Journal of Nematology 21(2):247–253. 1989.

Genetic Variability among Strains of the Entomopathogenic Nematode *Steinernema feltiae*[1]

RANDY GAUGLER,[2] TERRY MCGUIRE,[3] AND JAMES CAMPBELL[2]

Abstract: A systematic program of genetic improvement was initiated by assessing the phenotypic variation of *Steinernema feltiae* strains for two traits assumed to limit efficacy: ultraviolet tolerance and host-finding ability. All of the strains assayed showed both low ultraviolet tolerance and poor host-finding ability, indicating that the likelihood of improving these traits through more extensive population sampling is remote. Limited genetic variation was detected among the strains for tolerance to ultraviolet, suggesting that selective breeding for increased tolerance would be inefficient. By contrast, highly significant phenotypic differences were found with regard to host-finding ability, suggesting that this trait would be responsive to selection. A genetically heterogeneous population was constructed by round-robin mating of 10 strains; it will serve as the foundation population for selective breeding.

Key words: artificial selection, genetic improvement, host-finding ability, hybridization, *Steinernema feltiae* strains, ultraviolet radiation.

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae possess virtually every attribute of an "ideal" biological control agent (3), including safety (EPA exempt), ease of mass production, high virulence, and a broad host range. Field evaluations of these nematodes have often produced inconsistent results, however, with instances of ineffectiveness generally being attributed to nematode inactivation by environmental extremes (3,4,15). Infective stages are especially sensitive to desiccation (20,22) and the ultraviolet (UV) component of solar radiation (4).

Efforts to overcome the poor field persistence of entomopathogenic nematodes have focused on improved formulation including the use of evaporetardants (21), photoprotectants (6), encapsulation (16), and baits (8). Genetic improvement has been neglected as a means of increasing nematode environmental tolerance (4), although this method is commonly used to improve crops, livestock, bees, and silkworms. Selective breeding has also been applied to biological control agents, most often to increase the insecticide tolerance of predaceous mites (12,13). Genetic manipulation, however, remains controversial.

Entomopathogenic nematodes offer attractive advantages as subjects for genetic improvement, including a short generation time (7–10 days) and ease of culture and handling. Of particular significance is the fact that these parasites are intended primarily for inundative control (i.e., as biological insecticides). Genetic improvement has focused previously on natural enemies as inoculative agents, where "wild" properties involved in mating, dispersal, habitat selection, diapause, etc. must be preserved in the improved agent if establishment and recycling are to be expected. Hoy (13) notes that this constraint greatly increases the difficulty of improving natural enemies and speculates that selection programs using inundative agents might be more successful, since reduced fitness is not a serious disadvantage.

The framework for devising a selective breeding program for natural enemies originated with DeBach (1) and was subsequently revised and expanded by Messenger et al. (18) and Hoy (12). We have adapted Hoy's (12) design as a systematic plan to genetically improve entomopathogenic nematodes (Fig. 1). We have chosen

Received for publication 31 August 1988.

[1] New Jersey Agricultural Experiment Station Publication No. D-08251-22-88, supported by state funds. Funded in part by USDA Competitive Research Grant No. 87-CRCS-1-2298 and the International Society of Arboriculture Research Trust.

[2] Associate Professor and Senior Laboratory Technician, Department of Entomology, Rutgers University, New Brunswick, NJ 08903.

[3] Associate Professor, Department of Biology, Rutgers University, New Brunswick, NJ 08903.

We thank J. Oertli and D. Seckinger for technical assistance.

