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Phenotypic and genetic characterization of dumpy infective juvenile mutant in *Steinernema feltiae* (Rhabditida : Steinernematidae)

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Summary – The first morphological and behavioural mutant has been described in *Steinernema feltiae*. The mutation was spontaneous and occurred in a single gene locus designated as *Sfdpy-1*. Action of the new allele was clearly expressed in infective juveniles only. The resulting morphology has been classified as dumpy (dpy) due to significantly reduced ratio of the nematode body length to its maximum diameter. The identified gene was sex-linked and the new mutant allele remained recessive to the wild-type counterpart responsible for normal morphology. Besides altering the morphology, pleiotropic action of the dumpy allele affected the nematode movement activity and infectivity to insect hosts. The mean dispersion distance of mutant juveniles and their infectivity to *Galleria mellonella* and *Lycoriella solani* larvae were significantly reduced when compared with those of wild-type nematodes. Revertant individuals isolated occasionally from dumpy populations regained the ability of fast movement and dispersed even further than juveniles from parental strains. They were also more effective in penetration into the hemocoel of *L. solani* larvae. However, numbers of infected insects did not significantly differ from those observed for wild-type ScP and SN strains. Practical consequences of the reported mutation for biocontrol potential of *S. feltiae* have been discussed.

Résumé – ***Caractérisation phénotypique et génétique du mutant obèse des juvéniles infestants de* Steinernema feltiae *(Rhabditida : Steinernematidae)*** – Le premier mutant morphologique et comportemental de *Steinernema feltiae* est décrit. La mutation, spontanée, concerne un seul locus génique nommé *Sfdpy-1*. L'action de ce nouvel allèle ne s'exprime de façon nette que chez les juvéniles infestants. La morphologie du mutant – longueur du corps réduite par rapport au diamètre maximum – a conduit à le considérer comme un mutant « obèse ». Le gène identifié est lié au sexe et le nouvel allèle mutant demeure récessif par rapport à son homologue de type sauvage, responsable de la morphologie normale. En sus de changements dans la morphologie, l'action pléitrope de l'allèle « obèse » affecte la motilité et l'infestivité envers les insectes hôtes. La distance moyenne de dispersion des juvéniles mutants et leur infestivité vis-à-vis de *Galleria mellonella* et de *Lycoriella solani* sont significativement diminuées par rapport à celles des nématodes de type sauvage. Des individus revenus au type sauvage sont parfois isolés à partir de populations « obèses »; ils récupèrent la possibilité de mouvements rapides et se dispersent plus loin que les individus de leur souche parentale; ils pénètrent également plus efficacement dans l'hoemocèle des larves de *L. solani;* cependant le nombre d'insectes ainsi infectés ne diffère pas significativement de ceux observés dans le cas de types sauvages des souches ScP ou SN. Les conséquences pratiques de cette mutation pour les potentialités de biocontrôle par *S. feltiae* sont discutées.

Key-words : biological control, dumpy mutant, dumpy revertant, entomopathogenic nematode, genetics, *Steinernema feltiae*.

The infective juvenile is the only developmental stage of steinernematid and heterorhabditid nematodes that can live free in the soil environment, survive long storage, actively search for insect hosts, and penetrate into their hemocoel (Ishibashi & Kondo, 1990; Kaya, 1990). Therefore, characteristics of infective juveniles play the most important role in commercial handling and control effectiveness during the nematode field application against insect pests. Significant differences in biocontrol potential were recorded between nematode species and strains (Molyneux *et al.*, 1984; Shetlar, 1989; Klein, 1990). However, understanding of the genetic background for these differences still remains incomplete. Use of selective breeding to increase frequencies of advantageous characteristics, such as host-finding ability and infectivity, within nematode populations already has been validated for *Steinernema carpocapsae* (Gaugler *et al.*, 1989; Gaugler & Campbell, 1991), *S. feltiae* (Tomalak, 1989; Greval *et al.*, unpubl.; Tomalak, unpubl.) and *S. glaseri* (Selvan *et al.*, unpubl.). Genetic analysis of the improved strains was difficult, however, due to complexity of factors involved in expression, and apparently polygenic inheritance of the selected characteristics. Identification of genes controlling individual elements of the improved traits could help in understanding of the observed changes and would facilitate construction of the most desired phenotypes. Crossbreeding, mutagnesis and selection significantly contributed to the present

knowledge of genetic structure of a model, free-living nematode, *Caenorhabditis elegans* (Herman, 1988). Close taxonomic relation allows the methods developed for *C. elegans* to be effectively used in genetic analysis of nematodes in families Heterorhabditidae and Steinernematidae (Fodor *et al.*, 1990; Zioni *et al.*, 1992). Glazer *et al.* (1991) initiated genetic study on *Heterorhabditis bacteriophora* and subsequently induced and identified first mutations in this species. Mutants resistant to levamizole and avermectin have also been isolated in *S. carpocapsae* (Fodor *et al.*, 1990).

Recently, we started a detailed study on a genome of *S. feltiae* to facilitate our work on genetic improvement of the nematode performance as biocontrol agent against insect pests. Identification of existing alleles through analysis of intraspecific recombinants and induction of new mutant phenotypes through chemical mutagenesis have been undertaken as initial steps in this project. Extensive screening of infective juveniles from imbred ScP and SN strains of *S. feltiae* and from a series of hybrid populations between these strains revealed a number of individuals morphologically and behaviourally distinct from the wild-type nematodes. Subsequent crossbreeding of the collected forms between each other and backcrosses with individuals from parental populations allowed analysis and clarification of genetic status of some of the observed characteristics. The present paper reports results of the study on dumpy mutant isolated from among infective juveniles in one of the ScP × SN hybrid populations.

The main objectives of the conducted study were to *i)* characterize morphologically the dumpy infective juvenile mutant, *ii)* analyze genetic basis for the expression of dumpy characteristic in the population, and *iii)* determine the relationship between morphology of the mutant individuals, their movement activity and infectivity to insects.

Materials and methods

Insects and nematode strains

The greater wax moth, *Galeria mellonella* L. (Lepidoptera : Pyralidae), larvae were taken from a culture continuously reared in the laboratory for about 4 years. The culture of the mushroom fly, *Lycoriella solani* Winn. (Diptera : Sciaridae), originated from adult flies periodically collected in a commercial mushroom house, and was maintained for two or three generations on pasteurized mixture of peat, chalk and soy flour.

