Chromosome numbers of *Steinernema* and *Heterorhabditis* species

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SUMMARY

Chromosome number is conserved amongst species within the genera *Steinernema* (n = 5) and *Heterorhabditis* (n = 7), with the exception of the *Steinernema* sp. NC 513 group of isolates (n = 3). Interbreeding data and chromosome numbers indicate that the *S. glaseri* and *Steinernema* sp. NC 513 group of isolates should be considered separate species. Absence of pre-zygotic mating barriers, similar geographic distribution and similar morphology suggest that *Steinernema* sp. NC 513 may have evolved from a population of *S. glaseri* by chromosome fusion events. There is no difference in the ability of these species to grow, reproduce and retain the primary form of *Xenorhabdus nematophilus* subsp. *poinarii* NC 513 bacteria in the infective stage.

RÉSUMÉ

Nombres chromosomiques chez les espèces de Steinernema *et* Heterorhabditis

Le nombre de chromosomes est constant chez les espèces appartenant aux genres *Steinernema* (n = 5) et *Heterorhabditis* (n = 7), à l'exception des groupes d'isolats « *Steinernema* sp. NC 513 » (n = 3). Les données fournies par les essais de croisement et les nombres chromosomiques montrent que *S. glaseri* et *Steinernema* sp. NC 513 devraient être considérés comme des espèces distinctes. L'absence de barrières à une fécondation pré-zygotique, la répartition géographique similaire et une morphologie identique suggèrent que *Steinernema* sp. NC 513 peut avoir dérivé d'une population de *S. glaseri* à la suite de fusions chromosomiques. Il n'existe pas de différences entre ces deux espèces en ce qui concerne la croissance, la reproduction et la capacité de conserver chez les stades infestants la forme primaire de la bactérie *Xenorhabdus nematophilus* subsp. *poinarii* NC 513.

The increasing economic importance of insect-parasitic steinernematids and heterorhabditids has led to the collection of many new isolates from around the world. Unfortunately the taxonomic description of many of these nematodes has not kept pace with their acquisition. In part, this is due to the lack of general agreement on which morphological features constitute reliable diagnostic characters (Akhurst & Bedding, 1978; Akhurst, 1987). In other nematode groups the study of chromosomes has proved helpful in determining the evolutionary relationships between nematode taxa and provided useful diagnostic characters for species identification (Walton, 1959; Triantaphyllou & Hirschmann, 1980). The purpose of this study was to survey the available described and undescribed species of *Steinernema* and *Heterorhabditis* to determine if chromosome number could provide useful taxonomic information to supplement morphological data in the families Steinernematidae and Heterorhabditidae.

Materials and methods

The nematodes used in this study, listed in Table 1, were cultured *in vivo* in *Galleria mellonella* larvae or *in vitro* in monoxenic culture (Bedding, 1981). Gravid females of *Steinernema* species and first generation hermaphroditic adults of *Heterorhabditis* species were dissected from *G. mellonella* larvae or picked from the culture substrate and placed in Ringer's solution (150 mM NaCl, 4 mM KCl, 2 mM CaCl2). Adhering bacteria were removed by gentle agitation and the females transferred to a drop of fresh Ringer's solution on a glass slide. Excess Ringer's solution was removed with absorbent paper tissue until a thin surface film was left covering the nematode. The gonad was dissected out with the aid of a 27 G hypodermic needle, allowed to air dry rapidly on the slide, fixed in Carnoy's fluid (ethanol, chloroform, acetic acid:9:1:1) for 5-15 min and stained with aceto-orcein (1 g natural orcein dissolved in 45 ml glacial acetic acid, made up to 100 ml with distilled water and filtered) for 5-15 min. A glass coverslip was then placed over the specimen and gentle, even pressure applied with absorbent paper tissue to remove excess stain. Chromosome counts were made by counting the number of bivalents present in oocytes in *Steinernema* spp., and spermatocytes in *Heterorhabditis* spp. at prometaphase I. Counts are based on a minimum of five females or hermaphrodites and a total of 30 oocytes or spermatocytes at prometaphase I for each isolate.

