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Caenorhabditis elegans: A Genetic Guide to Parasitic Nematode Biology[1]

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Abstract: The advent of parasite genome sequencing projects, as well as an increase in biology-directed gene discovery, promises to reveal genes encoding many of the key molecules required for nematode-host interactions. However, distinguishing parasitism genes from those merely required for nematode viability remains a substantial challenge. Although this will ultimately require a functional test in the host or parasite, the free-living nematode *Caenorhabditis elegans* can be exploited as a heterologous system to determine function of candidate parasitism genes. Studies of *C. elegans* also have revealed genetic networks, such as the dauer pathway, that may also be important adaptations for parasitism. As a more directed means of identifying parasitism traits, we developed classical genetics for *Heterodera glycines* and have used this approach to map genes conferring host resistance-breaking phenotypes. It is likely that the *C. elegans* and *H. glycines* genomes will be at least partially syntenic, thus permitting predictive physical mapping of *H. glycines* genes of interest.
Key words: *Ancylostoma caninum, Caenorhabditis elegans,* dauer larva, genetics, *Heterodera glycines, Meloidogyne incognita,* molecular nematology, nematode, parasitism, synteny.

It is perhaps ironic that one of the major hurdles faced by biologists today is a surfeit of information. This is especially true for nematologists because the free-living nematode *Caenorhabditis elegans* has become the best-studied metazoan (Riddle et al., 1997; Wood, 1988). A challenge for plant-parasitic nematologists will be to exploit this information, as well as the tools developed by *C. elegans* researchers (Epstein and Shakes, 1995) to answer questions related to parasite biology and function (Blaxter and Bird, 1997). One powerful approach will be to exploit the *C. elegans* genome project (Waterston et al., 1997) as a point of comparison for genes identified in parasitic nematodes, particularly randomly sequenced cDNA clones termed ESTs (expressed sequence tags). However, this is not the only way in which *C. elegans* is immediately useful for studying parasite genes and their biology. In this paper we discuss some of the experimental strategies (based on *C. elegans* data and tools) we have begun or are planning to employ, to ask specific questions about plant-parasitic nematodes and also to reveal more general paradigms of parasitism. Readers are also directed to two recent, complementary discussions of these topics (Bürglin et al., 1998; Opperman and Bird, 1998). Although the use of the terms juvenile, J1, J2, J3, and J4 is specified by *Journal of Nematology* and several other nematological journals, we will use larva, L1, L2, L3, and L4 for *C. elegans* as these terms are prevalent in *C. elegans* literature.

Caenorhabditis elegans Homologues/Analogues of Parasite Genes

For convenience of discussion we use the term "homology" to denote common ancestry and "analogy" to describe genes with equivalent function. Analogy may or may not be derived by homology. DNA- or protein-sequence identity (deduced or determined) between a parasite and *C. elegans* can reflect either homology or analogy. Studying parasite genes with homology to *C. elegans* provides a tool for understanding evolution of parasitism, whereas studying analogous genes enables *C. elegans* to be used as a tool to study parasitic function.

Caenorhabditis elegans analogues of parasite genes: Several algorithms to compare a test sequence with those in the various public-domain databases have been written, includ-

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ing BLAST (Altschul et al., 1990), which is readily accessible via the Internet from remote servers, including those maintained at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). In addition to sequence alignment, the output from a BLAST search includes both a "score" and a "probability" (Altschul et al., 1990), which give an indication of similarity. Nevertheless, despite the power of BLAST for exposing identity between sequences, it is limited in its ability to quantify similarity and certainly gives no clue as to the biological relevance of the identity. However, once sequence similarity has been revealed, the aligned sequences can be subjected to phylogenetic analysis using maximum parsimony (Swofford, 1993) or neighbor-joining (Kumar and Nei, 1994) methods, permitting the construction of a phenogram. In the canonical phenogram depicted in Figure 1A genes or proteins 'a' and 'b' are "orthologous' to each other (as are 'c' and 'd'), whereas 'a' is "paralogous" to 'c' (as to 'b' to 'd'). Depending on the extent of divergence, orthologous genes have a reasonable likelihood of being analogous. As an example, we consider the *asp* genes, first identified in the hookworm *Ancylostoma caninum* (Hawdon et al., 1996).

