The soybean cyst nematode, *Heterodera glycines*: a genetic model system for the study of plant-parasitic nematodes

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Despite advances in understanding plant responses to nematode infection, little information exists regarding parasitic mechanisms. Recently, it has become possible to perform genetic analysis of soybean cyst nematode. Integration of classic and reverse genetics and genomic approaches for the parasite, with host genetics and genomics will expand our knowledge of nematode parasitism.

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**Abbreviation**

SCN soybean cyst nematode

**Introduction**

Nematodes are devastating parasites of plants and animals. World-wide, plant-parasitic nematode infections result in losses of more than $100 billion per year. Losses due to animal parasites are certainly greater, and are compounded by human diseases caused by nematode parasites. Despite their tremendous importance, the biology of both animal and plant parasitic nematodes is poorly understood. This deficit is largely due to the nature of nematode parasitism. In addition to being obligate, most parasitic nematodes complete complex life cycles within their hosts. It is very difficult, therefore, to directly observe and experiment upon the various life stages. Although little progress has been made studying nematode parasites directly, host plant responses to nematode infection have been characterized in detail. Numerous publications have described the latest work on host responses to nematodes [1,2], so it is not necessary to review this topic here. Instead, we focus on analysis of nematode parasitic abilities using a newly developed model genetic system.

The best understood and characterized animal is the free-living nematode *Caenorhabditis elegans*. In addition to the genome sequencing project (nearly complete, Table 1), detailed biochemical and genetic analyses over the past 30 years have provided a comprehensive picture of the complexities of behavior and development in a multicellular organism. It is somewhat ironic that this system is an extremely important model for human health, yet little of this knowledge has been applied to the study of parasitic species until recently [3••,4]. In large part, the utility of *C. elegans* as a model has derived from the ability to perform genetic analysis, something that has been lacking for parasitic nematodes. As previously alluded to, most important parasitic nematodes complete their life cycles within the host, making controlled crossing and analysis of progeny functionally impossible. A recently developed system, however, modeled after the *C. elegans* paradigm, now allows genetic approaches to answer questions not previously able to be posed, including identification of genes that play a direct role in nematode parasitism. As will be described in the following sections, the soybean cyst nematode (SCN), *Heterodera glycines*, has been developed as a model system to study parasitic nematode biology and behavior [5••].

**Soybean cyst nematode**

Economically, *H. glycines* is the major pathogen of soybean world-wide. This sedentary endoparasitic nematode is an obligate cross-fertile species with six life stages: the egg, four juvenile stages and the sexually dimorphic adult stage. The infective stage juvenile is a non-feeding, non-developing arrested stage that functions as a dispersal phase, and is functionally equivalent to a *C. elegans* dauer larvae. Similar to *C. elegans* dauers, the infective stage of SCN is resistant to detergent (SDS) treatment (Opperman CH, unpublished data) which is an indication of resistance to environmental perturbation. The infective dispersal stage penetrates the host plant root and migrates to an area near the vascular cylinder, where it establishes a complex feeding site [6,7]. Upon initiation of the feeding site, the nematode resumes its developmental cycle. Reinitiation of development includes degeneration of the somatic musculature, precluding further migration and resulting in the sedentary form of the parasite. Sexual differentiation also begins during the later stages of juvenile development. SCN males migrate out of the root within 10–15 days after infection for mating. As the adult female matures, her head remains embedded in the feeding site appressed to the root vascular bundle, while the posterior portion of the body erupts through the root surface and is exposed to the rhizosphere. The adult female nematode produces 50–400 eggs, which remain primarily in her swollen, hardened body, forming a cyst [8]. Each life cycle takes 25–40 days and there may be several generations in the field per growing season. Eggs are able to survive in the cyst for a number of years under very harsh environmental conditions [9,10], complicating pest management strategies. In the past several years, management options for SCN have been gradually eroded. Many effective nematicides either have been banned or restricted for environmental and toxicity reasons. Deployment of nematode-resistant, soybean germplasm
remains the principal control tool. Although management via the use of resistant soybean cultivars may be achieved, there are numerous genotypes (races) of SCN that may evade host resistance responses. The only alternative currently is crop rotation away from an SCN host, a practice that may not be economically feasible in many cases.