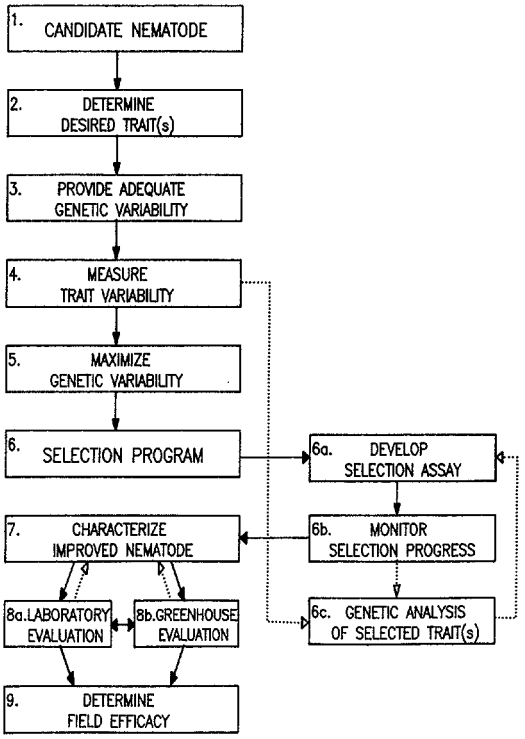

FIG. 1. A systematic plan for the design of a genetic improvement program for entomopathogenic nematodes. Adapted from Hoy (12).

Steinernema feltiae as our candidate species for genetic improvement (Fig. 1, no. 1) because it has been studied and field tested more extensively than any other nematode parasite of insects and because it possesses superior storage capabilities, compared with heterorhabditids.

The next consideration is nematode traits that limit field efficacy which should be genetically manipulated (Fig. 1, no. 2). The poor field persistence of *S. feltiae* is a critical limitation that might be attacked directly by enhancing juvenile tolerance to inactivation from UV radiation and indirectly by enhancing nematode host-finding ability. This latter trait is important because highly motile nematodes able to rapidly locate hosts from longer distances would be less vulnerable to environmental inactivation.

The next steps are to collect a number of ecologically diverse (and therefore, presumably, genetically diverse) populations (Fig. 1, no. 3) and assess their genetic potential for the desired traits (Fig. 1, no. 4). If only slight phenotypic differences are found for a trait, it is impractical to proceed to selective breeding. If there are substantial phenotypic differences, then one or more of the isolates might already have the desired phenotype, and it would not be necessary to carry out selective breeding. Instead, it would be desirable to stabilize the phenotype within an isolate by inbreeding. Finally, if there are genetic differences, but none of the isolates has the desired phenotype, the isolates (or a subset) can be used to breed a foundation strain with a high degree of heterozygosity (Fig. 1, no. 5) before proceeding to selective breeding for the desired phenotype (Fig. 1, no. 6). The principal foci of this paper are the collection and comparison of a number of geographical isolates and the construction and characterization of a foundation strain.

MATERIALS AND METHODS

Strain derivation: We obtained 21 geographical isolates of *S. feltiae* from four continents. The Mexican, All, and Soviet strains were obtained from Biosys (Palo Alto, CA); the Florida, Nachodka, Plou, Peridiarum, North Carolina 116, DD-136, Agriotos, and Breton strains came from R. Bedding (Hobart, Australia); the I-0, I-100, and I-165 strains from K. Deseö (Bologna, Italy); the Pennsylvania strain from Bio-Logic (Chambersburg, PA), the Ohio strain from M. Klein (Wooster, OH); the New Zealand strain from W. Wouts (Auckland, New Zealand); and the Zak strain from J. Kozodoi (Moscow, USSR). The Kapow strain was derived from the Mexican strain by selecting for rapid development (J. Lindegren, Fresno, CA). The NJ-41 and Griggstown strains were freshly isolated from soil samples in New Jersey. All procedures were conducted with nematodes freshly cultured in *Galleria mellonella* larvae.

The 22 strains were preliminarily screened for both host-seeking ability and UV tolerance using rapid test procedures. Ten strains representing the range of ge-

netic variability for the two traits were chosen from the top, middle, and bottom of the distributions for in-depth analysis (see next paragraph) and for hybridization resulting in the foundation strain. When choosing among strains with nearly identical phenotypes, we picked those that maximized geographical and ecological diversity. The 10 strains chosen were Agriotos, All, Breton, Florida, Griggstown, Kapow, Mexican, New Zealand, Plou, and Soviet.