The hybridization and selection study were conducted on ScP and SN strains of *S. feltiae* (Filipjev) (= *S. bibionis* Bovien). The ScP strain was derived from a Polish (PL) isolate of *S. feltiae* subsequently selected for 34 generations to improve its biocontrol efficacy against *L. solani* larvae in mushroom compost and casing (Tomalak, unpubl.). The SN strain, originally isolated in France, was obtained from Biosys (Palo Alto, Calif.). Both strains were extensively examined for their morphological, enzymatic and behavioural characteristics before the research reported here was conducted. They showed a number of distinct differences (Tomalak, unpubl.).

Rearing, hybridization and selection of nematodes

In relation to particular goals to be achieved, nematodes were culture-reared *in vitro* or *in vivo*. *In vitro* hybridization was conducted on Difco nutrient agar (NA) inoculated with a primary phase of a symbiotic bacteria, *Xenorhabdus bovienii*, isolated from the ScP or SN nematode strains. In short, the procedure was as follows : a heated, sterile NA was pipetted on cold Petri dishes to form small agar islets. Nine separate islets, each made of 4 NA drops, were prepared per 9 cm dish. A surface of each islet was smeared with a loopful of symbiotic bacteria taken from a pure culture on NA. Populations of nematode infective juveniles chosen for subsequent crossbreeding were surface sterilized with a sodium hypochlorite and washed in three changes of sterile distilled water. Then, the juveniles were individually transferred with a fine dissecting needle to separate islets and were incubated at 24 °C for 48-72 h, until they reached adult stage. Infective juveniles which occasionally managed to escape from the agar dried out on the glass surface separating the islets. Nematodes from different strains and lines were developed in separate dishes. After the incubation and identification of sexes adult parasites were individually transferred to fresh, 9 cm NA plates with 3-day-old culture of *X. bovienii* and set into pairs according to the hybridization scheme. The plates were incubated in upside-down position at 24 °C. In most cases none or only a few infective juveniles were produced in the first generation of crossbred nematodes. Therefore, incubation was continued for 2 weeks, until adequate numbers of infectives developed from the second-generation offspring. Then, the nematodes were washed off the agar and the obtained suspension cleaned of bacteria and dead individuals through a series of distilled water changes. The collected infective juveniles were conditioned at 20 °C for 2 days and then stored in distilled water at 4 °C, until use in subsequent experiments.

After 7 days of cold storage a part of each *in vitro* obtained nematode population was mass reproduced *in vivo*, in *G. mellonella* (Dukty *et al.*, 1964) or *L. solani* larvae (Tomalak, unpubl.). Significantly different sizes of both insects allowed the nematodes to develop only one or two generations within the host (*L. solani* and *G. mellonella*, respectively) before the emergence of new infective juveniles. This phenomenon was particularly advantageous during genetic analysis of the hybrid infective juvenile populations. The crossbreeding, and inbreeding procedures were periodically repeated according to the general breeding scheme.

Obtained nematodes were systematically screened for a morphology and behaviour of infective juveniles distinctively different from those observed in wild-type parental populations. In the study reported here only dumpy individuals with a characteristically reduced ratio of their body length to width were subjected to detailed analysis. Since no females were available among first isolated dumpy individuals of the F 8 ScP × SN hybrid generation, to produce homozygotic lines, the dumpy nematodes were backcrossed with parental strains and subsequently inbred within a group of individuals bearing the same characteristics.

Morphological analysis

Morphological analysis was conducted primarily on infective juveniles. Additional observations were carried out on adult individuals obtained from both *in vitro* and *in vivo* cultures. The nematodes were heat killed, fixed in TAF and processed using the glycerin-ethanol method (Seinhorst, 1959). Dehydrated and cleared specimens were mounted in glycerin and the cover slips were sealed with Glyceel®. The nematodes were examined under a compound microscope of magnification power 100 × to 1000 ×. The mutant measurements were presented according to the modified de Man's formula (Poinar, 1986) and compared with those recorded for ScP and SN strains. Mean values for each strain were calculated from measurements of 50 individuals randomly chosen from populations developed *in vivo,* in *G. mellonella* larvae.

Genetic analysis

The new dumpy infective juvenile mutant allele and its gene locus have been designated as *Sfdpy-1 (pn7ij) X.* In this paper it will be referred to as *Sfdpy-1.* The rules followed in construction of this code have been adapted from the genetic nomenclature system developed for *C. elegans* (Horvitz *et al.,* 1979). However, to avoid a possible confusion with names already used in other nematodes a specific prefix « *Sf* » standing for *S. feltiae* has been added to the gene designation. The « dpy » indicates dumpy phenotype, « *1* » first gene within this category, « *pn* » is a specific symbol used for the Institute of Plant Protection, Poznan, Poland where the mutant has been identified, « *7* » is the mutation number, « *ij* » is a descriptive suffix indicating expression of the mutant phenotype in infective juveniles only, and finally « *X* » indicates the linkage group.

Genetic analysis was based on phenotypic segregation ratio of wild-type to dumpy juveniles recorded in subsequent nematode generations obtained from individual backcrosses with parental strains and from lines inbred within morphological groups. Additional analysis was conducted on sex ratio of individuals developed from dumpy and dumpy revertant juveniles. Offspring of at least four similar crosses was analyzed in each generation group. In the method used, five 1 ml samples of live infective juveniles suspended in water were taken with a pipette from the stock. The samples were mixed together in 7 cm Petri dish and juveniles of both morphological forms of the nematode were withdrawn individually with a fine pipette and counted. In parallel, the ratios of females to males present in separately examined dumpy infective juvenile mutant *(Sfdpy-1)* and dumpy infective juvenile revertant *(Sfdpy-1R)* populations were calculated. In order to obtain adult nematodes, samples of infective juveniles were withdrawn from the stock suspension, surface sterilized in sodium hypochlorite, washed in three changes of sterilized, distilled water and transferred to NA plates with 3-day-old *X. bovienii* culture. After 3 days of incubation at 24 °C all nematodes were washed off the agar with M-9 buffer and numbers of males and females were counted under dissecting microscope.

