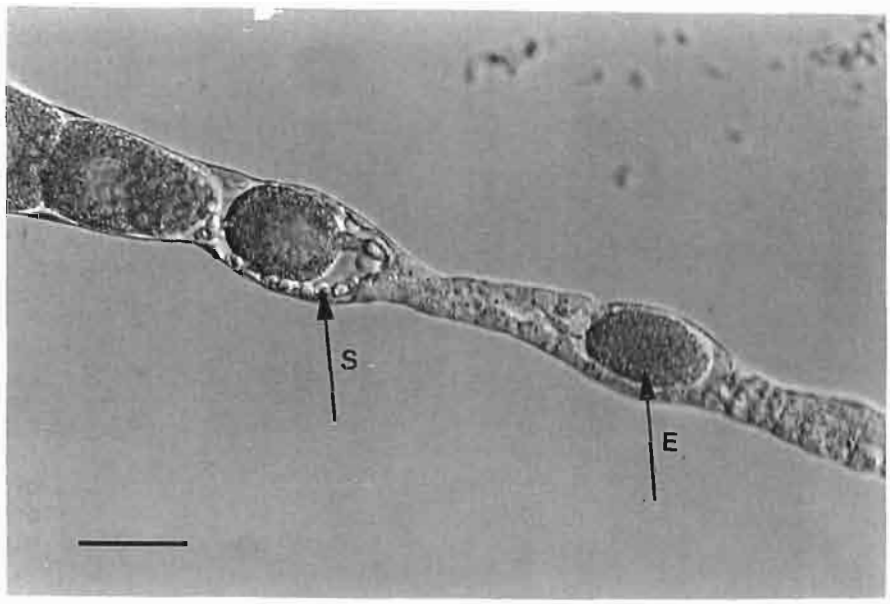

Fig. 3. *A portion of the ovotestis and oviduct of an unmated HP 88 hermaphrodite female. Arrows point to a fertilised egg (E) passing through the oviduct and to hermaphrodite sperm (S) collected in the proximal portion of the ovotestis* (Bar = 50 μm.)

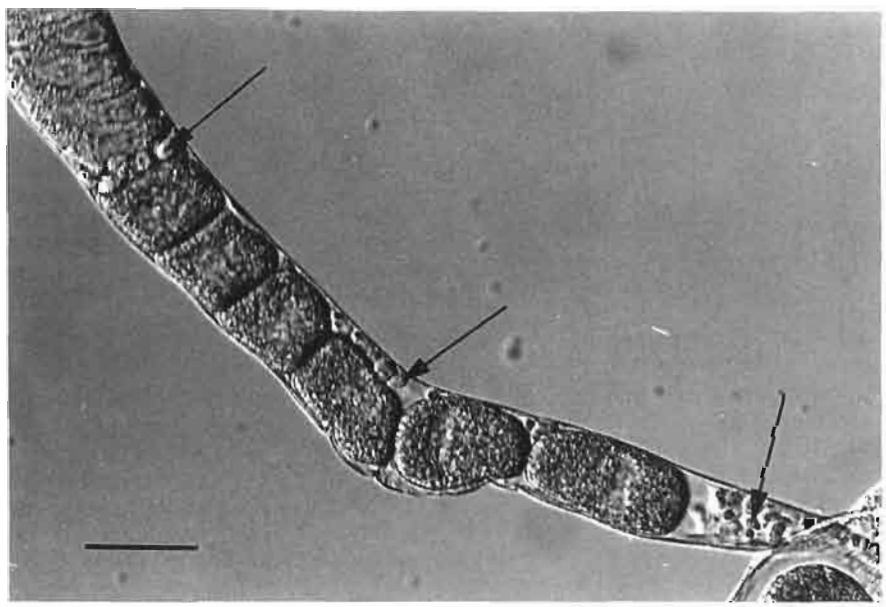

Fig. 4. *A portion of the ovotestis of an unmated* Heterorhabditis *HP 88 hermaphrodite. Hermaphrodite sperm (arrows) can be seen collected in the proximal end of the ovotestis, at the loop of the ovotestis and in a row between the mature and immature oocytes* (Bar = 50 μm.)