Host-finding assay: Procedures for measuring nematode host-finding abilities were based on modifications of Gaugler et al. (7). These assays were conducted in petri dishes (150 × 50 mm) containing a 3-mm layer of 2% agar. The dishes were left open after pouring until excess moisture on the agar surface evaporated. Dish tops were modified to hold two pipet tips (101–1,000 μl) fixed through holes located 2.5 cm on opposite sides of the dish point-of-center (i.e., the tips were 5 cm from each other). When the top was replaced, the pipet tips were perpendicular to, and 2 mm above, the agar surface. One pipet tip was designated as the experimental tip and the other as the control tip. Two last-instar *Galleria* larvae (0.2–0.25 g each) were placed into the experimental tip 24 hours before testing. Both ends of the tip were loosely packed with steel wool to prevent larval escape and the top end was sealed with plastic film. The control tip was similarly packed and sealed but did not contain larvae.

Approximately 1,000 infective-stage juveniles were placed with a fine brush onto a 1-cm-d inoculation site at the agar center, equidistant from the two pipet tips. The petri dish top was positioned over the agar, and the dish placed into an insulated box in a 25-C incubator for a 1-hour test period. The box minimized possible influences of temperature gradients and air movements. Nematode response was scored by recording the number of infectives from 1-cm-d agar cores taken directly below the experimental and control pipet tips. After removal of the cores the remaining nematodes were washed into a counting dish, enabling a precise count of total nematodes assayed, and host-finding and control responses were determined. Treatments and controls for each strain were replicated four times.

Ultraviolet tolerance assay: The UV source emitted medium UV radiation peaking at 302 nm, which is consistent with those wave lengths in natural sunlight believed to be principally responsible for nematode inactivation (5). The lamp was mounted in a cabinet and nematodes were placed 30 cm below the UV source, a distance producing an intensity of 60 $\mu W/cm^2$ as measured with a digital radiometer. Ultraviolet radiation intensity was monitored immediately before and after each exposure; trials varying from 60 $\mu W/cm^2$ were discarded.

Ultraviolet exposures were conducted by adapting the method of Gaugler and Boush (5,6). Approximately 500 infective-stage juveniles were pipetted onto 55-mm-d filter papers (Fisher Q2). Excess water was removed by vacuum filtration, and the filter paper was placed on a stack of moistened 47-mm cellulose fiber pads held in a 60-mm petri dish bottom for irradiation. The moist pads prevented nematodes from becoming desiccated during irradiation. After exposure, the backs of the Q2 filter papers were rinsed to remove nematodes that may have migrated to the underside of the papers and avoided UV exposure. The rinsed Q2 papers were placed face up in 60-mm petri dishes on another piece of filter paper. Five last-instar *Galleria* larvae were placed in each dish and set in darkness at 25 C for 4 days, after which larval mortality was determined. Treatments and controls for each nematode strain assayed were replicated nine times per exposure period.

Construction of foundation strain: The 10 strains were systematically mated in a round-robin design as shown in Figure 2 to obtain 10 F_1 populations. The 10 F_1 populations were mixed and allowed to infect and freely intermate within *Galleria* larvae to form the foundation strain.

Statistical analyses: Host-finding data were

FEMALE	X	MALE	=	F1
SOVIET	X	NEW ZEALAND	=	$S_{SO,NZ}$
NEW ZEALAND	X	BRETON	=	$S_{NZ,BR}$
BRETON	X	PLOU	=	$S_{BR,PL}$
PLOU	X	ALL	=	$S_{PL,AL}$
ALL	X	FLORIDA	=	$S_{AL,FL}$
FLORIDA	X	AGRIOTOS	=	$S_{FL,AG}$
AGRIOTOS	X	KAPOW	=	$S_{AG,KA}$
KAPOW	X	MEXICAN	=	$S_{KA,MX}$
MEXICAN	X	GRIGGSTOWN	=	$S_{MX,GR}$
GRIGGSTOWN	X	SOVIET	=	$S_{GR,SO}$

MASS MATING ⇨ FOUNDATION STRAIN

FIG. 2. Creation of a foundation strain of *S. feltiae* by round-robin mating of 10 strains followed by mass mating the resulting F_1 generation.

normalized using an arcsine transformation. Ultraviolet tolerance for each replicate was determined by probit analysis (LD_{50}) of the $\log_{10}$ tranformed data. Significance of main effects was determined by analysis of variance (ANOVA). The significance of individual strain scores was evaluated by a post-hoc Duncan's multiple-range test ($a = 0.05$).