Because *H. glycines* maintains an intimate parasitic relationship with its host, it is suspected that numerous nematode genes are involved in its parasitic abilities. The obligate parasitic nematode does not lend itself to classic mutagenesis strategies, however, as most morphological and behavioral mutations interfere with the host–parasite interaction. Rather, genetic mapping approaches approaches have been based on exploitation of natural variation. Inbred lines of soybean cyst nematode have been developed from a number of field isolates. Initially, these lines have been selected for their ability to parasitize resistant soybean cultivars [5**,], but other parasitism phenotypes are being evaluated, including host preference, hatching, entry and exit from diapause, and sex determination, to name a few.

**Genetic analysis of nematode parasitism**

A parasite must reproduce to successfully complete its life cycle. In this sense, the ability of an *H. glycines* individual to parasitize a soybean plant is measured by reproduction. In general, resistant hosts do not support female nematode development to reproductive maturity. Thus, parasitism of a particular host genotype is a qualitative trait that the individual nematode either possesses or does not. Nematode populations may be additionally described quantitatively by their level of reproduction on a given host plant. Field populations of *H. glycines* are mixtures of many genotypes, some of which may confer the ability to overcome host resistance genes. Selection pressure from growing resistant cultivars can alter the frequency of alleles in the population for reproducing on a resistant host.

To date, most progress on the genetics of parasitism in nematodes has been made in plant-parasitic species, particularly *Globodera rostochiensis* [11,12] and *H. glycines* [5**,13]. This is partly because these nematode are sexually dimorphic, obligate amphilic species, making them genetically tractable, but also because plants are experimentally more amenable as hosts than are many animals, especially in the numbers required for classic genetics. Importantly, it has proven possible to score for parasitism traits that enable particular nematode genotypes to evade host defense responses. A gene-for-gene relationship appears to be in operation in the case of the potato cyst nematode, (*G. rostochiensis*)–potato interaction [11,12]. Potatoes carrying the dominant *H1* gene are resistant to certain pathotypes of *G. rostochiensis*. Pure parasitic and nonparasitic lines of *G. rostochiensis* have been selected, and crosses using these lines have revealed that parasitism is inherited at a single locus in a recessive manner [11].

Results from reciprocal crosses suggested that there is no evidence for sex-linked inheritance of parasitism.

Because of the importance of soybean cyst nematode as a pathogen, and also the identification and utilization of host resistance by soybean breeders, a considerable body of literature exists on the genetic basis of parasitism in *H. glycines* [14*]. It is generally believed that both major and minor genes (including dominant, partially dominant and recessive alleles) are all involved to some degree in conferring resistance to *H. glycines* [15], although it is not clear which genes are essential and which are specific to certain nematode genotypes, if any. Interpretation is complicated by the use of *H. glycines* field populations to evaluate resistant soybean; field populations are highly heterogeneous, both among, and within isolates [15]. Results from population measurements usually are biased by this genetic variability, and the frequency of certain genes for parasitism (nematode genes necessary to overcome host resistance) may affect phenotypic designation of either parasitism or the levels of reproduction [16]. We, therefore, believe that the previous results are not accurate indications of the genetic basis of soybean parasitism in *H. glycines*.

Recently, pure lines of *H. glycines* that carry single genes for parasitic ability on soybeans have been developed, and used to demonstrate that *H. glycines* contains unlinked dominant and recessive genes for parasitism of various host genotypes [5**]. In this study, parasitism genes in *H. glycines* were analyzed by crossing two highly inbred lines (>29 generations). A nonparasitic *H. glycines* line, which fails to reproduce on the resistant soybean lines PI88788 and PI90763, was used as the female and recurrent parent, and was crossed to a parasitic line that does reproduce on these resistant hosts. The segregation ratio of the progeny lines developed by single female inoculation revealed that parasitism to these soybean lines is controlled by independent, single genes in the nematode. In accord with genetic nomenclature rules for parasitic nematodes [17], these loci were named *ror* for reproduction on a resistant host [5**]. In the inbred lines, *ror*−1(*kr1*) confers the ability to reproduce on PI88788 and is dominant. The recessive gene, *ror*−2(*kr2*) controls reproduction on PI90763. A second recessive gene, *ror*−3(*kr3*), controls the ability to parasitize the soybean line Peking. Although not verified, it is an intriguing possibility that some genes controlling parasitism may be acting additively. Examination of F1 data from controlled crosses (CH Opperman, unpublished data) reveals that the presence of two *ror* genes results in twice as many females being formed on PI88788 as when only one of these genes is present. This may explain varying levels of aggressiveness between different nematode populations on the same host genotype. It is particularly significant to note that these loci are entirely independent and do not appear to interact; that is to say, no novel host ranges are detected when combinations of *ror* genes are present.
in a particular nematode line. In addition to alleles for parasitism of resistant soybeans, there are SCN lines that have been selected to reproduce on tomato. The genes controlling this host acquisition remain to be characterized, either at the genetic or at the molecular level.