Table 1

Chromosome number, geographic origin and source of species of nematode

Species/Isolate	*Chromosome number*	*Geographical origin*	*Source**
Steinernema sp. NC 513/NC 17 A	3	North Carolina, USA	W. M. Brooks
Steinernema sp. NC 513/NC 18 A	3	North Carolina, USA	W. M. Brooks
Steinernema sp. NC 513/NC 19 A	3	North Carolina, USA	W. M. Brooks
Steinernema sp. NC 513/NC 20 B	3	North Carolina, USA	W. M. Brooks
Steinernema sp. NC 513/NC 513	3	North Carolina, USA	W. M. Brooks
Steinernema sp. ED/1	5	North Carolina, USA	A. T. Drooz
Steinernema sp. NC 270	5	North Carolina, USA	CSIRO
Steinernema sp. C 85011	5	South China	GEI
Steinernema sp. C 2 B 2	5	North China	CAAS
Steinernema sp. C-Zuhai	5	South China	GEI
Steinernema sp. CWL 05	5	South China	GEI
S. affinis/DAN	5	Denmark	A. E. Pye
S. anomali	5	USSR	S. Spiridonov
S. bibionis/T 335	5	Tasmania	CSIRO
S. carpocapsae/ALL	5	Florida, USA	K. V. Deseo
S. glaseri/KG	5	North Carolina, USA	H. K. Kaya
S. glaseri/NC 32	5	North Carolina, USA	CSIRO
S. glaseri/NC 34	5	North Carolina, USA	CSIRO
S. glaseri/NC 40	5	North Carolina, USA	CSIRO
S. glaseri/NC 50	5	North Carolina, USA	CSIRO
S. glaseri/NC 52	5	North Carolina, USA	CSIRO
Heterorhabditis sp. D 1	7	Northern Territory, Aus.	CSIRO
Heterorhabditis sp. NZ	7	Auckland, New Zealand	W. Wouts
Heterorhabditis sp. V 16	7	Victoria, Aus.	CSIRO
Heterorhabditis sp. HW 79	7	Netherlands	W. R. Simons
Heterorhabditis sp. NC 162	7	North Carolina, USA	CSIRO
H. heliothidis	7	North Carolina, USA	W. Wouts
H. bacteriophora	7	South Australia, Aus.	W. Wouts
H. megidis	7	Ohio, USA	T. Jackson
Undescribed genus P., Q 1	5	N. Queensland, Aus.	CSIRO
Undescribed genus P., Q 617	5	N. Queensland, Aus.	CSIRO

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Species determinations in the genus *Steinernema* were made by the cross-breeding technique of Akhurst and Bedding (1978), with appropriate controls including the injection of single nematodes into *G. mellonella* to test for parthenogenetic or self-fertilizing hermaphroditic nematode isolates.

Results

The chromosome numbers of 31 species/isolates of *Steinernema, Heterorhabditis* and an undescribed new genus of steinernematid (Q 1, Q 617; Bedding, pers. comm.) are presented in Table 1. All *Heterorhabditis* species have n = 7; *Steinernema* species have n = 5, with the exception of *Steinernema* sp. NC 513 which has n = 3. The two species Q 1 and Q 617 of the undescribed genus have n = 5. Examples of each chromosome complement are presented in Fig. 1. There was no variation of chromosome number within a species or isolate. Single nematodes of all *Heterorhabditis* isolates injected into *G. mellonella* produced fertile offspring; no progeny were obtained from single nematodes of any *Steinernema* isolate.