Movement activity test

Movement activity of ScP, SN, *Sfdpy-1* and *Sfdpy-1R* infective juveniles was examined on agar surface and compared according to the following method. Nine cm Petri dishes were filled with 2 % Difco agar at the rate of 25 ml of agar per dish. The plates were left uncovered for 1 h to let the medium cool off and solidify. Two-hundred nematode infectives were pipetted onto the surface of 1 cm filter paper disc placed on paper towels. After brief removal of excess water the disc was immediately transferred to the agar surface and deposited in the center. The experimental dishes were left uncovered for 60 min to allow the infectives to move freely. Dispersion from the disc was examined under dissecting microscope and numbers of nematodes moved from filter paper as well as their positions on the agar surface after 30 and 60 min were marked with different colours at the dish bottom. After the second observation dishes were covered, placed in upside down position and left undistrubed for additional 23 h. After that period of time the nematode positions on the agar surface were marked again and all juveniles remaining on the filter paper were washed into a 5 cm Petri dish and counted. After the experiment straight line distances from the disc edge to the colour marks were measured and mean dispersion distances reached in time variants were calculated for each nematode strain. The experiment was replicated six times.

Infectivity tests

Infectivity of ScP, SN, *Sfdpy-1* and *Sfdpy-1R* strains to *G. mellonella* larvae was assessed in 5 cm Petri dishes with a double layer of filter paper. The nematodes suspended in distilled water were pipetted onto the filter paper at a dose of 100 IJ per dish. The host larvae were individually placed into the dishes where the nematodes had been inoculated and incubated at 20 °C. After 48 h all insects were dissected and numbers of recovered nematodes counted under a dissecting microscope.

The nematode infectivity in L3 and L4 instar larvae of *L. solani* was examined in a mushroom substrate. Five cm Petri dishes were filled up with a mature mushroom casing (peat-moss and chalk mixture), supplemented with a trace of soy flour. After a gentle down-pressing of the substrate, 25 L3 and L4 instar larvae of *L. solani* were placed into each dish and allowed to disperse. Two hours later 500 infective juveniles were pipetted into each dish. The substrate moisture was adjusted to 1:2.5 of dry substrate weight to water content weight, which ratio was found to create optimal conditions for *S. feltiae* to infect *L. solani* larvae (Tomalak & Lipa, 1991). After 24 h of incubation at 20 °C, all insect larvae were recovered from the substrate, transferred to 2 °C and dissected within the following 6 h. Both the prevalence and intensity of infection were recorded for each tested variant. The experiment was replicated six times.

Statistics

The data obtained in dispersion and infectivity tests was normalized through Freeman-Tukey transformation and subjected to ANOVA. The significance of difference was tested using Tukey's multiple range test at 0.05 significance level.

Results

Nematode hybridization and isolation of the *Sfdpy-1* mutant

In this study breeding of nematodes was conducted according to the general schedule shown in Fig. 1. The initial series of pair-wise crosses between ScP and SN strains of *S. feltiae* produced a number of hybrid popu-

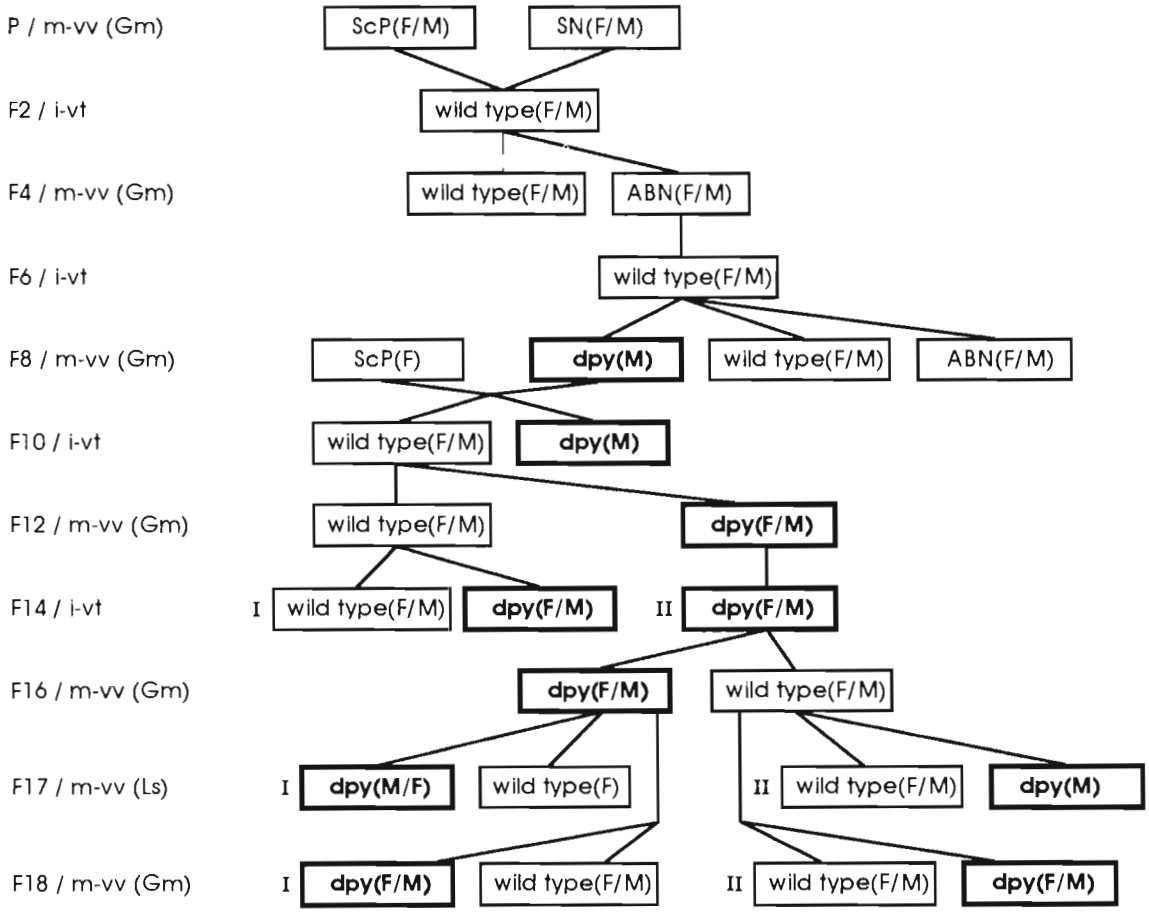

Fig. 1. *Graphic representation of* Steinernema feltiae *breeding program for homozygosity in the* Sfdpy-1 *gene locus. Codes represent abnormal (ABN) and dumpy (dpy) phenotypes; female (F); male (M); individual crossbreeding or inbreeding* in vitro *(i-vt), and mass inbreeding* in vivo *(m-vv) in* Galleria mellonella *(Gm), or in* Lycoriella solani *(Ls).*