A physical genetic map
Classic genetic approaches to parasitism permit the mapping and hence isolation of any genes that exhibit a scorable phenotype, including developmental, behavioral and parasitism traits, and this strategy will prove to be a powerful tool to characterize many aspects of the host–parasite interaction. A complementary approach is based on a physical map of *H. glycines* currently under development (B Sosinski, J Heer, D McK Bird and CH Opperman, unpublished data). The physical map will be constructed by fingerprinting BAC and cosmid clones, and overlapping clones will be assembled into contigs [18]. Clones received from outside sources (ie. genes cloned in other labs, new markers, etc.) will be placed on the map via fingerprinting.

The construction of a physical map will enable rapid mapping and isolation of genes with scorable phenotypes in the absence of a mutagenesis system. As alluded to earlier, it is difficult to perform classical mutagenesis on an obligate parasite with any degree of success. Strategies based on transposon mutagenesis are in their infancy, as we have only recently isolated transposable elements from SCN (SJ Hogarth, D McK Bird, and CH Opperman, unpublished data). Although gene isolation based solely on a genetic location (ie., map-based cloning) is quite possible, it may be a formidable task. Because the *C. elegans* genome sequence has nearly been completed, one tool that might readily be exploited is that of conserved synteny. The genome size and complexity of SCN is also strikingly conserved across wider evolutionary distances. Rice, maize and wheat, species believed to share a common ancestor 400 million years since vertebrate diversification [20], exhibit sufficient co-linearity to permit the reconstruction of a hypothetical, ancestral grass genome [19], and significant blocks of synteny have been conserved during the 400 million years since vertebrate diversification [20]. Just as evolutionary relationships can be reconstructed by comparing the DNA sequence of homologous loci, mapping the chromosomal rearrangements that have led to breakage of synteny might prove to be a powerful tool in understanding nematode evolution and phylogenetic relationships. Both the genetic and the physical maps of the genome of *H. glycines* might readily be exploited is that of conserved synteny. The genome size and complexity of SCN is also strikingly conserved across wider evolutionary distances. Rice, maize and wheat, species believed to share a common ancestor 400 million years since vertebrate diversification [20], exhibit sufficient co-linearity to permit the reconstruction of a hypothetical, ancestral grass genome [19], and significant blocks of synteny have been conserved during the 400 million years since vertebrate diversification [20]. Just as evolutionary relationships can be reconstructed by comparing the DNA sequence of homologous loci, mapping the chromosomal rearrangements that have led to breakage of synteny might prove to be a powerful tool in understanding nematode evolution and phylogenetic relationships. Both the genetic and the physical maps of the genome of *H. glycines* are currently under development (B Sosinski, J Heer, D McK Bird and CH Opperman, unpublished data).

### Table 1

<table>
<thead>
<tr>
<th>Property</th>
<th><em>C. elegans</em></th>
<th><em>H. glycines</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Life cycle</td>
<td>Free living</td>
<td>Plant parasitic</td>
</tr>
<tr>
<td>Chromosomes</td>
<td>six</td>
<td>nine</td>
</tr>
<tr>
<td>Reproduction</td>
<td>Hermaphroditic</td>
<td>Amphimictic</td>
</tr>
<tr>
<td>Genome size</td>
<td>100 Mb</td>
<td>92.5 Mb*</td>
</tr>
<tr>
<td>Genome complexity†</td>
<td>83% unique</td>
<td>82% unique</td>
</tr>
<tr>
<td>G–C content</td>
<td>36%</td>
<td>36%</td>
</tr>
<tr>
<td>% of genome sequenced</td>
<td>98%</td>
<td>&lt;0.0005%</td>
</tr>
</tbody>
</table>

* Determined by flow cytometry (SJ Hogarth, D Mck Bird, and CH Opperman, unpublished data). † Percent of nonrepetitive DNA determined by C<sub>t</sub> analysis (SJ Hogarth, D McK Bird, and CH Opperman, unpublished data), *C. elegans* data from [26].

for *H. glycines* will permit these types of analyses to be performed for a parasitic nematode. A more compelling motive in searching for synteny is to be able to make predictions about gene position in *H. glycines* on the basis of the location of the homologous gene in *C. elegans*. This approach will be especially powerful for the identification of homologous genes with low levels of DNA sequence identity.