Mated hermaphrodites

Recently mated hermaphrodites can be recognised by the presence of a mating plug and of a large number of mature sperm in their uteri (Fig. 5). A few hours after mating, only a small number (five to ten) of sperm remain in the uterus. The movement of mature sperm up through the oviduct to the proximal part of the ovotestis, was observed (Fig. 6) and male sperm intermingled with the hermaphrodite sperm in the proximal part of the ovotestis. The pseudopods on the male sperm looked more pronounced than those on hermaphrodite sperm and male sperm appeared to move more rapidly. In addition to the pseudopodial movement of the sperm itself, the male sperm was also transported to the proximal part of the ovotestis by peristaltic movements of the oviduct (Fig. 6 A-D). The increased mobility of male sperm relative to that of hermaphrodite sperm might explain why male sperm have greater success in fertilisation in the first days following copulation than has the hermaphrodites' own sperm.

Mated second generation amphimictic females

In contrast to the situation in first generation hermaphrodites, a spermatheca was observed at the proximal end of the oviduct in both mated and unmated second generation females (Fig. 7 A, B). By 2-3 hours after copulation, the sperm deposited in the uterus of the amphimictic female had migrated to the spermatheca. Sperm were never observed in the proximal part of the ovary, contrary to our findings for mated hermaphrodites. Small numbers of sperm were frequently observed among the developing eggs in the uterus. These sperm are presumed to have been swept out of the spermatheca along with the fertilised eggs.

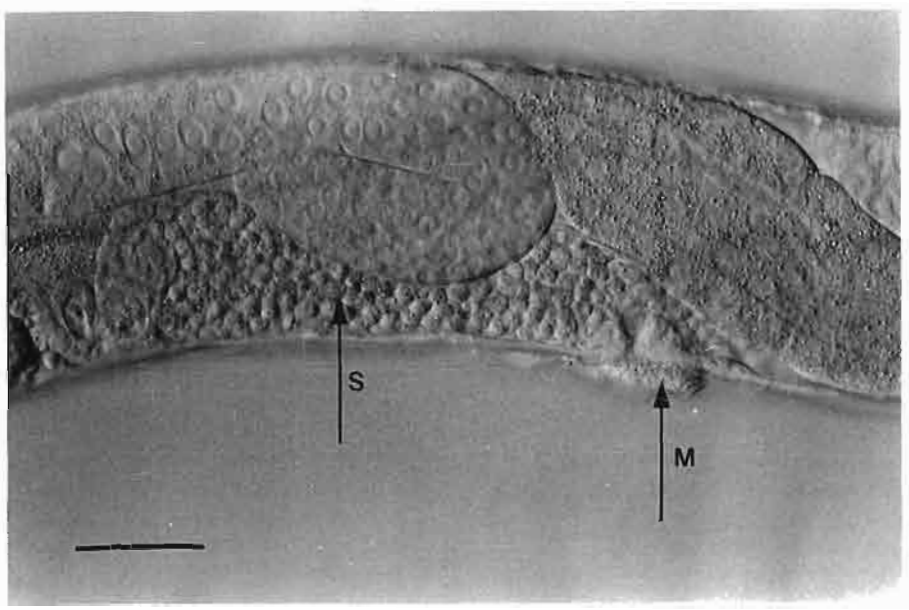

Fig. 5. *A recently inseminated young* Heterorhabditis *HP 88 hermaphrodite. Arrows show the position of the mating plug (M) and of the male sperm in the uterus (S)* (Bar = 50 μm.)