RESULTS AND DISCUSSION

Efforts to screen for natural isolates possessing the desired trait improvements were unsuccessful: none of the strains evaluated displayed a useful level of either UV tolerance (Fig. 3) or host-finding ability (Fig. 4). While it is always preferable to exhaust natural sources of variation for a desired trait before undertaking a selection program, the overall phenotypes recorded here were so low that the likelihood of improving these traits through more extensive population sampling is remote.

The data suggest low genetic variability and, therefore, perhaps inadequate genetic potential to efficiently select for UV tolerance (Fig. 3). From the most to the least tolerant strain, we observed an LD_{50} range of 5.71 (Agriotos) to 7.26 minutes (Mexican), a difference of only 27%. This difference is statistically significant ($P > 0.05$), but it is clouded by a greater variance between blocks than strains (block $F = 5.73$, $P = 0.0001$; strain $F = 2.92$, $P = 0.0039$). The controls performed as expected: nematodes not exposed to UV killed 100% of *Galleria* larvae, whereas *Galleria* not inoculated with nematodes showed nearly 100% survival. Selection can be effective when there is a pool of variation to act on, but it will be ineffective when populations approach genetic uniformity. The data suggest a small genetic effect with regard to UV tolerance, but response to selection on a trait with so much environmental variation and so little genetic variation would be slow at best. With only a 27% difference in UV tolerance and large block effects, it may be more feasible to reduce nematode UV vulnerability by formulation or evening application.

While it is questionable whether a program of genetic selection should proceed for UV tolerance, host-finding ability showed a fourfold difference between the New Zealand and Florida strains (Fig. 4), and there was a much smaller block effect (block $F = 3.09$, $P = 0.032$; strain $F = 23.96$, $P = 0.0001$), suggesting that there is sufficient variability to expect a strong response to selection pressure. Differences in host-finding ability were not due to differences in activity among strains, since no significant differences were detected among the 11 strain controls ($P > 0.74$); the controls were subsequently pooled for presentation in Figure 4.

What is less clear is why, even with the "best" strain (i.e., Florida), no more than 8% of infectives were able to locate a highly

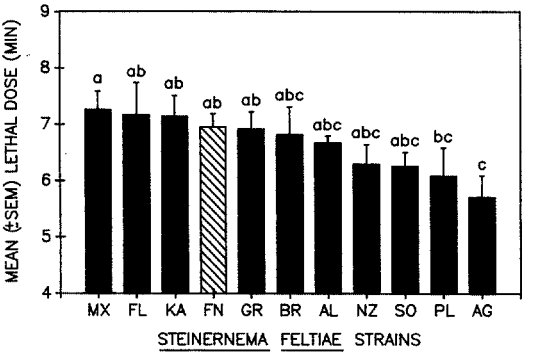

FIG. 3. Comparison of the ultraviolet tolerance of *S. feltiae* strains. Bars bearing the same letter are not significantly different ($a = 0.05$; Duncan's multiple-range test). AG = Agriotos strain, AL = All, BR = Breton, FL = Florida, GR = Griggstown, KA = Kapow, MX = Mexican, NZ = New Zealand, PL = Plou, SO = Soviet, FN = foundation.

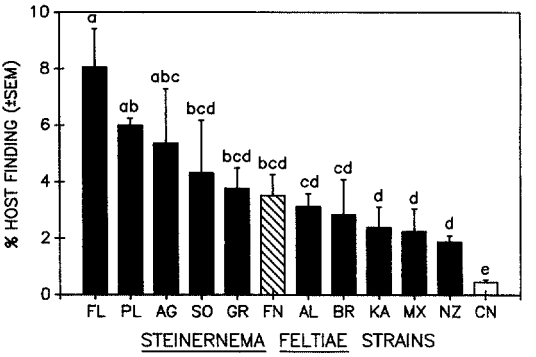

FIG. 4. Comparison of the host-finding abilities of *S. feltiae* strains. Bars bearing the same letter are not significantly different ($a = 0.05$; Duncan's multiple-range test). AG = Agriotos strain, AL = All, BR = Breton, FL = Florida, GR = Griggstown, KA = Kapow, MX = Mexican, NZ = New Zealand, PL = Plou, SO = Soviet, FN = foundation, CN = control.

