

THE *CAENORHABDITIS ELEGANS* GENOME: A Guide in The Post Genomics Age

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■ **Abstract** The completion of the entire genome sequence of the free-living nematode, *Caenorhabditis elegans* is a tremendous milestone in modern biology. Not only will scientists be poring over data mined from this resource, but techniques and methodologies developed along the way have changed the way we can approach biological questions. The completion of the *C. elegans* genomic sequence will be of particular importance to scientists working on parasitic nematodes. In many cases, these nematode species present intractable challenges to those interested in their biology and genetics. The data already compared from parasites to the *C. elegans* database reveals a wealth of opportunities for parasite biologists. It is likely that many of the same genes will be present in parasites and that these genes will have similar functions. Additional information regarding differences between free-living and parasitic species will provide insight into the evolution and nature of parasitism. Finally, genetic and genomic approaches to the study of parasitic nematodes now have a clearly marked path to follow.

INTRODUCTION

It has been argued (52) that the age of molecular biology began when the structure of DNA was solved. Whether or not a date can be assigned to the origin of “genomics” is questionable, but nevertheless, the decision by John Sulston and colleagues to construct a physical map of the entire genome of the free-living nematode

TABLE 1 Electronic sites for the *C. elegans* genome

URL	Site
www.sanger.ac.uk	Sanger Centre, Hinxton, UK
genome.wustl.edu/gsc/gschmpg.html	Washington University sequencing center
www.ddbj.nig.ac.jp/htmls/c-elegans/html/CE_INDEX.html	<i>C. elegans</i> EST database
http://helios.bto.ed.ac.uk/mbx/fgn/filgen1.html	Filarial nematode genome project
http://genome.wustl.edu/gsc/blast/blast_servers.html	Washington University BLAST servers
http://elegans.swmed.edu/	<i>C. elegans</i> WWW server
ftp://ncbi.nlm.nih.gov/repository/acedb	Source code and data for ACeDB

Caenorhabditis elegans in the mid-1980s (17), and the culmination of this project some 15 years later with publication of the complete *C. elegans* genomic sequence (16), appropriately opens genomics as a discipline, and concomitantly closes the “molecular-cloning” epoch in its current guise. A happy outcome is being able to return to addressing biological questions rather than occupying one’s time with the technical necessity of isolating individual genes. A very readable scientific history of the *C. elegans* sequencing project has recently been published (15).

Although we direct the reader to electronic resources (Table 1) and comprehensive texts of *C. elegans* biology (46, 65) and methodology (24), it is not our intent in this article to provide a comprehensive review of *C. elegans*, nor indeed to reiterate what can easily be read in the description of the genomic sequence (16) and the accompanying articles (1, 4, 13, 14, 48). Nor do we intend to review the discipline of genomics, as the literature is replete with recent articles on this topic [e.g. (9, 10, 38, 43, 64, 66)], including an excellent series of essays and a glossary on that aspect likely to be the most daunting to biologists, namely bioinformatics (7). There is no straightforward definition of genomics, and it is perhaps best viewed as a gene discovery/gene analysis approach predicated on three elements: (a) a well-characterized model organism to serve as a reference, (b) an approach to obtain genetic map data and/or DNA sequence information from organisms of interest, and (c) computer programs to integrate data from the model and test organisms. Using the *C. elegans* genome project as an exemplar, we hope to demonstrate the power of genomics approaches per se, and specifically how this approach can be brought to bear in studying plant-parasitic nematodes.

Relevance of the *C. elegans* Genome Project

Aside from any historical relevance, why should the genomic sequence of a free-living nematode be of interest to plant pathologists? One obvious answer is that the *C. elegans* sequence is an immensely powerful tool for the study of plant- (and

animal-) parasitic nematodes (5), and we elaborate on this point below. Although there is some controversy as to the relationship of the phylum Nematoda to other metazoans [see (4)], phylogenetic relationships within the phylum, particularly of the major parasitic groups and *C. elegans*, appear to be well established (4, 6). Such information may prove helpful in understanding the evolution of parasitic traits.