Discussion

Since first generation *Heterorhabditis* females are protandrous hermaphrodites, a single IJ invading an insect host can give rise to a successful infection. In *Steinernema* where the IJs develop into an amphimictic generation of males and females, at least one IJ of each sex must enter the host if the life cycle of the nematode is to be completed. Hermaphroditism thus confers a distinct survival value to heterorhabditid nematodes, but at the risk of inbreeding. The data presented in this study and by Zioni *et al.* (1992 *a*) demonstrate that first generation hermaphrodite females can be fertilised by males. Our data also show that for both mated and unmated hermaphrodites, over half of the males arise from eggs laid on the first day of egg laying. Thus in cases of multiple infection, these males can outcross with other hermaphrodites. Since Zioni *et al.* (1992 *b*) have shown that the development time for young adult males of *Heterorhabditis* HP 88 is 52 hours at 25 °C and we have shown in this study that hermaphrodites can continue to lay eggs for up to 96 hours, such males could also inseminate their hermaphrodite parent.

The predominant mode of reproduction in nematodes is by amphimixis, although protandrous hermaphroditism and meiotic or mitotic parthenogenesis may also occur (see reviews by Triantaphyllou and Hirschmann, 1964, and by Poinar and Hansen, 1983). Protandrous hermaphrodite females of *Caenorhabditis ele-*

gans can be mated by males, which occur at a low frequency in natural populations. By using morphological mutants, Ward and Carrel (1979) have demonstrated that in *C. elegans,* male sperm has a competitive advantage at fertilisation over hermaphrodite sperm. Within a few hours of mating, many of the mated hermaphrodites produced only outcross progeny. Some hermaphrodites continued to produce only outcross progeny, whereas others subsequently resumed production of self-progeny. In all cases however, the maximum proportion of outcross progeny was produced within 20 h of mating. Although none of the mated hermaphrodites in this present study produced outcross progeny exclusively, it is clear from Fig. 2 A, B that nearly 70 % of the progeny produced on the first day of egg laying resulted from outcrossing with male sperm. Using a fluorescent vital stain Ward and Carrel (1979) found evidence that in *C. elegans* hermaphrodites, the male sperm were able to displace hermaphrodite sperm from the wall of the spermatheca. In addition to the displacement mechanism, these authors also hypothesised that other mechanisms might also exist : e.g. the male sperm might have a higher affinity for the oocytes or might release an inhibitory factor which inactivates the hermaphrodite sperm. A spermatheca is absent in *Heterorhabditis* hermaphrodites and both hermaphrodite sperm and inseminated male sperm are located in the proximal part of the ovotestis. Our light microscope observations would suggest that the greater degree of activation and/or mobility of inseminated sperm relative to that of hermaphrodite sperm may be the basis for the competitive advantage of the male sperm at fertilisation.