lations with normal (wild-type), and abnormal (ABN) individuals among infective juveniles of the F4 generation. The ABN category was heterogeneous and comprised nematodes with altered morphology and behaviour. Locally widened diameter of the infective juvenile body was the characteristic most frequently observed in that group. Abnormal nematodes inbred for additional generations continued to produce populations with variable phenotypes, including wild-type individuals. A completely new and morphologically distinct dumpy phenotype was recorded among infective juveniles in F8 generation of a 92.10.30/8 hybrid line. The first twelve dumpy infectives were recovered from a mixed population of about 78×10^3 wild-type and abnormal individuals. No similar phenotypes could be found in parental populations or in any other hybrid line under study.

The observed distinctive morphology and uniformity within the group, very low number of recovered individuals and apparent lack of similar forms among infectives from earlier generations suggested that the dumpy phenotype was related to a spontaneous mutation which took place within a single nematode in F6 or F7 generation of the hybrid line.

Mutant morphology

Morphology of the dumpy mutant infective juveniles significantly differed from those of wild-type infectives recorded in parental SN and ScP strains and in other ScP × SN hybrid populations. The nematode body measurements are presented in Table 1. Most of the examined characters, including body length, maximum diameter, distances from anterior end to excretory pore and anterior end to nerve ring, tail length and a, b, c and d ratios of dumpy individuals fell outside the ranges recorded for wild-type nematodes. Dumpy mutant infective juveniles were much shorter and wider than wild-type individuals, characteristics which made both forms easy to distinguish in mixed populations (fig. 2 A, B) Dumpy infectives harvested from NA/*X. bovienii* plates were generally smaller than those from *G. mellonella* (L = 495 μm, w = 31 μm *vs* L = 620 μm, w = 36 μm).

Table 1. *Comparison of body measurements between dumpy mutant* (Sfdpy-1) *and wild-type infective juveniles from various populations of* Steinernema feltiae *(all measurements in μm). Ratio a = length divided by maximum body diameter; b = length divided by distance from anterior end to pharynx base; c = length divided by tail length; d = distance from anterior end to excretory pore divided by distance from anterior end to pharynx base; e = distance from anterior end to excretory pore divided by tail length.*

Character	Nematode population				
	ScP wild type	SN wild type	92.10.30/8 ScP × SN F12 wild type	*Sfdpy-1* mutant	*Sfdpy-1R* revertant
Body length	825 (660-920)	843 (770-980)	905 (820-980)	620 (510-710)	811 (660-990)
Maximum body diameter	28 (24-30)	27 (25-30)	29 (26-30)	36 (34-38)	27 (24-31)
Anterior end to excretory pore	58 (53-63)	59 (55-65)	65 (63-70)	47 (43-53)	56 (49-56)
Anterior end to nerve ring	91 (85-98)	90 (83-93)	98 (95-110)	80 (75-83)	89 (93-98)
Anterior end to pharynx base	139 (128-150)	139 (135-145)	142 (133-153)	135 (130-143)	135 (130-150)
Tail length	78 (60-88)	75 (66-88)	85 (75-90)	64 (58-70)	74 (63-88)
Ratio a	30 (24-31)	31 (28-36)	31 (29-33)	17 (15-19)	30 (26-33)
Ratio b	5.9 (4.9-6.6)	6.1 (5.6-6.8)	6.4 (5.6-7.1)	4.6 (3.9-5.4)	6.0 (5.0-7.1)
Ratio c	10.6 (9.5-11.6)	11.3 (10.3-12.7)	10.7 (9.9-12.8)	9.7 (8.4-10.9)	10.9 (9.8-12.3)
Ratio d	0.41 (0.39-0.43)	0.43 (0.39-0.46)	0.46 (0.42-0.49)	0.35 (0.32-0.40)	0.42 (0.37-0.47)
Ratio e	0.74 (0.68-0.90)	0.79 (0.68-0.90)	0.77 (0.68-0.90)	0.74 (0.61-0.83)	0.76 (0.70-0.84)

Besides the body measurements some other characteristics such as a morphology of head and tail regions were distinctive for the dumpy phenotype. The nematode head was abruptly narrowed at the mouth region and resembled a short snout (Fig. 2 C, D). The body line was irregularly wavy throughout the whole nematode length, while in wild-type individuals it was regular and smooth (Fig. 2 E, F). The mutant tail appeared to be compressed along the body axis and it had numerous transversal or oblique folds located below and above the anus (Fig. 2 G, H). However, the tail end was smooth and similar to those of wild-type infectives.