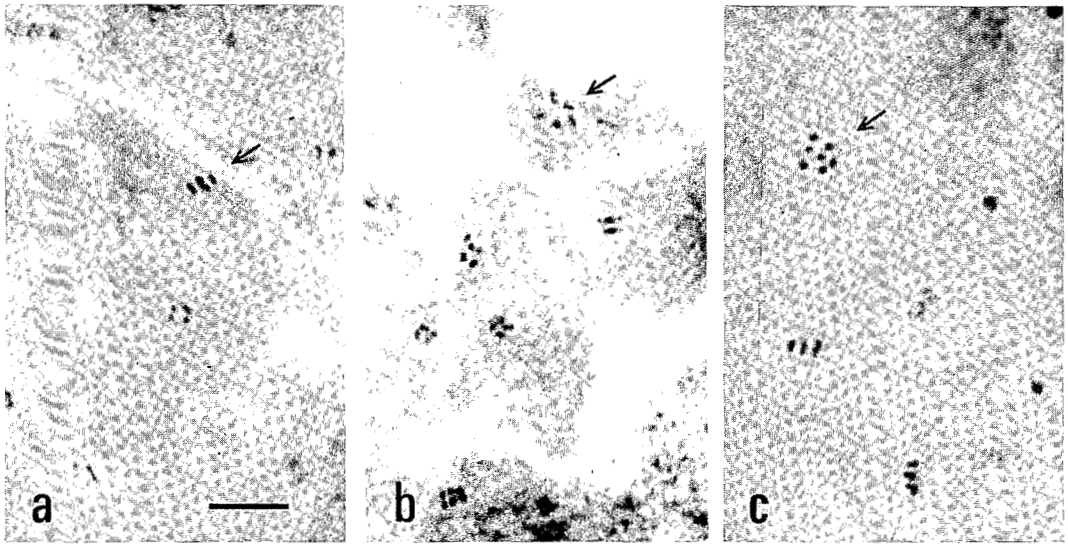

Fig. 1. Examples of aceto-orcein stained prometaphase I chromosomes of *Steinernema* spp. oocytes and *Heterorhabditis* spp. spermatocytes — (a) *Steinernema* sp. NC 513, n = 3; (b) *S. glaseri* NC 32, n = 5; (c) *H. heliothidis*, n = 7. Note that all bivalents are not in the same focal plane in all metaphase plates shown. (Bar = 8 µm.)

The *Steinernema* spp. listed in Table 1 together with Q 1 and Q 617 did not produce fertile offspring when mated to the morphologically most similar nematode species using the cross-breeding method of Akhurst and Bedding (1978) (Bedding, pers. comm.). Interbreeding tests proved the conspecificity of isolates *Steinernema* NC 17 A, 18 A, 19 A, 20 B with *Steinernema* sp. NC 513. In reciprocal crosses of *S. glaseri* NC 34 and *Steinernema* sp. NC 513 progeny were produced only in the NC 34 female × NC 513 male cross; all progeny (51 and 2 from two crosses) developed into phenotypic females which were infertile in backcrosses to both NC 34 and NC 513 males (four females : five males, three females : five males respectively). Examination of PO females revealed chromosomal abnormalities in developing, fertilized eggs, though sperm transfer and egg penetration appeared normal; in the F 1 (i.e. the females used in the backcross) gonad development was abnormal with degeneration of the lower reproductive tract and lack of developing oocytes. Abundant sperm were present.

The undescribed *Heterorhabditis* spp. listed in Table 1 were recognized as being different from other described species by morphological (Bedding, pers. comm.) and/or repetitive DNA restriction fragment length differences (Curran, unpubl.).

Discussion

Chromosome number is conserved amongst species within the genera *Heterorhabditis* (n = 7) and *Steinernema* (n = 5), with the exception of the *Steinernema* sp. NC 513 group of isolates (n = 3). This conservation of chromosome number within each genus minimizes the general taxonomic value of this character for species identification. However, chromosome number is extremely useful in distinguishing the morphologically similar *S. glaseri* and *Steinernema* sp. NC 513. The observed chromosome numbers (n = 3 to 7) are within the range previously reported for species of Rhabditida (n = 3-9; Walton, 1959; Triantaphyllou & Hirschmann, 1980), and in agreement with the chromosome number n = 5 or 2 n = 9 previously illustrated for *S. carpocapsae* males (Poinar, 1967), and n = 7 for hermaphrodites of *H. heliothidis* (Khan, Brooks & Hirschmann, 1976). All *Steinernema* isolates required the presence of both sexes to reproduce whilst infective stage *Heterorhabditis* nematodes developed into hermaphroditic adults and were able to reproduce when a single nematode was present in the host (Poinar, 1983; data from this study).

One question posed by this study is the relationship of *S. glaseri* to the *Steinernema* sp. NC 513. The latter species is morphologically similar to *S. glaseri* and, in the literature, has been referred to as an isolate of this species (Couche *et al.*, 1987). Interbreeding data and chromosome number indicate that the *S. glaseri* and *Steinernema* sp. NC 513 group of isolates should be considered separate species. The absence of pre-zygotic mating barriers in these two morphologically similar *Steinernema* species in the same geographic area (near Raleigh, North Carolina) suggests that the two species recently diverged from each other. Furthermore, the

occurrence of a chromosome number of n = 5 throughout *Steinernema* suggests that n = 5 is the base chromosome number for the genus. *Steinernema* sp. NC 513 may have evolved by chromosome fusion events from a population of *S. glaseri*. Interestingly, in the laboratory, both species grow and reproduce with greater than 90 % of the infective stage juveniles retaining the primary form of *Xenorhabdus nematophilus* subsp. *poinarii* NC 513 bacteria. The ability of two separate *Steinernema* spp. to utilize the same *Xenorhabdus* symbiont, without obvious deleterious effects, has not been reported previously (Akhurst, 1983).

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