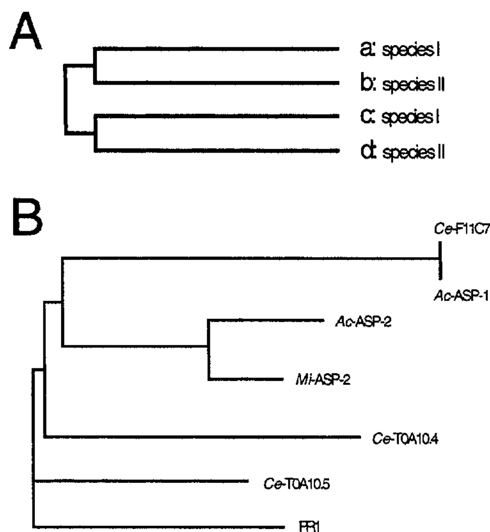

FIG. 1. A) Canonical form of a phenogram determined from an alignment of the nucleic acid or protein sequence of genes a, b, c, and d from two species, I and II. Sequences a and b (and c and d) are orthologous; a and c are paralogous as are b and d. The length of the horizontal lines indicates relatedness. B) Maximum parsimony phenogram of protein sequences deduced from the *Caenorhabditis elegans* cosmids F11C7 and T0A10 (*Ce*-T0A10.4 and *Ce*-T0A10.5), the *Ancylostoma caninum* ASP-1 and ASP-1 genes (*Ac*-ASP-1 and *Ac*-ASP-2), and the *Meloidogyne incognita* ASP-2 gene (*Mi*-ASP-2). The *nicotiana tabacum* PR1 gene was used as an outgroup.

Ancylostoma-secreted protein (ASP): Like many parasitic nematodes, the infective stage of hookworm is a dauer larva, which is specialized for dispersal and long-term survival (Riddle et al., 1987). Resumption of development occurs only following entry into the definitive host. This transition to parasitism, which requires a stimulatory signal from the host, is marked by release of two proteins, ASP-1 (Hawdon et al., 1996) and ASP-2 (J. Hawdon, pers. comm.), that show similarity to the antigen 5/antigen 3 family of proteins from hymenopteran venom. Compounds such as 4,7-phenanthroline that inhibit resumption of larval development (and hence block establishment of the parasitic interaction) also inhibit release of ASP, and targeting the ASPs in a vaccine strategy appears to be a viable control approach. Together, these data suggest that ASPs might occupy a critical and central role in the transition from the external environment to parasitism. If that is the case, then the role played by the ASPs might be generally conserved in parasitic nematodes, and they presumably will have either homologues or analogues (or both) in *C. elegans.*

Meloidogyne ASPs: Using oligonucleotide primers designed from the *Ancylostoma caninum asp* genes, we amplified products from *M. incognita* genomic DNA by PCR (K.-C. Lin and D. Bird, unpubl.). BLAST analysis of the sequenced products revealed identity with the hookworm *asp* sequences, and also with *C. elegans* sequences (Waterston et al., 1997). Deduced protein sequences of *A. caninum asp-1* and *asp-2* (Hawdon et al., 1996; J. Hawdon, pers. comm.), *M. incognita asp-2* (K.-C. Lin and D. Bird, unpubl.), and three *C. elegans* genes with the most identity to the hookworm sequences were manually aligned and subjected to maximum parsimony analysis (Swofford, 1993). This tree (Figure 1B), which describes the relatedness

of the homologous nematode genes, was rooted using the tobacco PR1 sequence as an outgroup. PR1, which shares 33% sequence identity with the *M. incognita asp-2* protein (over more than 100 amino acids), is thought to play a role in the defense reaction against pathogens and belongs to a class that includes mammalian SCP/TPX1, insect AG3/AG5, and fungal SC7/SC14.