C. elegans is, however, more than just a model nematode. Genetic elucidation of *C. elegans* developmental pathways for processes as diverse as vulva formation (26), developmental arrest via the dauer pathway (44), and programmed cell death (29) has shown that the underlying biochemical machinery is conserved among the nematode, insects, mammals, and probably more generally. This is dramatically seen in the Ras signaling pathway, in which the perception of a growth factor is transduced through a cascade of intermediate proteins to the nucleus to effect gene transcription, thus specifying cell fate during development (53, 54). For *C. elegans* vulval induction (a Ras-mediated process), the growth factor is encoded by *lin-3* (30), which is homologous to mammalian epidermal growth factor and the *Drosophila* BOSS product. Similarly, each of the seven subsequent steps of the cascade has a clear nematode, fly, and mammalian counterpart, and in fact the colinearity of these pathways was used to predict the existence of at-the-time unidentified components in each system [reviewed by (54)]. The predictive power of *C. elegans* to fill in gaps in mammalian and *Drosophila* pathways has been exploited in the study of programmed cell death (29) and in dissecting the insulin pathway (28), and it is likely that many more cases will be revealed in the future, including examples from plants (49) and their pathogens. The importance of the finding that pathways are conserved across vast evolutionary distances cannot be overstated, because it not only validates the use of model organisms to study less-tractable systems, but also gives credence to the notion that conserved gene sequence is reflective of conserved function. This theme is extensively developed by Ruvkun & Hobert (48).

Other features of *C. elegans* biology (46, 65) make it an experimentally very amenable organism (24). Thus, not only is the *C. elegans* genome sequence a powerful tool for suggesting identity of genes sequenced from other organisms (revealed by sequence comparison), but the nematode itself is well suited for the experimental testing of gene function in vivo. Although the experimental details are beyond the scope of this article, and the general approaches are described elsewhere (3, 11), we briefly mention two strategies for functional gene analysis in *C. elegans*. The first is based on the discovery that the introduction of double-stranded RNA into the nematode, complementary to an endogenous gene, causes a transient inactivation of expression of that gene (25). Remarkably, the source of dsRNA for this process, which is termed RNA interference (RNAi), can simply be in the bacteria used to feed the nematode culture (56). An approach complementary to the transient gene-knockout strategy of RNAi is based on microinjection of DNA, typically a cosmid from the genome sequencing project, for construction of semistable transgenic nematode lines (39). The transgenes in these lines can

then be crossed into different genetic backgrounds for complementation tests. In this way, the relationship between a DNA sequence and a genetic locus defined by mutation can be established, thus permitting *C. elegans* to be used as a surrogate for organisms in which genetics is not established.

Finally, as the primordial genomics project (55, 62), what has been learned about the technical and philosophical challenges of the approach serves as a map to successful completion of other genomes. Perhaps the most important concept articulated by the *C. elegans* genome project is that the data should be freely and immediately available, with no constraints on their subsequent use (16, 61). This notion, formally expressed as the "Bermuda statement" (63), has been adopted by the public genomics community at large. Although the bulk of the *C. elegans* genomic sequencing has been done either at the Sanger Centre in the United Kingdom or at Washington University, St. Louis, and many of the cDNA sequences obtained by Y Kohara at the National Institute of Genetics, Mishima, Japan, the entire *C. elegans* research community has indirectly contributed through the free exchange of information, particularly genetic map data. Obviously, many of the technical developments achieved for the *C. elegans* project (including computer software) are directly applicable to analyzing the genomes of other organisms, but the *C. elegans* paradigm is more expansive than just that. For example, the need to couple a large-scale genomic sequencing effort with a genome map of the organism in question is strongly argued by Waterston & Sulston (62). The lessons learned in determining the *C. elegans* sequence (16) need not be relearned for other genome projects.

THE *C. ELEGANS* GENOME

At the time of writing (November 1998), the sequencing of the 97-megabase (mbp) *C. elegans* genome is essentially complete (16), with an estimated 98% of the sequence available at the genome center databases (Table 1). Although deciphering the entire information content will likely be a long-term and on-going process, a remarkable amount has already be discerned. A key tool for locating genes is the program GENEFINDER (P Green & L Hillier, unpublished). Briefly, this algorithm exploits statistical criteria to recognize the structural makeup of genes in the raw sequence data [splice sites, open reading frames, etc; see (16) for more details], and has revealed an estimated 19,141 protein-coding genes. The likelihood of the predictions being correct is strengthened by finding homology with proteins from other organisms, and nearly 50% of the predicted proteins show an informative similarity to proteins in the public databanks to the extent that some functional information can be inferred (16). Additional confidence is provided by identifying *C. elegans* cDNA sequences corresponding to predicted exons, which thus confirm that the postulated genes indeed are transcribed. Approximately 40% of the predicted genes encode one of the more than 75,000 cDNA sequences obtained by single-pass sequencing, which are termed ESTs (expressed sequence

tags). The 60% of the genes for which a corresponding EST remains to be identified are presumably expressed in a temporally or spatially restricted fashion such that their transcripts are rarely cloned. This is dramatically seen for the 650-member, "7 transmembrane G-protein coupled receptor" (7-TMG) family of genes (16), which are transcribed at such low levels as to be almost absent in the current EST databases. Also interesting are wide variations between chromosomal regions from which ESTs have been derived. For example, of the predicted genes on the right arm of chromosome V, only 19.5% are currently described by an EST, whereas on the left arm of chromosome I, 57% of genes encode an EST. It is not clear whether this is because the genome has become organized with respect to the transcriptional level of genes, or by the more random expansion of certain gene families; chromosome V does have a large number of 7-TMG clusters.