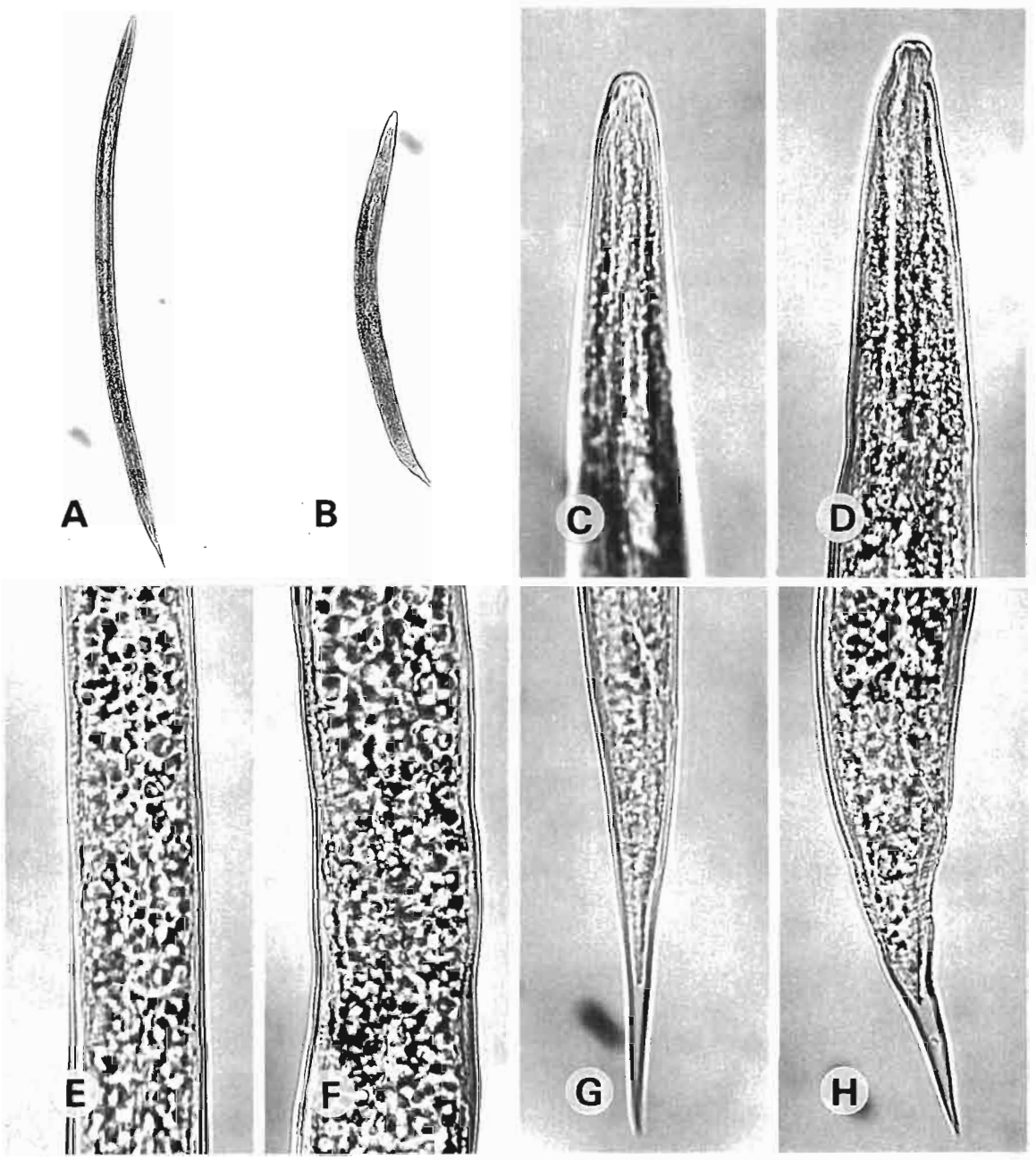

Fig. 2. *Comparison of morphological characteristics between wild-type (A, C, E & G), and* Sfdpy-1 *(B, D, F & H) infective juveniles of* Steinernema feltia. *A-B : General body view; C-D : Head region; E-F : Mid-body; G-H : Tail region.*

A general microscopic examination failed to reveal significant differences between adult nematodes developed from wild-type and dumpy infective juveniles. The only exception observed among mutant individuals was the greater proportion of males with rounded tail end, although this was still variable in different populations. In such individuals the tail mucron was almost completely reduced. In various populations ranges of body measurements of adult nematodes developed from both juvenile morphological groups often overlapped and they seemed to depend more on the nematode rearing conditions than on the original phenotype of infective juveniles.

The obtained data showed that the reported dumpy mutant could be distinguished morphologically only in the infective juvenile stage.

Development and reproduction

The mutation did not noticeably affect the nematode development and reproduction potential. Dumpy juveniles matured at comparable time to wild-type individuals in both *in vitro* and *in vivo* cultures. Since sizes of adults nematodes developed from both juvenile morphological forms were similar, no physical barriers within dumpy, between dumpy and wild-type phenotype groups were observed during mating. Eighty-four per cent of crosses involving one or two dumpy partners were successful *in vitro*. In order to complete two consecutive generations and produce infective juveniles *in vitro* both, mutant and wild-type nematodes required 14 days at 24 °C, on average.

Genetic analysis of the *Sfdpy-1* mutant

A number of individual crosses between dumpy nematodes and backcrosses with parental wild-type strains (Fig. 1) allowed clarification of the genetic status of the reported mutation. Proportions of morphological forms observed in resulted populations and sex ratios recorded within each mutant group are presented in Table 2.

All dumpy mutants originally isolated from F 8 generation of the ScP × SN hybrid population developed into males. Therefore, no direct inbreeding within the dumpy morphological group was possible. Large populations of infective juveniles (n >1000) obtained in F2 (F10) generation of *in vitro* backcrosses between ScP and SN females and mutant males consisted of nematodes of both forms. The phenotypic segregation rate of normal to dumpy individuals was 3.54 ± 0.22:1 for ScP × *Sfdpy-1,* and 3.21 ± 0.30:1 for SN × *Sfdpy-1* crosses. All dumpy infectives from that generation again developed into males only. F10 juveniles obtained from mass reproduction in *G. mellonella* gave rise to mixed populations of normal and dumpy individuals at the rate of 3.36 ± 0.14:1, respectively, in F2 (F12) generation. Both females and males developed from dumpy infectives of that generation. However, the number of males recorded was much higher than expected and gave an overall rate of 1:83 ± 6.46 of females to males. The dumpy individuals inbred within the morphological group produced populations of dumpy juveniles only, in F2 (F14) generation. In those populations the rate of females to males was, however, higher on the female side and averaged 1.92 ± 0.24:1, respectively. In parallel breeding wild-type infective juveniles from F12 generation inbred within the group continued to produce both morphological forms in F2 (F14) and subsequent generations.