susceptible insect host from a distance of 2.5 cm on a simple two-dimensional substrate over a 1-hour test period. Particularly noteworthy is that a mere 4% of All strain infectives, one of the most widely field tested of *S. feltiae* strains and the only nematode commercially available on a large scale, were successful in finding the host. This observation may explain, in part, why dosages of nearly 800 All strain infectives/cm^2 provide on average little more than 50% control of Japanese beetle larvae in the field (Georgis and Gaugler, unpubl.). The available data suggest that an inadequate host-finding ability may limit the field efficacy of these nematodes, further increasing the importance of this trait as a target for genetic improvement. Doutt and DeBach (2) regard a high host-finding ability to be the single most important attribute of an effective natural enemy.

Our finding that *S. feltiae* performs poorly at host finding was unexpected, since rapid host finding should be a highly adaptive trait. Infectives have been shown to migrate 14 cm through soil columns to infect *Galleria* larvae (9), but a tendency for most infectives to remain near the point of application has also been noted (9,19). Ishibashi and Kondo (14) hypothesized that this nematode enters a quiescent state following soil applications. In this study we observed that many infectives remain motionless at the point of inoculation throughout the test period.

The data suggest that *S. feltiae* populations may consist of a small proportion of infective stages that aggressively search for hosts and a larger proportion of more passive infectives that take an energy conserving approach. This polymorphism would serve to maximize host contact, and could be genetically predetermined, as in the case of *Meloidogyne incognita* diapause (10). Alternatively, infective stages may show different latencies in moving from the application site, a difference that might again be brought under genetic control. Our screening assay permitted classification as host finders only those nematodes that moved and located the host within the first hour. In either case, it should be relatively easy to shift the proportion of nematodes that are active searchers or that move with a short latency, since we would be selecting to change only the frequency of phenotypes already represented in the population.

The significant phenotypic differences observed between strains in host-finding ability reflect underlying genetic differences. The nature of these genetic differences is unknown. One possibility is that the strains are segregating for identical al-

leles at the same set of genes. The only differences between strains would be the allelic frequencies at those loci. Another possibility is that the strains are segregating at the same genes but have different alleles at those loci. A third possibility, is that the nematodes are segregating for different genes, all of which affect host seeking. Most likely, all three are true. If the first reason accounts for all of the genetic variation, there would be no reason to construct a foundation strain, as selection on any noninbred strain would have the same result. If reasons two or three are important, however, selection on a single strain would eliminate important alleles and even important genes from selection and might ultimately limit the results of selection.

We maximized genetic variation by creating the foundation strain from a series of round robin matings (Fig. 2). This method of intraspecific hybridization maximizes variability, tends to break down behavioral reproductive isolation, increases the probability of preserving rare alleles and obtaining new genetic combinations, and assures equal representation from all 10 parental strains. Although hybridization sometimes results in hybrid vigor or heterosis that yields improvements over parental stock (11,17), our foundation strain did not perform any better than did the 10 parental strains (Figs. 3, 4). This strain contains all of the genetic variability contained in all 10 of the geographical isolates, however, so we anticipate a much greater response to selection than would be possible with any single strain. In short, we anticipate that imposing selection on our foundation strain will increase the likelihood of achieving desired trait improvements.

The primary goal of this study was to provide a systematic approach for genetic improvement and to determine the feasibility of such a program. We believe that we now have a firm basis for proceeding to the next phase in our genetic improvement scheme: to devise a selection program (Fig. 1, no. 6) to enhance host finding in the foundation strain, in hopes of obtaining a new strain with superior field efficacy.

Literature Cited

1. DeBach, P. 1958. Selective breeding to improve adaptations of parasitic insects. Proceedings of the Tenth International Congress of Entomology 4: 759–768.

2. Doutt, R. L., and P. DeBach. 1964. Some biological concepts and questions. Pp. 118–142 *in* P. DeBach, ed. Biological control of insect pests and weeds. New York: Reinhold Publishing Co.