Although GENEFINDER can identify putative genes, additional analyses using protein alignment tools such as PFAM (51) provide better predictions about function by aligning function-domains within proteins. Examination of the relative frequency of function-domains in *C. elegans*, deduced from PFAM alignments (16), shows that the genome has a higher proportion of proteins involved in transcriptional control, regulatory proteins such as kinases and phosphatases, and proteins involved in cell-cell signaling than is observed in the genome of the single-celled eukaryote yeast (13). Of the genes that are found in clusters and therefore proposed to have arisen through recent duplication events, the 7-TMGs are most prominent, which suggests that this is the most rapidly evolving family in *C. elegans*. This is perhaps not surprising as many of these genes are likely to encode chemoreceptors (58). By specifying components of the machinery for environmental perception, it is likely that the 7-TMG proteins will be involved in the role of *C. elegans* as a predator and in the avoidance of being preyed upon itself; the evolution of this family might play an important role in the ability of *C. elegans* to invade new niches.

EXPLOITING *C. ELEGANS* FOR GENE DISCOVERY IN PARASITIC NEMATODES

EST Projects

A fruitful mode of investigation will be to identify genes of interest in other nematodes and then identify the homologue in *C. elegans*. Subsequent study of the gene can take place within the more genetically amenable organism. The most rapid approach for gene discovery initially is through sequencing ESTs, although as demonstrated for *C. elegans* (see above), rarely expressed transcripts may not be present in the cDNA clones selected. Additionally, unless methods are employed to normalize the cDNA libraries used, many genes will become overrepresented in the resulting EST data. Despite these shortcomings, this strategy has been brought to bear on a number of parasitic nematode species. Table 2 lists the ESTs registered

TABLE 2 Number of ESTs registered in the Genbank dbest database from nematodes other than those in the genus *Caenorhabditis*

Nematode species	Number of ESTs
<i>Dictyocaulus viviparus</i>	2
<i>Angiostrongylus cantonensis</i>	3
<i>Trichostrongylus vitrinus</i>	9
<i>Meloidogyne javanica</i>	22
<i>Strongyloides stercoralis</i>	57
<i>Wuchereria bancrofti</i>	125
<i>Toxocara canis</i>	516
<i>Pristionchus pacificus</i>	703
<i>Onchocerca volvulus</i>	3988
<i>Brugia malayi</i>	16,515

in the Genbank dbest database from nematodes other than those in the genus *Caenorhabditis*, and there are some additional cDNA sequences from parasitic nematodes in other public databases. Clearly, the largest data set to date has been obtained by the Filarial Nematode Genome Project (Table 1) for *Brugia malayi* (a causative agent of elephantiasis) (5).

Obtaining large numbers of EST sequences is a technically straightforward process, primarily constrained by the cost of DNA sequencing. However, making sense of the sequences (i.e. actually identifying genes) requires a set of well-characterized genes for comparison, and the fact that the genome of *C. elegans* is now essentially complete greatly enhances our ability to utilize ESTs from other nematodes. To examine the ability to utilize ESTs derived from *Brugia* to determine putative homologues in *C. elegans*, we searched 5555 random *Brugia* EST sequences against the database of 19,099 predicted *C. elegans* proteins (including products from alternatively spliced genes) using the WUBLASTX program. Despite the wide range of scores (Table 3), the deduced proteins from 86% of the ESTs (4791) showed a match with the *C. elegans* proteins, which confirms the utility of the *C. elegans* sequence as a tool for brute-force gene discovery in parasitic nematodes.