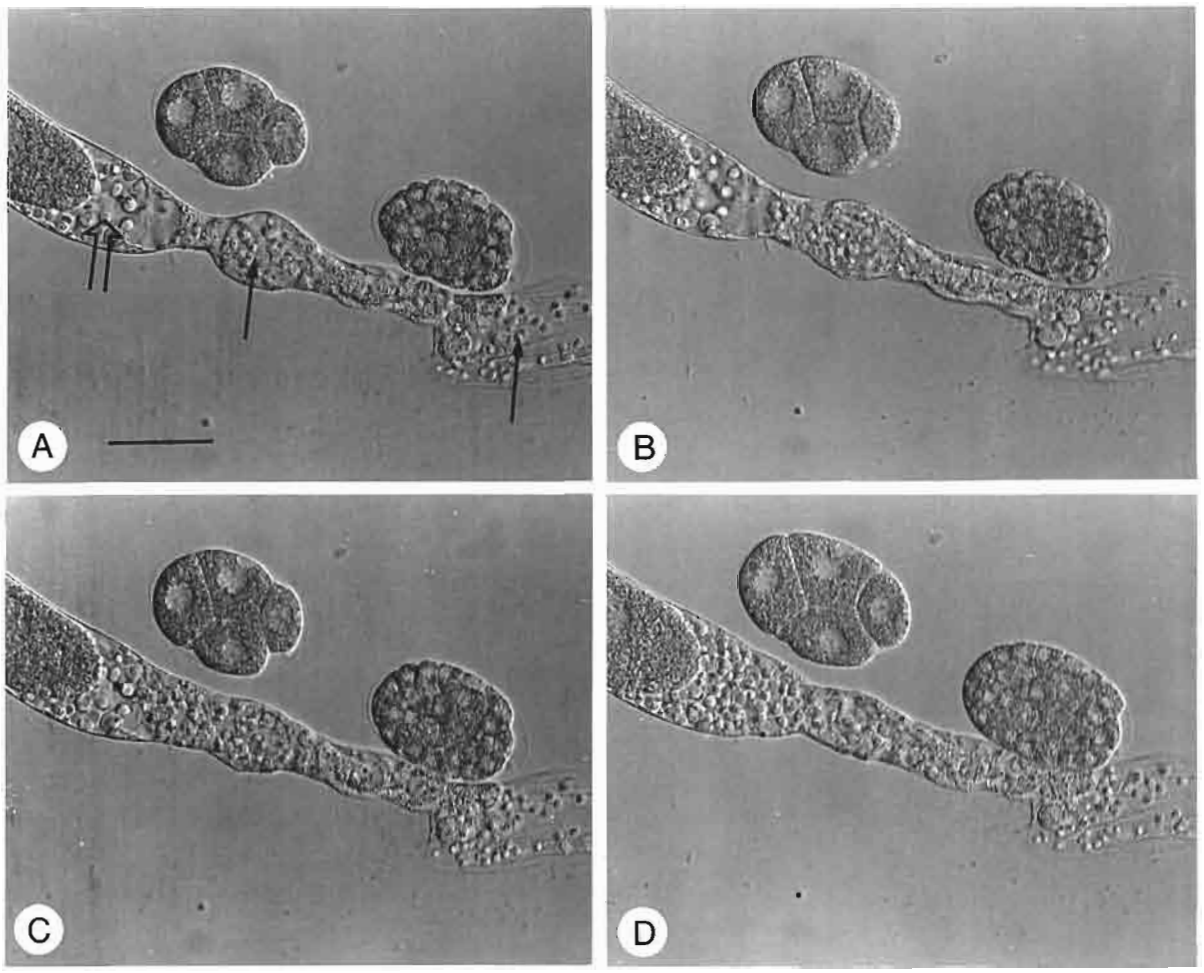

Fig. 6. *The progress of a peristaltic wave of contraction carrying sperm from the uterus to the proximal portion of the ovotestis in an inseminated HP 88* Heterorhabditis *hermaphrodite. A : Hermaphrodite sperm (open arrow) can be seen in the proximal part of the ovotestis, inseminated sperm can be seen in the distal portion of the uterus and in the portion of the oviduct which is in the relaxation phase of peristalsis (closed arrows); B : Relaxation of the muscle at the distal end of the oviduct allows the first of the inseminated sperm to reach the proximal part of the ovotestis; C : Hermaphrodite sperm and inseminated sperm intermingle in the proximal part of the ovotestis; D : The oviduct lumen becomes constricted again* (Bar = 50 µm.)