3. Gaugler, R. 1981. Biological control potential of neoaplectanid nematodes. Journal of Nematology 13:1–13.

4. Gaugler, R. 1987. Entomogenous nematodes and their prospects for genetic improvement. Pp. 457–484 *in* K. Maramorosch, ed. Biotechnological advances in invertebrate pathology and cell culture. New York: Academic Press.

5. Gaugler, R., and G. M. Boush. 1978. Effects of ultraviolet radiation and sunlight on the entomogenous nematode, *Neoaplectana carpocapsae.* Journal of Invertebrate Pathology 32:291–296.

6. Gaugler, R., and G. M. Boush. 1979. Tests on materials as ultraviolet protectants of an entomogenous nematode. Environmental Entomology 8:810–813.

7. Gaugler, R., L. LeBeck, B. Nakagaki, and G. M. Boush. 1980. Orientation of the entomogenous nematode *Neoaplectana carpocapsae* to carbon dioxide. Environmental Entomology 9:649–652.

8. Georgis, R. 1987. Nematodes for biological control of urban insects. American Chemical Society Meeting (Division of Environmental Chemistry), New Orleans, LA, 27:816–821 (Extended abstr.).

9. Georgis, R., and G. O. Poinar, Jr. 1983. Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). Journal of Nematology 15:308–312.

10. de Guiran, G. 1979. A necessary diapause in root-knot nematodes. Observations on its distribution and inheritance in *Meloidogyne incognita.* Revue de Nematologie 2:223–231.

11. Hoy, M. 1975. Forest and laboratory evaluations of hybridized *Apanteles melanoscelus* (Hym.: Braconidiae), a parasitoid of *Porthetria dispar* (Lep.: Lymantriidae). Entomophaga 20:261–268.

12. Hoy, M. A. 1979. The potential for genetic improvement of predators for pest management programs. Pp. 106–115 *in* M. A. Hoy and J. J. McKelvey, Jr., eds. Genetics in relation to insect management. New York: Rockefeller Foundation Press.

13. Hoy, M. A. 1986. Use of genetic improvement in biological control. Agriculture, Ecosystems and Environment 15:109–119.

14. Ishibashi, N., and E. Kondo. 1986. A possible quiescence of the applied entomogenous nematode, *Steinernema feltiae,* in soil. Japanese Journal of Nematology 16:66–67.

15. Kaya, H. K. 1985. Entomogenous nematodes for insect control in IPM systems. Pp. 283–302 *in* M. Hoy and D. C. Herzog, eds. Biological control in agricultural IPM systems. New York: Academic Press.

16. Kaya, H. K., and C. E. Nelsen. 1985. Encapsulation of steinernematid and heterorhabditid nematodes with calcium alginate: A new approach for in-

sect control and other applications. Environmental Entomology 14:572–574.

17. Legner, E. F. 1972. Observations on hybridization and heterosis in parasitoids of synanthropic flies. Annals of the Entomological Society of America 65:254–263.

18. Messenger, P. S., F. Wilson, and M. J. Whitten. 1976. Variation, fitness, and adaptability of natural enemies. Pp. 68–92 *in* C. B. Huffaker and P. S. Messenger, eds. Theory and practice of biological control. New York: Academic Press.

19. Moyle, P. L., and H. K. Kaya. 1981. Dispersal and infectivity of the entomogenous nematode, *Neoaplectana carposcapsae* Weiser (Rhabditida: Steinernematidae), in sand. Journal of Nematology 13:295–300.

20. Simons, W. R., and G. O. Poinar, Jr. 1973. The ability of *Neoaplectana carpocapsae* (Steinernematidae: Nematodea) to survive extended periods of desiccation. Journal of Invertebrate Pathology 22: 228–230.

21. Webster, J. M., and J. F. Bronskill. 1968. Use of Gelgard M and an evaporation retardant to facilitate control of larch sawfly by a nematode-bacterium complex. Journal of Economic Entomology 61:1370–1373.

22. Welch, H. E., and L. J. Briand. 1961. Field experiment on the use of a nematode for the control of vegetable crop insects. Proceedings of the Entomological Society of Ontario 91:197–202.