Without the complete genome sequence the crucial step of homologue identification was often problematic, and somewhat arbitrary decisions were required in assessment of the match between the EST and the putative *C. elegans* homologue. This was especially true for matches defined by low BLAST values. One obfuscating factor is that ESTs represent only segments of the entire transcript and, as such, may encode regions of the protein not under strong selection pressure. Also, EST sequences are error prone, frequently leading to frameshifts in the deduced protein sequences, which reduce the potential aligned score. Therefore, in many

TABLE 3 Homologies revealed by searching 5555 random *Brugia malayi* ESTs against the entire *C. elegans* protein complement using WUBLASTX

BLAST score	Number of <i>Brugia</i> ESTs matching <i>C. elegans</i>
>500	138
475–500	43
450–475	45
425–450	41
400–425	57
375–400	87
350–375	78
325–350	92
300–325	96
275–300	80
250–275	128
225–250	103
200–225	147
175–200	132
150–175	165
125–150	173
100–125	176
75–100	290
50–75	1571
<50	1149

cases it was difficult to determine whether a true homologue had been determined or a stronger match remained undiscovered and unsequenced in the genome. With the availability of the complete *C. elegans* genomic sequence, this last concern is negated; the most likely candidate for a *C. elegans* homologue simply becomes the protein providing the best match for the *B. malayi* EST.

It should also be noted that although 14% of the *Brugia* ESTs failed to match the *C. elegans* protein set, this does not necessarily indicate the lack of a homologue. The *C. elegans* protein set was derived from the incomplete genome (95 mbp) and up to 5% of the total protein set may not be represented. It is also possible that the portion of the protein represented by the EST has evolved/drifted rapidly and any similarity is no longer detectable using WuBLAST, or that the EST sequence derives from either a 3' or 5' untranslated region and so no similarity would be

expected. Nevertheless, parasitic nematodes will have genes with no clear analogue in *C. elegans* (i.e. a gene encoding an equivalent function) and, indeed, because such genes are candidates for encoding functions associated with specific adaptation to parasitism, they might be of most interest to parasitologists. The endoglucanase genes (presumed to encode cellulases) identified in plant-parasitic nematodes (50) are a good example. Using the deduced ENG-1 protein from *Globodera rostochiensis* as a query does identify two candidates from the entire *C. elegans* protein set (with BLAST scores of 55 and 46), but examination of these sequences indicates that neither encodes a cellulase nor a related protein. Indeed, based on the similarity of the nematode cellulases to those from bacteria, it has been proposed that, although examination of the genes revealed typical eukaryotic structure, they were acquired from bacteria via horizontal gene transfer (67).

Large data sets from other nematodes will permit the number of nematode-specific genes to be accurately determined, and one of the obvious goals of parasite genomics will be identification of the subset of nematode-specific sequences that reflect unique adaptations to exploit particular parasitic niches. PFAM analysis of the deduced *C. elegans* protein set (WormPep, release 14) by one of us (SJM Jones) revealed approximately 400 apparently nematode-specific protein domains, and to date more than half of the entire *C. elegans* genetic complement appears to be nematode-specific. Some of these are clearly part of the unique biology of nematodes (4), although some of these domains will be shared with non-nematodes, and will be revealed when more sequences are available or when comparisons are made at the level of protein tertiary structure. Eddy (21) provides a useful example of avoiding misidentification of a domain as being nematode-specific, emphasizing the point that our ability to generate raw data can easily outpace our ability to appreciate the biological relevance.

Conserved Synteny

One of the most useful features of the *C. elegans* sequencing project is that it is a genomic sequence, and thus it identifies the position of each gene along each of the six chromosomes. Further, a remarkably powerful suite of genetic tools has been developed for *C. elegans* and a densely marked linkage map constructed (31). Although EST projects define genes, they provide no information about location. However, the *C. elegans* genetic map and the physical map (i.e. the sequence) can be exploited to study parasitic nematodes by exploiting conserved synteny, as long as a physical and/or genetic map has been developed for the parasite in question (see below). Conserved synteny describes the colinearity of homologous loci between different species. The shorter the evolutionary distance between species, the lower the likelihood that linkage disruptions will have occurred to break synteny for any given genes. Thus, as they are separated by only 40–60 million years, it is not surprising that *C. elegans* and *C. briggsae* exhibit a high degree of synteny.

To date, more than 6 million nucleotides of *C. briggsae* genomic sequence have been determined (M Marra, personal communication), and this sequence can be searched using the BLAST server available at the St. Louis Genome Sequencing Center (Table 1). We have examined one region of over 70 kilobases of *C. briggsae* genomic sequence, in which 19 genes are discernible. All but one of these genes have homologues in *C. elegans*, and all of these homologues lie on two overlapping cosmids in *C. elegans*. The chromosomal order of these 18 genes is conserved between the two species, as is their orientation. The one *C. briggsae* gene that lacks a homologue appears to have been inserted between two other genes without disruption of the flanking sequence.