Further mass inbreeding of the F14 dumpy lines produced a few wild-type individuals among large populations of dumpy infectives in F2 (F16) generation. Dumpy individuals reproduced for one generation in *L. solani* larvae gave rise to populations consisting of normal and dumpy infectives at the rate of 1:136, respectively. In contrast, 2.51 ± 0.32:1 of normal to dumpy nematodes were recorded in F1 (F17) population obtained from wild-type individuals. The former proportion suggests a random reversion from dumpy form in one of the parent individuals, whereas the latter indi-

Table 2. *Phenotypic segregation, and sex ratios recorded in subsequent generations of* Steinernema feltiae *intraspecifically crossbred and inbred for homozygosity in the* Sfdpy-1 *gene locus.*

Generation		Ratios		
		Total offspring wild type :dpy	*Sfdpy-1* mutant females : males	*Sfdpy-1R* revertant females : males
P (SN; Scp)		no dpy	-	-
F2		no dpy	-	-
F4		no dpy	-	-
F6		no dpy	-	-
F8		6500 : 1 (78000 : 12)	males only (n = 12)	-
F10		3.54 : 1 (1727 : 488)	mals only (n = 452)	-
F12		3.36 : 1 (638 : 190)	1 : 83 (4 : 331)	-
F14	I	3.17 : 1 (1163 : 367)	1 : 3.86 (118 : 456)	-
	II	dpy only (n = 22000)	1.92 : 1 (230 : 119)	-
F16		1 : 119 (16 : 1897)	1.11 : 1 (863 : 780)	3.11 : 1 (28 : 9)
F17	I	1 : 136 (7 : 953)	1.04 : 1 (264 : 254)	females only (n = 91)
	II	2.51 : 1 (596 : 237)	males only (n = 87)	-
F18	I	1 : 104 9 : 932	1.24 : 1 (241 : 195)	2.05 : 1 (41 : 20)
	II	3.10 : 1 (911 : 294)	1 : 4.46 (70 : 312)	-

cates the presence of both homozygotic and heterozygotic genotypes among morphologically wild-type individuals. Observed in the latter crosses, the proportion of dumpy nematodes was, however, greater than the expected one, which should here be 5:1 of normal to dumpy infectives. Examination of the sex ratio of adult nematodes in both morphological mutant groups revealed that only females developed from F1 (F17) wild-type infectives were obtained from dumpy parents. Among the dumpy offspring females and males were represented in almost equal proportions of 1.04 ± 0.08:1, respectively. In contrast, only males developed from dumpy infectives of the F1 (F17) offspring were produced through inbreeding of wild-type nematodes.

Movement activity

Movement activity of dumpy infective juveniles was significantly reduced when compared with normal wild-type individuals (Fig. 3). In the Petri dish experiment only 15.0 ± 2.3 % of mutant nematodes moved from the 1 cm filter paper disc to the agar surface within 30 min and 18.5 ± 2.7 % within 60 min. At the same time, 24.0 ± 3.4 and 30.4 ± 2.6 % of SN, and 41.5 ± 2.9 and 53.9 ± 3.6 % of ScP infectives, respectively, moved to the agar surface. The greatest activity was, however, recorded in the dumpy revertant population where as many as 96.0 ± 1.4 and 92.5 ± 1.8 % of applied infectives were recorded on the agar surface after 30 and 60 min, respectively. Within 24 h 92-98 % of wild-type nematodes moved to the agar surface, while 54.4 ± 3.7 % of dumpy individuals remained still on the filter paper disc.

The mean distance between moving dumpy nematodes and the disc edge was 1.8 ± 0.2 mm after 30 min and 2.2 ± 0.2 mm after 60 min (Fig. 4). Wild-type infectives moved further from the disc and they were recorded 6.0 ± 0.8 and 7.5 ± 0.7 mm away from the disc in the SN strain, and 6.0 ± 0.5 and 6.3 ± 0.6 mm in the ScP strain, at respective time. The dispersion of dumpy revertant wild-type infectives was even greater than that of the SN and ScP strains. The mean distance from the disc edge was 9.1 ± 0.4 after 30 min. and 9.5 ± 0.5 mm after 60 min. Observations carried out after 24 h were inconclusive since a large proportion of wild-type infectives moved away from the agar surface and dried up on the dish edge. In contrast, within the 24 h period none of the dumpy juveniles reached the dish edge and they aggregated mostly within a 20 mm distance from the filter paper disc.

Infectivity to insect hosts

Infectivity of dumpy juveniles to *G. mellonella* and *L. solani* larvae was significantly lower than that of wild-type nematodes. Only occasional infections of *G. mellonella* larvae were recorded when the insects were exposed to dumpy juveniles isolated from F2 (F10) offspring of the ScP × *Sfdpy-1* backcross. Due to the lack of females among dumpy individuals of F10 generation no reproduction took place in insects infected with those nematodes. Infectivity to insects improved in F16 generation, where both males and females were present in the dumpy population. However, it still remained much lower than that of wild-type individuals. An average of 4.50 ± 0.5 % of dumpy infective juveniles penetrated the hemocoel of *G. mellonella* larvae when the nematodes

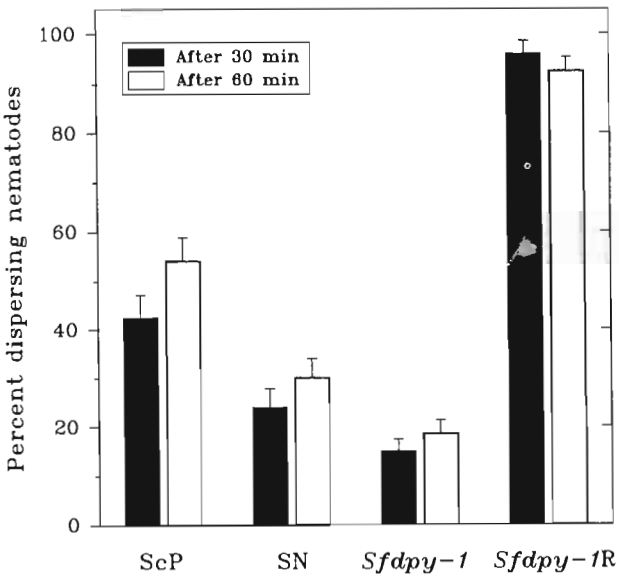

Fig. 3. *Dispersion of* Steinernema feltiae *infective juveniles on agar surface : percentage of actively moving nematodes in ScP, SN,* Sfdpy-1 *mutant and* Sfdpy-1R *revertant populations.*