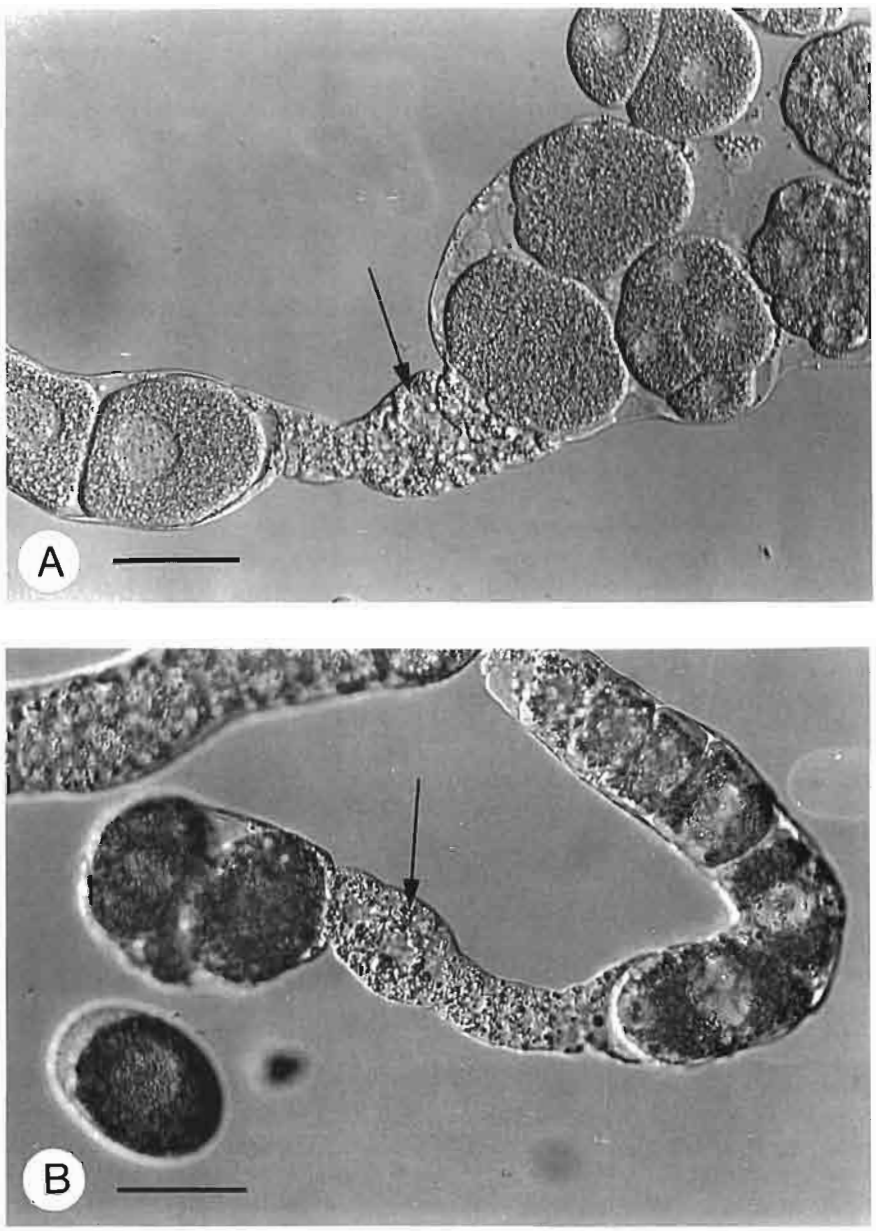

Fig. 7. *Portions of the dissected reproductive systems from A : mated and B : unmated second generation amphimictic* Heterorhabditis *HP 88 females in each of which a spermatheca (arrow) can be seen in the proximal part of the oviduct* (Bar = 50 μm.)

Hess and Poinar (1986) noted the absence of a spermatheca in second generation amphimictic females of the Brecon strain *H. bacteriophora*. The spermatheca is a specialised sperm storage chamber which, in rhabditids, is derived from the oviduct (Geraert, 1983). Hess and Poinar found that sperms which were inseminated into amphimictic females of the Brecon strain migrated to the expanded distal portion of the uterus and thus the oocytes were fertilised as they left the oviduct. In this report we describe the presence of a specialised spermatheca in the distal part of the oviduct of the amphimictic females of the HP 88 strain of *Heterorhabditis* and we observed that inseminated sperm were stored there rather than in the uterus. The cross-breeding data of Dix *et al.* (1992) indicated that the HP 88 strain of *Heterorhabditis* was not conspecific with the Brecon strain of *H. bacteriophora*, thus data which we now present provide evidence of a morphological difference be-

tween these two distinct biological species. The presence of a spermatheca in the amphimictic female and the absence of a spermatheca in the hermaphrodite female of the HP 88 strain is indicative of the differing reproductive specialisations of these two stages, one (the hermaphrodite female) being predominantly self fertilising, and the other being exclusively amphimictic.