Conservation of synteny within *Caenorhabditis* has been exploited in gene cloning experiments [e.g. (2)], and provides a powerful tool for the investigation of potential gene regulatory regions in the gene under examination. Such sites may well be conserved in other nematodes. However, a more utilitarian use for synteny may be to permit predictions to be made about gene position in parasites based on 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. To give a hypothetical example, consider a parasite gene involved in environmental sensing (e.g. a 7-TMG gene). Such a gene might have evolved to respond to specific chemical cues, thus diverging to such a degree that DNA hybridization with its *C. elegans* homologue can no longer be experimentally observed. However, it might be possible to identify a nearby gene (in *C. elegans*) that has not diverged as much (e.g. encoding a metabolic enzyme), and which could be used to isolate its homologue from the parasitic species; if synteny is conserved in that region, then sequencing the region around the parasite's metabolic enzyme gene should reveal the parasite 7-TMG gene. Further, just as evolutionary relationships can be reconstructed by comparing the DNA sequences 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.

Currently, the amount of synteny between *C. elegans* genes and those of plant-parasitic nematodes is difficult to estimate. Blaxter's group (4) has examined a 65-kb genomic region from *B. malayi* and found conserved synteny with *C. elegans*. *Brugia*, a spirurid nematode, appears to be evolutionarily more diverged from *C. elegans* than do the Tylenchida (6), which includes both the root-knot (*Meloidogyne*) and cyst (*Heterodera* and *Globodera*) genera of plant parasites. Indirectly, this implies that substantial synteny might be found between the tylenchids and the rhabditids. At least consistent with this hope are findings of conserved gene order and orientation across hundreds of millions of years since vertebrate diversification (23). Although the fossil record for nematodes is poor (42), it would not be surprising if plant-parasitic nematodes coevolved with their terrestrial hosts, which were established by 400 MYA. Assuming the same evolutionary rate of globins in nematodes as in mammals, molecular phylogenetic analysis points to divergence of the rhabditids from the tylenchids at between 500

and 350 MYA (6, 59). It seems that the vertebrates shared a common ancestor at approximately the same time as did the tylenchids and *C. elegans*, and we are thus hopeful that the latter will exhibit a degree of conservation of synteny similar to that shown by the former (23).

An obvious prerequisite for establishing long-range synteny between *C. elegans* and a parasite is information about gene order in the parasite, i.e. a map. Constructing a physical map of the parasite of interest (the ultimate form being an entire genomic sequence) is sufficient to identify and exploit whatever degree of conserved synteny that might exist. Construction of a genetic map, on the other hand, permits the identification of parasitism traits based on phenotype. The appeal of a genetic approach is that identification of genes is not predicated on any a priori knowledge of their function. This strategy is thus complementary to the strictly DNA sequence-based approaches outlined above.

PARASITE GENETICS

There are several approaches to identifying genes potentially involved in nematode parasitic abilities. For example, as mentioned above, putative cellulase genes have recently been isolated from several plant-parasitic nematodes (50). However, the function of the cellulases in the nematode-plant interaction is not yet known. Although plausible models for the role of these enzymes in the parasitic interaction can be envisaged, merely demonstrating that a protein is present at a particular stage of the life cycle of an obligate parasite is insufficient proof that it is involved in a parasitism function; these genes were isolated by screening an expression library with an antibody directed to nematode secretions. Even demonstration that ablation of the putative parasitism gene (e.g. in a transgenic nematode) abolishes the parasitic interaction is insufficient proof that the gene in question truly is a parasitism gene. 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 & Baillie (34) estimate that from 15% to 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, because the functions encoded by these genes appear to be dispensable for reproduction per se, they are not classified as essential. However, this assignment is, to a large degree, an artifact of the way *C. elegans* is maintained in the laboratory. For example, based on genetic analysis, the second largest class of genes in *C. elegans* is that in which mutation gives an uncoordinated (Unc) phenotype. Because coordinated 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 nonessential. In contrast, the equivalent genes (and many others as well) are almost certainly essential for plant-parasitic nematodes. For these obligate parasites to reproduce, they must locate a host, invade, and select and

establish a feeding site, events that certainly require coordinated movement and behavior.