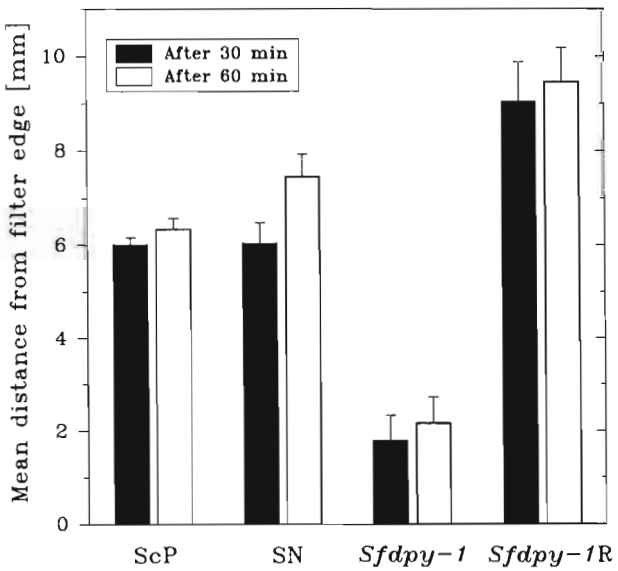

Fig. 4. *Dispersion of* Steinernema feltiae *infective juveniles on agar surface : Mean distance moved from the place of allocation.*

were applied at a dose of 100 individuals per insect. At similar dose rate 19.3 ± 1.2 % of SN, 18.0 ± 1.3 % of ScP and 19.8 ± 1.2 % of dumpy revertant juveniles penetrated the insect hemocoel.

Assessment of the nematode infectivity to *L. solani* larvae further supported observations made for *G. mellonella*. Only as few as 12.0 ± 1.4 % of the insect individuals were infected with dumpy nematodes, within 24 h. (Fig. 5). Wild-type infectives performed much better and 84.4 ± 1.8, 68.1 ± 2.4 and 81.4 ± 2.6 % of the pest larvae were infected by ScP, SN and dumpy revertant juveniles, respectively. Significant differences were also recorded in percentage of nematodes successfully penetrating the host hemocoel (Fig. 5). The dumpy juveniles were least effective with only 1.2 ± 0.6 % of successful individuals. Surprisingly high infectivity was presented by the dumpy revertant nematodes. As many as 48.2 ± 1.8 % of the originally applied individuals penetrated the insect larvae. That was almost twice as many nematodes as recorded for ScP strain and over three times as many as for SN strain. All successful parasites initiated further development within the 24 h experimental time.

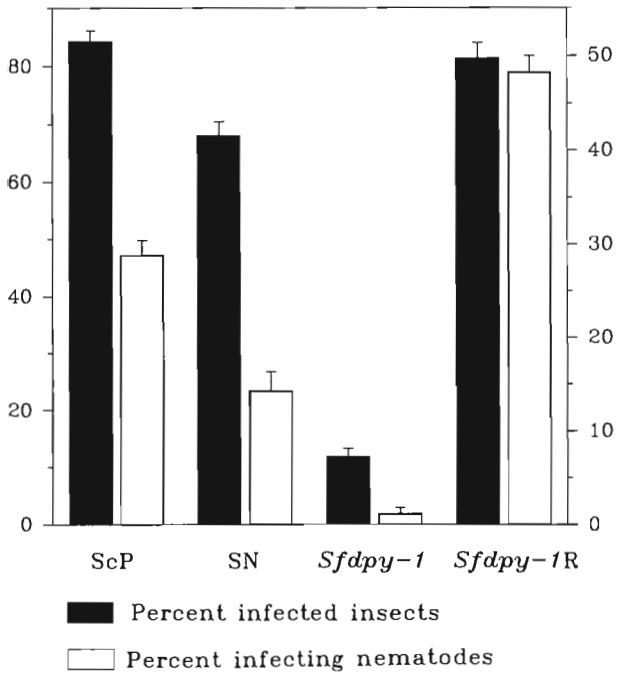

Fig. 5. *Infectivity of ScP, SN,* Sfdpy-1 *mutant and* Sfdpy-1R *revertant* Steinernema feltiae *to* Lycoriella solani *larvae in mushroom casing mixture, after 24 h exposure.*

Discussion

Growing economic importance of entomopathogenic nematodes as biocontrol agents has meant that species in families Steinernematidae and Heterorhabditidae have become one of the most extensively studied groups of parasites. Pest control level achieved with natural isolates is, however, sometimes disappointing (Georgis & Gaugler, 1991). Artificial selection, hybridization, and genetic engineering are postulated as available approaches to improve biocontrol efficacy of the nematodes (Gaugler, 1987). Abundance of existing steinernematid and heterorhabditid isolates from all over the world, short generation time and relative ease in obtaining large populations, make them a very convenient subject for genetic study. Nevertheless, basic research on genetics of these organisms has been generally neglected. The comprehensive model system developed for analysis of *C. elegans* genome (Herman, 1988) can be readily adapted for study on entomopathogenic nematodes (Fodor *et al.*, 1990). However, due to production of hermaphrodites *Heterorhabditis* is more likely than *Steinernema* to become a convenient target candidate for such adaptations.

Recently conducted work on genetic selection of *S. carpocapsae* (Gaugler *et al.*, 1989; Gaugler & Campbell, 1991), *S. feltiae* (Tomalak, 1989; Tomalak, unpubl.) and *S. glaseri* (Selvan *et al.*, unpubl.) revealed a strong need for better insight into genetics of *Steinernema*. Therefore, basic research on identification and linkage of easily recognizable genes affecting beneficial traits in these nematodes seems to be the most important task for the near future.

This paper reports results of systematic study on the first spontaneous morphological and behavioural mutant in *S. feltiae*. The isolated mutant can be easily distinguished from wild-type individuals by shortened length and widened diameter of its body, and according to the classification applied for *C. elegans* (Brenner, 1974) it can be described as dumpy. Action of the gene was pleiotropic, affecting both morphology and behaviour of the nematode. Penetrance of the mutant allele was complete in infective juveniles only. Therefore, the name " dumpy infective juvenile " *(Sfdpy-1 [pn7ij] X)* has been suggested for designation of the newly described nematode mutant.