Ward and Carrel (1979) demonstrated that mated *C. elegans* hermaphrodites produce more progeny than unmated hermaphrodites. This is because the hermaphrodites produce more that 100 excess oocytes relative to the amount of sperm which they produce and store as L 4 larve, so sperm is limiting in these hermaphrodites. Such apparent wastage of metabolic energy in unfertilised oocytes does not occur in *Heterorhabditis* as after 3-4 days of egg laying, the remaining larvae develop *in utero,* utilising the remaining food reserves of the mother. In this study we did not observe an increase in the number progeny produced by mated hermaphrodites as compared with unmated hermaphrodites. Our light microscopic observations suggest that intermittent spermatogenesis may occur in adult hermaphrodites and this may be the reason why unmated hermaphrodites produced as much progeny as mated hermaphrodites. In *C. elegans* spermatogenesis occurs only in the L 4 larval stage and not in adult hermaphrodites, although sperm maturation does continue into the adult stage. In some other rhabditid species, however, sperm production may occur either intermittently or continuously in the adult hermaphrodites (Runey *et al.,* 1978).

The male/female ratio among the outcross progeny of mated *C. elegans* is 1:1 or close to it, as would be expected in a species where the male is the heterogametic sex and where sex determination is of the XX/XO type (Nigon, 1949). Although first generation *Steinernema* females are slightly more numerous than males, the male/female ratio is close to 1:1 (Bednarek *et al.,* 1986; Gaugler *et al.,* 1990; Nguyen & Smart, 1992). In *Heterorhabditis,* by contrast, less than 0.7 % males were observed among the outcross progeny of hermaphrodites and amphimictic females in the study reported here. Poinar (1967) observed that *Steinernema carpocapsae* (DD-136) males contained four bivalents and a single univalent chromosome (2 n = 9), and on this basis proposed that the karyotype of *S. carpocapsae* females was 2 n = 10. Guohan *et al.* (1989) have described the karyotypes of three species of *Steinernema* and in all cases they found that the males are 2 n = 9 and the females 2 n = 10. This indicates that in *Steinernema* the male is the heterogametic sex and sex determination is of the XX/XO type. A karyotype of n = 7 has been observed in first generation *Heterorhabditis* hermaphrodites from a range of species and isolates (Khan *et al.,* 1976; Curran, 1989) however no data are available on the karyotype of *Heterorhabditis* males.

Sex determination in nematodes is typically of the XX/XO type (or less commonly of the XX/XY type), with the male as the heterogametic sex. In some species of nematodes however, sex determination is the result of environmental factors such as crowding, temperature or nutritional depletion (reviewed by Poinar and Hansen, 1983; see also Clark, 1978; Bull, 1983). In this study we have observed that the source of the sperm, whether from males or hermaphrodites, greatly influences sex ratios. In marked contrast to *C. elegans,* the proportion of males is extremely low among outcross progeny and this proportion is twenty to thirty times lower than the proportion of males which results from hermaphrodite self fertilisation. It is possible that the deviation from the 1:1 ratio expected among the outcross progeny of heterogametic males may be the result of meiotic drive resulting from a reduced fitness of nullo-X sperm. An alternative explanation is that sex determination in the HP 88 strain of *Heterorhabditis* is influenced by environmental factors. In the absence of karyotype data, it is difficult to ascertain whether sex determination in *Heterorhabditis* is mediated via the karyotype or the environment. In future experiments we plan to carry out karyotype analyses and to investigate sex ratios among outcross progenies from a range of *Heterorhabditis* strains and species.