The most effective approach to understanding parasitic behavior in nematodes is through genetics. Genetic approaches are extremely powerful because they do not rely upon preconceived assumptions about how something might work. Instead, they make use of observations regarding the important traits or behaviors. To date, most progress on the genetics of parasitism in nematodes has been made in plant-parasitic species, particularly *Globodera rostochiensis* (47) and *Heterodera glycines* (19, 41). This is partly because these nematodes are dioecious, obligate amphimictic 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 classical genetics. During our development over the past ten years of the genetics of the obligate plant-parasitic nematode *H. glycines* (soybean cyst nematode: SCN), *C. elegans* has not only served as a model nematode, but the *C. elegans* system has been used as a model to develop SCN as a model parasite.

A parasite must reproduce to successfully complete its life cycle. As such, the ability of an *H. glycines* individual to parasitize a soybean plant is measured by reproduction. In general, resistant hosts do not permit the female nematode to develop to reproductive maturity. Parasitism is a qualitative trait that the individual nematode either possesses or does not. In addition, nematode populations may be 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. Race designation of a field population is based upon the prevalent phenotype in the population. Selection pressure from growing resistant cultivars often alters the frequency of alleles in the population for reproducing on a resistant host.

Importantly, it has proven possible to score for parasitism traits that enable particular nematode genotypes to evade host defense responses. In the case of the *G. rostochiensis*–potato interaction, nematode genes for parasitism are recessive. Potatoes carrying the dominant H1 gene are resistant to certain pathotypes of *G. rostochiensis*, but those nematodes carrying recessive parasitism genes can reproduce. 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 (32, 33). Results from reciprocal crosses suggested that there is no evidence for sex-linked inheritance of parasitism. The expected segregation patterns of 3:1 nonparasitic to parasitic combined with the dominant nature of the H1 resistance gene suggest that this interaction functions in a classical gene-for-gene type of mechanism (33).

Research on the genetic basis of parasitism in *H. glycines* is complicated by several factors. Results from population measurements usually are biased by genetic variability among and within *H. glycines* populations, and the frequency of a certain gene for parasitism (nematode genes necessary to overcome host resistance) may affect phenotypic designation of either parasitism or the levels of

reproduction (18, 35, 41). Single-pair mating and F1 hybrid host range tests of *H. glycines* populations suggested that the parasitism gene(s) in these populations of races 2 and 4 were partially dominant to the parasitism gene(s) in races 1 and 3. Single cyst selection and inbreeding on a resistant host for many generations indicated that this nematode would tolerate concurrent selection and inbreeding (20). Secondary selection of these inbred lines on a different resistant host resulted in suppressed cyst development on the previous selection host, which suggests that alleles of parasitism genes exist for some hosts (36, 37). However, other studies demonstrated that continuous selection of *H. glycines* on a resistant soybean increased frequency of parasitism genes in that group, but the frequencies of parasitism genes in other groups were not affected, which suggested that the parasitism genes in the PI88788 and PI90763 host groups are not allelic, but are independent loci (57). Reciprocal crosses between field populations indicated that the parasitism genes were not sex linked in the progeny (57).

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 (19). 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. These loci were designated *ror* for reproduction on a resistant host (19). 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(kr5)*, controls the ability to parasitize the cultivar '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 reveals that the presence of two genes results in twice as many females being formed on PI88788 as when only one gene 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; i.e. no novel host ranges are detected when combinations of *ror* genes are present in a particular nematode line.

This classical genetic approach can be applied to identify any parasitism genes for which a phenotype can be observed. For example, SCN lines able to reproduce on tomato have been selected; identifying the genes controlling this host acquisition would be an especially interesting subject of genetic analysis.

Linkage and Physical Genetic Mapping

Classical 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. During the genetic analysis of *H. glycines* *ror* genes, several groups of mapping lines were constructed and approximately 1600 progeny lines derived from our mapping crosses. We have subjected these lines to several molecular mapping approaches, including RAPD-PCR bulk-segregant analysis and AFLP- and EST-mapping. We conducted a modified bulk-segregant analysis to detect RAPD markers linked to a dominant parasitism gene of PI88788, *ror-1*, in a recombinant inbred mapping population. We have screened approximately 1000 RAPD 10-mer primers against the 3 SCN inbred parents and the progeny lines; ~400 revealed robust, usable polymorphisms. Based on the data generated to date from these reactions, we constructed a RAPD-linkage map of the *H. glycines* genome. Of particular significance in this result is the identification of two flanking markers linked to one of the PI88788 parasitism loci (*ror-1*). Closer analysis of this locus reveals that one marker is located less than 2.3 map units from *ror-1* (K Dong & CH Opperman, manuscript submitted). This particular marker has homology to a family of transposable elements and has proved to be unstable (CH Opperman, JA Heer, BR Sosinski, HE Burgwynn, M Dhandaydham & T Pedersen, unpublished data), perhaps indicative of active transposition. A genetic map of the *H. glycines* genome was constructed by RAPD marker segregation and linkage analysis (CH Opperman, unpublished data). The map consists of 9 linkage groups spanned by 41 markers (*H. glycines* has 9 chromosomes). A second-generation linkage map using AFLP markers has also been constructed. This map has more than 200 markers over 9 linkage groups, and merger with the RAPD map is under way (JA Heer, HE Burgwynn, BR Sosinski & CH Opperman, unpublished data). In addition, microsatellite analysis has been performed and several families of dinucleotide and trinucleotide microsatellites are being added to the map (BR Sosinski & CH Opperman, unpublished data). Finally, an EST project has been initiated to add cDNA markers to the consensus map.