A simplified interpretation of the admittedly limited data set shown in the phenogram is that the gene on the *C. elegans* cosmid T0A10.4 is closest to the basal nematode gene from which the *asp* genes may have evolved. ASP-1 and ASP-2 appear to be distinct classes, with the *C. elegans* cosmid F11C7 product being both orthologous and similar to *A. caninum* ASP-1, suggesting that these proteins might have a related function. The lack of an obvious *C. elegans asp-2* ortholog (Fig. 1) implicates ASP-2 as being a parasitism function. Because the *C. elegans* genes T0A10.4 and T0A10.5 are more diverged, it will be interesting to determine their function, particularly as they are adjacent in the genome and appear to be organized in an operon (i.e., with a shared promoter). Even if it is later demonstrated that they are not analogous, the fact that they are homologous might shed some light on the origin of the ASPs as proteins involved in parasitism, and the molecular evolution of parasitism genes.

Establishing analogy: Demonstrating that a parasite gene and a *C. elegans* gene exhibit similar spatial and temporal expression profiles in vivo is consistent with the genes being analogous, although it is not proof. Conversely, different expression patterns suggest that they do not share a common function, although they might indicate an evolutionary origin of the parasitism gene.

The robust *C. elegans* DNA transformation/reporter gene system (Mello and Fire, 1995) permits the expression profile of virtually any *C. elegans* gene to be determined, using the strategy conceptually outlined in Figure 2. Candidate analogues of parasitism genes are identified readily by computer examination of the soon-to-be completed *C. elegans* genomic sequence (Waterston et al.,

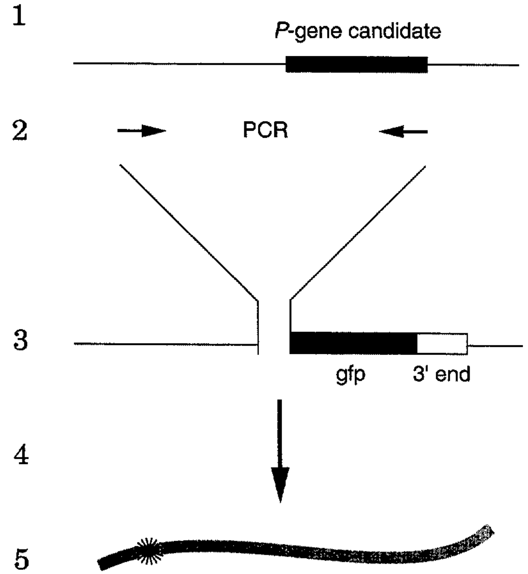

FIG. 2. Strategy to determine the temporal and spatial expression of *Caenorhabditis elegans* genes. Step 1: The DNA sequence of the transcribed region (solid bar) and upstream sequences likely to encompass the promoter (e.g., 1 to 3 kb) of a gene of interest (e.g., with identity to a candidate parasitism gene: *P*-gene) is identified by database analysis. Step 2: Oligonucleotide primers (arrows) are designed and used to amplify the gene and its promoter using the polymerase chain reaction. Step 3: The amplified product is inserted into a promotorless, *C. elegans* expression vector to generate a translational fusion of the *P*-gene with the gene encoding green fluorescent protein (*gfp*) and a generic 3′-end. Step 4: Nematode lines transgenic for the *P*-gene:*gfp* fusion are generated. Step 5: Progeny nematodes are scored for GFP expression (represented as a starburst, localized to one region in this example).

1997; <http://genome.wustl.edu/gsc/gschmpg.html>) and may be obtained from the genome by PCR amplification. Careful design of the amplification primers permits direct insertion of the gene into a promotorless expression vector such that a translational fusion is made with the coding region of a reporter gene, ideally the green fluorescent protein (GFP). Although promoter elements are not necessarily apparent merely by inspection of the DNA sequence (Bird et al., 1997), inclusion of up to several kilobases of 5′-flanking sequence along with as much of the transcribed region as possible (ideally the entire coding region) increases the likelihood of obtaining correct expression from the transgene. Expression of the GFP protein in a transgenic nematode pro-

vides a spatial and temporal mirror of expression of the gene being tested, although there are certainly many subtle points to consider in interpreting the data (Mello and Fire, 1995).