Reverse genetics
Construction of transgenic nematodes will undoubtedly prove valuable for the cloning of dominant genes (such as *ror-1*), or the transgenic complementation of recessive mutations. Transgenic SCN have been generated in our laboratory via sperm microinjection and subsequent mating (T Pedersen, D McK Bird, and CH Opperman, unpublished data). This approach will also permit ablation of cloned parasitism gene candidates. For example, a small family of putative cellulase genes have recently been isolated from several cyst nematodes, including SCN [21]. Although cellulases have long been known to be secreted by plant parasitic nematodes [22–24], their role in nematode parasitism has remained speculative. They are potentially involved in nematode migration through plant tissues, as well as a possible role in feeding site establishment. Because these genes were isolated by screening a cDNA expression library with an antibody directed to nematode gland secretions, there is no function as yet ascribed. Use of the transgenic nematode technologies will permit knockout of these genes, potentially generating a phenotype helpful in defining their role in the parasitic interaction. Such functional analysis will be essential for confirmation of a direct role in parasitism, although care will need to be exercised in interpreting these results; merely demonstrating that genetic ablation of a particular gene disrupts the life cycle of an obligate parasite is insufficient proof that the gene encodes a parasitism
function. This is because parasitic nematodes are likely to have significantly more essential genes (i.e., genes with products necessary for the nematode to complete its life cycle) than free-living organisms, such as *C. elegans*.

Although many parasitism genes will be essential genes, the converse is not the case. Johnsen and Baillie [25] estimate that 15–30% of *C. elegans* genes are essential, and this is the largest single class in *C. elegans*. Although mutations at many other loci can give drastic phenotypes, the functions encoded by these genes appear to be dispensable for reproduction per se, so they are not classified as essential. This assignment is, however, to a large degree, an artifact of the way *C. elegans* is maintained in the laboratory. For example, the second largest class of genes in *C. elegans* is that in which mutation gives an unco-ordinated (Unc) phenotype. Because co-ordinated movement is dispensable for a free-living nematode lying on a petri plate in a sea of bacteria, the unc loci are considered to be non-essential. In contrast, the equivalent genes (and many others as well) are almost certainly essential for obligate parasites such as *H. glycines*. For these nematodes to reproduce they must locate a host, invoke, and select and establish a feeding site, events that certainly require co-ordinated movement and behavior. Thus, correct interpretation of genetic ablation experiments requires an assay that accurately scores disruption of equivalent function, and underscores the power of classical genetics to study parasitism.

**Conclusions**

The development of a model system to study parasitism will have significant impact on our understanding of nematode biology and behavior. This system will enable the direct testing of gene function in relation to parasitic ability and overcoming host resistance. In addition, the ability to compare *H. glycines* to *C. elegans* will provide insight into the evolution of parasitism and the sedentary life-style of plant nematodes.

**Acknowledgements**

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as follows:

- of special interest
- of outstanding interest


This paper describes how the well-characterized free living nematode, *C. elegans*, can be exploited as a tool to study parasitism. Computational and experimental approaches using *C. elegans* to identify and analyze cloned parasitism genes are presented, and the utility of *C. elegans* biology as an approach to reveal pathways likely to be important for parasites is discussed.


Here the basic tools for performing genetics on soybean cyst nematode, including development of breeding lines, crossing methodologies and strategies, are presented. It is shown that parasitic ability is inherited in a Mendelian fashion, and that multiple independent dominant and recessive nematode loci condition host resistance breaking of distinct soybean genotypes.


An extensive review of the race concept in soybean cyst nematode and host resistance.


This is the first report of a cloned animal cellulase gene, and confirms biochemical studies of the nematode protein from the 1970s. In light of a recent phylum-wide phylogeny [27], it is interesting to note the proposition that these genes were acquired from bacteria via horizontal gene transfer.