Although genetic drift may have affected the frequency of a certain allele within each line, the use of multiple lines allowed us to correct for this potential problem. Recombination events that could cause linkage equilibrium within individual lines did not affect our genetic mapping purpose because we used data from groups of lines. Host range test results among these lines combined with marker analysis have provided information to detect markers physically linked to parasitism genes as a prerequisite for map-based cloning of the parasitism gene(s).

A complementary approach is based on a physical map of *H. glycines*, currently under development (JA Heer, BR Sosinski & CH Opperman, unpublished data). BAC and cosmid libraries have been constructed in preparation for development of overlapping physical regions (contigs). The construction of a physical map, and its subsequent merger with the genetic linkage map, will enable rapid mapping and isolation of genes with scorable phenotypes in the absence of a mutagenesis system. As alluded to earlier, the large number of essential genes in an obligate parasite hampers the ability to perform classical mutagenesis. Strategies based on transposon mutagenesis are in their infancy, as we have only recently isolated

transposable elements from SCN (SJ Hogarth, DMcK Bird & CH Opperman, unpublished data). Although gene isolation based solely on a genetic location (i.e. map-based cloning) is quite possible, it may be a formidable task. However, as outlined above, one potentially very powerful use of the physical map will be to identify and exploit conserved synteny with *C. elegans*. The genome size and complexity of SCN is similar to that of *C. elegans* (40), and despite evolutionary distance, it may be possible to find synteny between the two nematode species. 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 physical maps 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* based on 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.

PARASITISM ROLES FOR GENES PRESENT PHYLUM-WIDE

There are several key processes that all parasites must undergo to successfully complete their life cycles. These include hatching in the presence of a potential host, location and penetration of a host, migration to an appropriate feeding site, development to maturity, and reproduction. In addition, all parasites must evade host defense responses. Because all parasites must function in a similar manner, we believe that the basic biological mechanisms used will be conserved between plant- and animal-parasitic nematodes, although the direct host signals and corresponding nematode reactions may be different. The conservation of the basic mechanisms makes these parasites vulnerable to the use of their own biology against them in designing new control methods. Although *C. elegans* is a nematode, it is not a parasitic species. However, many of the traits that make a successful parasite may also be found in free-living nematodes such as *C. elegans*.

C. elegans Pathways in Parasites: An Example

Originally described by Fuchs [cited in (44)] as an adaptation to parasitism, the dauer larva has been best characterized in *C. elegans*, where it serves as an environmentally resistant, dispersal stage (44). An extensive genetic analysis (44) has revealed numerous genes controlling dauer formation (*daf* genes). By testing for epistasis of various pairwise combinations of *daf* genes, Riddle's group (27) has defined a pathway through which environmental signals are perceived and processed into developmental (e.g. dauer entry/exit) and behavioral (e.g. egg-laying) changes, and antigenic switching on the nematode surface. Microscopy of *daf*

mutants and cellular localization of *daf* gene expression (44) have demonstrated that the dauer pathway is primarily a neuronal one, making it an ideal conduit for a rapid response to the environment.

The dauer pathway plays a pivotal role in linking a wide range of developmental and behavioral responses of the nematode to changes in the environment (44), which suggests that rather than being a specialized adaptation to the *C. elegans* lifestyle, the dauer pathway is a fundamental aspect of nematode biology. Indeed, the dauer pathway might have afforded nematodes the adaptability (in real time, as well as in evolutionary terms) that has permitted them to occupy many parasitic niches.