Acknowledgements

This research was carried out in part with the generous support of the European Community (ECLAIR Grant 151).

References

Akhurst, R. J. (1980). Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *J. gen. Microbiol.,* 121 : 303-309.

Akhurst, R. J. (1986). *Xenorhabdus,* a genus of bacteria symbiotically associated with insect pathogenic nematodes. *Inst. J. Syst. Bacteriol.,* 33 : 38-45.

Bednarek, A., Nowicki, T. & Wojic, W. F. (1986). Sex structure in populations of *Steinernema feltiae*. *Sezs. Probl. Postepox Nauk Roln.,* 323 : 213-223.

Bull, J. J. (1983). *Evolution of sex determining mechanisms.* Menlo Park, California, The Benjamin/Cummings Publishing Co. Inc., 316 p.

Clark, W. C. (1978). Metabolite-mediated density dependent sex determination in a free-living nematode, *Diplenteron potohikus*. *J. Zool. London,* 184 : 245-254.

Curran, J. (1989). Chromosome numbers of *Steinernema* and *Heterorhabditis* species. *Revue Nématol.,* 12 : 145-148.

Dix, I., Burnell, A. M., Griffin, C. T., Joyce, S. A., Nugent, M. J. & Downes, M. J. (1992). The identification of biological species in the genus *Heterorhabditis* (Nematoda : Heterorhabditidae) by cross-breeding second-generation amphimictic adults. *Parasitology,* 104 : 509-518.

Dunphy, G. B. & Webster, J. M. (1989). The monoxenic culture of *Neoaplectana carpocapsae* DD 136 and *Heterorhabditis heliothidis*. *Revue Nématol.,* 12 : 113-123.

EDGAR, L. G. & MCGHEE, J. D. (1986). Embryonic expression of a gut specific esterase in *Caenorhabditis elegans*. *Develop. Biol.*, 114 : 109-118.

GAUGLER, R. & CAMPBELL, J. F. (1989). Selection for host-finding in *Steinernema feltiae*. *J. Invert. Pathol.*, 54 : 363-372.

GAUGLER, R., CAMPBELL, J. F. & MCGUIRE, T. (1990). Fitness of a genetically improved nematode. *J. Invert. Pathol.*, 56 : 106-116.

GAUGLER, R., CAMPBELL, J. F. & GUPTA, P. (1991). Characterisation and basis of enhanced host-finding in a genetically improved strain of *Steinernema carpocapsae*. *J. Invert. Pathol.*, 57 : 234-241.

GERAERT, E. (1983). The use of the female reproductive system in systematics. *In :* Stone, A. R., Platt, H. M. & Khalil, L. F. (Eds). *Concepts in Nematode Systematics*. London, Academic Press :73-84.

GLAZER, I., GAUGLER, R. & SEGAL, D. (1991). Genetics of the entomopathogenic nematode *Heterorhabditis bacteriophora* (Strain HP 88) : The diversity of beneficial traits. *J. Nematol.*, 23 : 324-333.

GUOHAN, W., JUNLIE, L. & ZHENGSUN, W. (1989). Comparative studies on the karyotypes of three species of nematodes in the genus *Neoaplectana*. *Zool. Res.*, 10 : 71-77.

HESS, R. & POINAR, G. O. (1986). Ultrastructure of the genital ducts and sperm behavior in the insect parasitic nematode *Heterorhabditis bacteriophora* Poinar (Heterorhabditidae : Rhabditida). *Revue Nématol.*, 9 : 141-152.

KHAN, A., BROOKS, W. M. & HIRSCHMANN, H. (1976). *Chromonema heliothidis* n. gen., n. sp. (Steinernematidae, Nematoda), a parasite of *Heliothis zea* (Noctuidae, Lepidoptera) and other insects. *J. Nematol.*, 8 : 159-168.