The near Mendelian 3:1 phenotypic segregation ratios of normal to dumpy individuals recorded in F2 (F10) generation obtained from wild type (ScP, SN) × *Sfdpy-1* crosses indicate that the dumpy morphology is related to a single mutation in one gene locus and the mutant allele remains recessive to the wild type. Moreover, the complete lack of females among individuals developed from the F2 (F10) infective juveniles suggests that the gene affected by the reported mutation is located in a sex chromosome. To be morphologically expressed in so few infectives (n = 12) of the F8 generation, the mutation had to take place in a single female of F7, or in a male of F6 hybrid generation. Otherwise, both dumpy females and males would be present in the F8 generation, if the change had occurred in an F6 female, or no dumpy individuals would be recovered

from among F8 generation juveniles, if the mutation had occurred in an F7 male.

Obvious discrepancy between expected (1:4) and recorded (1:83) ratios of females to males developed from dumpy juveniles of F12 generation is difficult to interpret. The observed over twenty-fold increase in proportion of males in the examined population cannot be explained by any potential lethal effect arising from homozygous condition of recessive dumpy alleles present in females. Sex ratio recorded in later generations was much closer to expected values and in homozygotic dumpy populations it reached near 1:1 equilibrium. Also the ratio of normal to dumpy individuals recorded in the F12 population showed higher than expected (12:1, respectively) proportion of dumpy juveniles in the population. A possible, yet somewhat speculative explanation for these discrepancies could be a preferential mating of wild-type males with heterozygotic females in F11 that in extreme situations would lead to a segregation ratio of 3:1 of wild type to dumpy juveniles (observed : 3.36:1, respectively) and only males would develop from dumpy individuals (observed : 1:83 females to males). All differences between recorded and expected data could be attributed to concurrent, although less frequent, mating between nematodes with remaining genotypes.

Analysis of the nematode offspring obtained through a separate inbreeding of dumpy and wild-type individuals that appeared in the F2 (F16) generation provided evidence for spontaneous, unusually frequent, reversion of the *Sfdpy-1* mutation. The numerical data recorded in F1 (F17) indicates that the reversion occurred in a male nematode and induced return of the recessive, sex-linked dumpy mutant allele to wild-type, dominant form. If the reversion had occurred in a female, both wild-type females and males in equal proportions should be observed in the F1 (F17) populations. Conversely, in the F1 (F17) offspring obtained through inbreeding of wild-type nematodes, all dumpy infectives developed into males. Such a situation was possible only because of sex-linkage of the mutant allele. The recessive dumpy allele could not be expressed in heterozygotic females.

In the family Steinernematidae only the dauer third-stage juvenile can actively search for, and infect the insect. This is also the only stage of the nematodes that can survive outside the host without further development. Therefore, physiological and behavioural characteristics of infective juveniles play a crucial role in overall effectiveness of the nematodes in commercial biopesticides. The described spontaneous mutation affects the juvenile morphology, movement activity and infectivity to insect hosts, leaving the reproductive potential of adult individuals apparently unchanged. The pleiotropic action of the dumpy allele alone could reduce efficacy of the nematode infective juveniles from 68-84 to 12 % with respect to numbers of infected insects and from 14-48 to 1 percent with respect to numbers of infecting juveniles successfully penetrating the host hemocoel, as recorded in *L. solani* larvae. Conversely, the spontaneous reversion of the mutation restored the nematode morphological quality and even improved its movement activity and infectivity over the original parental strains. Whether the regaining of wild-type phenotype was caused by a true reversion of the earlier mutational events or by a suppression resulting from secondary site mutation remains to be determined in later study. The improved performance of the dumpy revertant over parental strains strongly suggests, the second alternative however. If this is true, we may have a powerful allele with a great potential for further study on genetic improvement of *S. feltiae*. Pure dumpy revertant strains have already been obtained and incorporated into the nematode breeding programme for improved infectivity to the Western Flower Thrips, *Frankliniella occidentalis* (Pergande) (Tomalak, unpubl.).

In natural conditions most of the dumpy juveniles would be eliminated from the population due to reduced ability to disperse and infect insect hosts. The flow of dumpy allele to the next generation would, therefore, depend mostly on wild-type heterozygotic female juveniles. Since the major prerequisite for the Hardy-Weinberg equilibrium cannot be fulfilled, such a situation should lead to gradual reduction of overall frequency of the dumpy allele in the population. The segregation ratio of wild-type to dumpy juveniles recorded in F10 and subsequent generations of *in vivo* reproduced wild-type individuals does not follow the expected trend and remains fairly constant at the level of 3:1, respectively. This phenomenon again could be explained by preferential mating of wild-type males with heterozygotic females, as suggested earlier.

The described mutation was spontaneous, thus it could be expected in any population, including those reproduced in millions of individuals by *in vitro* liquid cultures. In such a situation presence of dumpy allele in the nematode inoculum could significantly reduce overall biocontrol potential of the obtained nematode product due to very low infectivity of the dumpy portion of the population. Nematode recycling in the host following the field application would conserve the dumpy allele in heterozygotic females. If the preferential mating of heterozygotic females and wild-type males is true the parasites developing inside the insect host would continue to produce offspring with one-quarter of the population dumpy. The obtained data shows that once present in the population the dumpy allele cannot be eliminated by natural selection and retains its large share in the offspring of following generations.

A potential role of the characterized dumpy mutant in further genetic analysis of *S. feltiae* genome will be gradually verified as other new mutants are subjected to detailed studies. The most important finding of the research reported here is the discovery of a major gene that

affects the size of the nematode juveniles, movement activity and infectivity characteristics which usually behave in populations as polygenic traits. Further study should explain the genetic basis for such dramatic change caused by mutation in just a single gene locus.

Despite a strong criticism morphological characteristics of infective juveniles and those of male adults still play an important role in separation of *Steinernema* species (Poinar, 1990). General morphology and body measurements of dumpy juveniles significantly differ from those set for type specimens of *S. feltiae* (Poinar, 1986). Dumpy individuals retain, however, their ability to interbreed successfully with individuals from various *S. feltiae* strains. The date presented shows that even a single spontaneous mutation can significantly alter body measurements of nematode individuals and if conditions allow the entire population can be transformed into a completely new morphological entity. Therefore, these findings provide further evidence that morphological characteristics may not be satisfactory for taxonomic determination of steinernematids and, as it was suggested earlier by Akhurst and Bedding (1978), crossbreeding and analysis of fertility in the F1 and F2 progeny should be considered as a minimum standard test for identification of *Steinernema* species.

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