More direct proof of analogy can be obtained by using a cloned parasite gene to rescue a mutation in *C. elegans*. In this approach, the *C. elegans* genetic map is examined to identify a pre-existing mutation in the gene analogous to a cloned parasite gene. The parasite gene is then injected into the mutant *C. elegans* strain and the progeny are scored to see if the transgene has restored the function abolished or altered by the mutation. Despite some obvious exceptions (e.g., involving genes with multiple copies or related family members), this strategy provides compelling evidence that the genes are functionally analogous. Although a mutation in the *Ce*-F11C7*asp-1* ortholog is yet to be identified in a phenotypic screen, a partial deletion has been obtained with a reverse genetics approach and phenotypic analysis of the mutant is in progress (L. Liu, pers. comm.). If this mutation confers a discernible phenotype, it is a candidate for testing analogy with the *M. incognita asp* genes.

Despite the power of the transgenic approaches, even demonstrating mutant rescue using a parasite gene does not guarantee that the biological process (e.g., a behavioral response) will be the same in the parasite as in *C. elegans*. Even if it were found that cloned *Ac-asp-1* could rescue a *C. elegans* mutation with a tantalizing phenotype, and despite the correlation between the transition to parasitism in the *Ancylostoma* life cycle and the secretion of ASPs, determining whether these proteins play a direct role in the parasitic interaction will require a functional test. This is true for any parasitism gene candidate identified by cloning or PCR (e.g., encoding a secreted or nematode-surface protein). The most direct approach is to abolish the postulated parasitism function, either by mutagenesis or reverse genetics, and then to score the parasitic ability of the nematode. Powerful strategies for reverse genetics based on identifying deletions in specific genes (Jansen et al., 1997), as well as transgenic inactivation of gene function by double stranded RNA, termed RNAi for RNA interference (Fire et al., 1998), have been developed for *C. elegans*. Development of a genetic system for *Heterodera glycines* (Dong and Opperman, 1997), coupled with a transformation system (T. Pedersen, D. Bird and C. Opperman, unpubl.), permits these types of tests to be performed for any identifiable parasitism gene candidate. The genetics of *H. glycines* are detailed below.

Distinguishing parasitism genes from essential genes: Genes with products necessary for the nematode to complete its life cycle are termed "essential genes." Although many parasitism genes will be essential genes, the converse is not the case; the majority of essential genes will play no role in parasitism per se. Johnsen and Baillie (1997) estimate that 15–30% of *C. elegans* genes are essential, and this is the largest single class of *C. elegans* gene. Mutations at many other loci can give drastic phenotypes, but, because the functions encoded by these genes appear to be dispensable for reproduction, they are not classified as essential genes. However, this assignment is, 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 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 non-essential. In contrast, the equivalent genes (and many others as well) are almost certainly essential for obligate parasites such as *Heterodera* and *Meloidogyne* spp. For these nematodes to reproduce they must locate a host, invade a root, and select and establish a feeding site, events that certainly require coordinated movement and behavior. Thus, parasitic nematodes are likely to have significantly more essential genes than a free-living nematode such as *C. elegans*. Consequently, merely demonstrating that genetic ablation of a particular gene disrupts the life cycle of a parasite is insufficient proof that the gene encodes a parasitism function. Correct interpretation of genetic ablation experiments

requires an assay that accurately scores disruption of the specific parasitic interaction being tested. Paradoxically, the more one knows about the role played by the parasitism gene candidate being ablated, the more robust can be the assay that one develops. It is in this area that the data gained from experiments in *C. elegans* may prove to be the most useful.

Caenorhabditis elegans Pathways in Parasites

Nematodes exhibit striking, phylum-wide morphological conservation (Bird and Bird, 1991), and it would indeed be surprising if such conservation did not extend to the underlying biochemistry. Extensive genetic analysis (Riddle et al., 1997; Wood, 1988) has permitted the detailed dissection of many of the pathways controlling nematode development and behavior. Two pathways in particular, those controlling sex determination and dauer transformation are of interest to parasitologists because these pathways play key roles in the parasitic life cycle.