Plant Parasites as Dauers Although facultative in *C. elegans*, the dauer stage is typically the obligate, infective stage for many parasitic nematodes. Importantly, there is no strict, phylum-wide definition of a dauer. Rather, particular developmental stages have historically been described as being dauer larvae if they exhibit a preponderance of dauer characteristics. Although certain features, such as the lateral alae of *C. elegans* dauers, might be highly specific adaptations, there are a suite of attributes that all dauers might be expected to possess, and which can be considered diagnostic. Dauers are developmentally arrested, long-lived, environmentally resistant, and motile. Their metabolism is based on stored lipids, and they are nonfeeding until the cue(s) that signals resumption of development is perceived, at which point their metabolism reverts to one based on the TCA cycle (60). It is important to draw a distinction between merely failing to develop because of lack of food (such as occurs when *C. elegans* eggs are hatched into a simple salt solution) and the genetically programmed, developmental arrest of a true dauer.

Some plant-parasitic nematode species, such as *Anguina agrostis*, clearly form dauers, and these are termed DJ2 (45). However, the J2 stage of other plant-parasitic nematodes, including *Meloidogyne* and *Heterodera* spp., exhibits all the principal characteristics of dauers and also should be considered to be dauer larvae. A convenient assay for the unique dauer cuticle is to measure susceptibility to detergents such as SDS (12). Like *C. elegans* and *A. agrostis* dauers, the J2 of *M. incognita*, *H. glycines*, and *Radopholus similis* were SDS-resistant (DT Kaplan, CH Opperman, unpublished data).

The predominant cue for *C. elegans* dauer exit is a high food-signal to pheromone ratio, and this is presumably the same for root-knot and cyst nematodes. The nature of the food-signal is arcane, but presumably occurs only at the feeding site, and may be the same cue used by the parasite to select a cell from which to establish a feeding site. Initiation of feeding has not been studied in planta, but presumably supervenes the commitment to recover (i.e. to resume development). It is perhaps for this reason (i.e. the necessity to perceive a recovery cue prior to feeding) that attempts to establish ex-planta systems in which sedentary endoparasites are able to feed and develop have been unsuccessful (8).

Other Roles for the Dauer Pathway One key to successful parasitism is the ability of the parasite to couple its development to that of the host. Thus, like

C. elegans, parasitic nematodes need to make developmental decisions based on environmental cues. A role of the dauer pathway in processing such cues has previously been suggested for animal-parasitic nematodes (44), and the same is probably true for plant-parasitic nematodes. Both root-knot and cyst nematodes base developmental decisions on as yet unidentified host signals. Sex determination for parthenogenetic *Meloidogyne* species is based on perception of host status, a character that is conceptually equivalent to the food-signal perceived by *C. elegans*. Similarly, *H. glycines* couples progeny-diapause with host senescence. In both cases, it is likely that the dauer pathway mediates between the host cue and the developmental outcome. Obviously, the chemical nature of those cues will differ from species to species, and indeed such differences may play a central role in determining the host range of any given parasite or species. Understanding such complex phenomena is a major promise of the genomics era.

CONCLUSION

Although predicting the future is risky, genomic approaches will inevitably greatly expand our biological horizons. This is especially true in plant pathology, where many of the organisms have proven difficult to examine by traditional technologies but are amenable to genomics approaches. We have discussed lessons learned from the *C. elegans* genome project, and how *C. elegans* can be used as a model for plant-parasitic nematodes. The development of *H. glycines* as a genetic system will greatly enhance the utility of the model, and permit the direct identification of parasitism genes, which in turn can be modeled in *C. elegans*.

As more sequence data are obtained, our ability to identify parasitism loci solely by bioinformatic approaches will be enhanced, and some of the algorithms developed for gene identification in large DNA sequence data sets, including GENEFINDER and PFAM, have been mentioned above. However, although these programs are very powerful, development of tools to extract information from DNA sequence has clearly lagged behind the technical advances in obtaining that sequence. A perhaps telling example of this is that the most widely used informatics tool for gene discovery, i.e. BLAST, was developed to infer evolutionary relationships between DNA sequences. In addition to improved algorithms for gene discovery, better tools are also needed for displaying, integrating, manipulating, and sharing large amounts of disparate information. Although not very intuitive for the novice user, ACeDB (**a C. elegans database**) is a paradigm for such programs [for a comprehensive description see (22)]. ACeDB displays and links virtually any type of data, including the genetic and physical maps, sequence, microscopy images, bibliographical data, etc, and versions that run on a variety of computer platforms can be obtained electronically (Table 1). Whether ACeDB, (or, more likely, a subsequent iteration of ACeDB), or some entirely novel program emerges as the principal tool for genomic data management, an essential feature must be the open and free exchange of data. Ultimately, the most important lesson

that the *C. elegans* genome project reinforces is that progress on large questions is greatly enhanced by cooperation; “together we can do more” (15).

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