KLEIN, M. (1990). Efficacy against soil-inhabiting insect pests. *In :* Gaugler, R. & Kaya, H. K. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton, Fl, USA, CRC Press : 195-214.

NIGON, V. (1949). Les modalités de la reproduction et le déterminisme de sexe chez quelques nématodes libres. *Ann. Sci. nat. Zool.*, 11 : 1-132.

NGUYEN, K. B. & SMART, G. C. (1992). Life cycle of *Steinernema scapterisci* Nguyen & Smart, 1990. *J. Nematol.*, 24 : 160-169.

POINAR, G. O. (1967). Description and taxonomic position of the DD 136 nematode (Steinernematidae, Rhabditoidea) and its relationship to *Neoaplectana carpocapsae* Weiser. *Proc. helminth. Soc. Wash.*, 34 : 199-209.

POINAR, G. O. (1975). Description and biology of a new insect parasitic rhabditoid *Heterorhabditis bacteriophora* n. gen., n. sp. (Rhabditida; Heterorhabditidae n. fam.). *Nematologica,* 21 : 463-470.

POINAR, G. O. (1990). Taxonomy and biology of Steinernematidae and Heterorhabditidae. *In :* Gaugler, R. & Kaya, H. K. (Eds). *Entomopathogenic nematodes in biological control :* Boca Raton, Fl, USA, CRC Press : 23-61.

POINAR, G. O. & HANSEN, E. (1983). Sex and reproductive modifications in Nematodes. *Helminth. Abstr. Ser. B.*, 52 : 145-163.

POINAR, G. O. & GEORGIS, R. (1990). Characterization and field application of *Heterorhabditis bacteriophora* strain HP 88 (Heterorhabditidae : Rhabditida). *Revue Nématol.*, 13 : 387-393.

POINAR, G. O., JACKSON, T. & KLEIN, M. (1987). *Heterorhabditis megidis* sp. n. (Heterorhabditidae : Rhabditida), parasitic in the Japanese beetle *Popillia japonica* (Scarabaeidae : Coleoptera), in Ohio. *Proc. helminth. Soc. Wash.*, 54 : 53-59.

POINAR, G. O., KARUNAKAR, G. K. & HASTINGS, D. (1992). *Heterorhabditis indicus* n. sp. (Rhabditida : Nematoda) from India : separation of *Heterorhabditis* spp. by infective juveniles. *Fundam. appl. Nematol.*, 15 : 467-472.

RUNEY, M. W., RUNEY, G. L. & LAUTER, F. H. (1987). Gametogenesis and fertilisation in *Rhabdias ranae* Walton 1929, 1. The parasitic hermaphrodite. *J. Parasitol.*, 64 : 1008-1014.

TRIANTAPHYLLOU, A. C. & HIRSCHMANN, H. (1964). Reproduction in soil and plant nematodes. *Ann. Rev. Phytopath.*, 2 : 57-80.

WARD, S. & CARREL, J. S. (1979). Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. *Develop. Biol.*, 73 : 304-321.

WOODRING, J. L. & KAYA, H. K. (1988). *Steinernematid and Heterorhabditis nematodes : A handbook of biology and techniques.* Arkansas agric. Exp. Stn., Fayetteville, Arkansas, Southern Cooperative Series Bulletin, 331, 30 p.

ZIONI (COHEN-NISSAN), S., GLAZER, I. & SEGAL, D. (1992 *a*). Phenotypic and genetic analysis of a mutant of *Heterorhabditis* bacteriophora strain HP 88. *J. Nematol.*, 24 : 359-364.

ZIONI (COHEN-NISSAN), S., GLAZER, I. & SEGAL, D. (1992 *b*). Life cycle and reproductive potential of the nematode *Heterorhabditis bacteriophora* strain HP 88. *J. Nematol.*, 24 : 352-358.