The dauer pathway: Originally described by Fuchs (cited in Riddle and Albert, 1997) as an adaptation to parasitism, the dauer larva has been best characterized in *C. elegans,* where it serves as an environmentally resistant, dispersal stage (Riddle and Albert, 1997). An extensive genetic analysis (Riddle and Albert, 1997) has revealed numerous genes controlling dauer formation (*daf* genes). By testing for epistasis of various pairwise combinations of *daf* genes, Riddle and coworkers have defined a pathway through which environmental signals are perceived and processed into developmental (e.g., dauer formation and resumption of development) and behavioral (e.g., egg-laying) changes, and antigenic switching on the nematode surface (Grenache et al., 1996). Microscopy of *daf* mutants and cellular localization of *daf* gene expression (Riddle and Albert, 1997) has 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 (Riddle and Albert, 1997), suggesting that rather than being a specialized adaptation to the *C. elegans* life style, 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 dauer larvae: Although the dauer stage is facultative in *C. elegans,* it is typically the obligate, infective stage for many parasitic nematodes (Riddle and Georgi, 1990). 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 is a suite of attributes that all dauers might be expected to possess and that can be considered diagnostic. Dauers are developmentally arrested, long-lived, environmentally resistant, and mobile. Their metabolism is based on stored lipids, and they are non-feeding until the cues that signal resumption of development are perceived, at which point their metabolism reverts to one based on the TCA cycle (Wadsworth and Riddle, 1989). 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 (Riddle and Bird, 1985). However, the J2 of other plant-parasitic nematodes, including *Meloidogyne* and *Heterodera* spp., exhibit 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 (Cassada and Russell, 1975). Like *C. elegans*

and *A. agrostis* dauers (Riddle and Bird, 1985), the J2 of *M. incognita, H. glycines,* and *Radopholus similis* were found to be SDS-resistant (D. Bird and C. Opperman, unpubl.).

Temporal regulation of dauer formation: Caenorhabditis elegans dauer larvae correspond to an alternative third-stage larva (L3) and represent the canonical developmental stage for a dauer. Thus, compared to *C. elegans,* the J2 dauers of *Meloidogyne* and *Heterodera* spp. reflect precocious dauer development. Conversely, species such as *Bursaphelenchus xylophilus,* which make J4 dauer larvae, are considered retarded in comparison with *C. elegans* (Riddle and Georgi, 1990). Although such changes in developmental timing seem extreme, there are numerous examples in *C. elegans* of genes that control temporal regulation of developmental pathways, including the dauer pathway. These loci are termed "heterochronic" genes (Ambros, 1997) of which a good example is *lin-14.* Excess *lin-14* results in L4 (i.e., retarded) dauers, whereas reduced *lin-14* activity gives precocious L2 dauers (Ambros, 1997; Riddle and Albert, 1997).

Teleologically, it makes sense for a plant parasite to hatch as a dauer (i.e., the environmentally resistant, dispersal stage), and this presumably has been selected by evolution. Given that such heterochronic changes are possible, it might seem evolutionarily more adaptive for the nematode to hatch as a J1 dauer. Put another way, why does the first molt occur in the egg? One answer lies in the ontogeny of the J1. Like the *C. elegans* dauer, the *Meloidogyne/Heterodera* J2 is derived from a molt, whereas the J1 is generated by embryogenesis. This difference might indicate a barrier over which heterochronic regulators of the dauer pathway are unable to act; reprogramming a molt to precociously form a dauer is probably a simple evolutionary task, whereas reprogramming embryogenesis to form a dauer might be more difficult. Thus, the *Meloidogyne/Heterodera* J1 might represent an evolutionary relic that serves no function other than to molt to an obligate dauer.

Entry into dauer stage: Although the precise nature of the molecules involved remains elusive, it is well established that *C. elegans* integrates the environmental cues of nematode-produced pheromone, food signal, and temperature to control entry into, and exit from, the dauer stage (Golden and Riddle, 1984). These cues permit individual nematodes to predictively assess whether sufficient resources are and will remain available to complete the next life cycle. The obligate formation of a dauer stage by root-knot and cyst nematodes implies that either the entry cue is constitutively provided or that there is no a priori need for an entry cue per se.

Based on data from laser ablation experiments in *C. elegans* (Bargman and Horvitz, 1991), it can be argued that the amphidial neurons ADF, ASG, ASI, and ASJ function to suppress the dauer pathway, although it is now clear that the neuronal responses are very complex (Riddle and Albert, 1997). This model, in which chemosensory input is required to override dauer development, is at least consistent with the notion that the dauer pathway is the default nematode pathway, and so perhaps the need for an entry cue is a special adaptation employed by *C. elegans* (and presumably other nematodes with similar life histories). Alternatively, if an entry cue is obligatory, then species that are obligate dauers may have adaptations to ensure that the correct cue is available. In *C. elegans,* all stages secrete dauer pheromone, but the decision to initiate dauer development is made prior to the L1 molt. As noted above, this molt occurs in the egg for root-knot and cyst nematode species, a condition that would likely ensure a low food-signal-to-pheromone ratio (if, indeed, a dauer pheromone is produced), which is the predominant cue for *C. elegans* dauer formation. Thus, hatching as a J2 might be an adaptation to ensure that the correct dauer-promoting cue is available.

Exit from dauer stage: The predominant cue for *C. elegans* dauer exit is a high food-signal: pheromone ratio, and this is presumably the same for root-knot and cyst nematodes. For plant parasites, 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 for establishment of a feeding site. Initiation of feeding has not been studied in planta, but presumably it 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 (Bolla, 1987).

Other roles for the dauer pathway: One of the keys 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 for the dauer pathway in processing such cues has previously been suggested for animal-parasitic nematodes (Riddle and Albert, 1997), 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* spp. 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.

Genetic Analysis of Parasitic Nematodes

We have outlined the necessity of a genetic approach to reveal parasitism genes and to confirm the function of parasitism gene candidates, and discussed the utility of *C. elegans* as a surrogate to examine parasite gene function. To date, most progress on the genetics of parasitism in nematodes has been made in plant-parasitic species, particularly *Globodera rostochiensis* (Rouppe van der Voort et al., 1994) and *Heterodera glycines* (Dong and Opperman, 1997). This is partly because these nematodes are dioecious, obligately 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 to perform parasite genetics. Importantly, it has proven possible to score for parasitism traits that do not encode essential functions.

Heterodera glycines genetics: For the data to be interpretable, it is essential that genetically homogeneous lines are used as the parents for genetic crosses. *Heterodera glycines* lines have been established in which the many polymorphisms found in field isolates have been fixed as homozygous alleles (Esbenshade and Triantaphyllou, 1988). Dong and Opperman (1997) subsequently developed these lines by single-cyst descent inbreeding, and designed a crossing strategy that permitted the construction of a linkage map for *H. glycines* (Dong and Opperman, 1997; C. Opperman, unpubl.). This map currently has 239 markers mapped to 9 linkage groups, which correspond to the 9 chromosomes. Through the crosses, genes that control host-resistance breaking were identified and mapped (Dong and Opperman, 1997; C. Opperman, unpubl.). These genes, which are identified by phenotype, are named *ror* for reproduction on a resistant host (Bird and Riddle, 1994), define bona fide parasitism functions, and are the targets of an intensive cloning effort.

The genetic strategy developed for *H. glycines* (Dong and Opperman, 1997) will permit the mapping and, hence, isolation of any genes that exhibit a scorable phenotype, including developmental, behavioral, and parasitism traits. Accordingly, this approach will prove to be a powerful tool to characterize many aspects of the host-parasite interaction. However, even with the molecular tools currently under development, including a physical map of *H. glycines* (D. Bird and C. Opperman, unpubl.), gene isolation based solely on a genetic location (i.e., map-based cloning) is a formidable task. Construction of transgenic nematodes has been discussed previously and will undoubtedly

prove valuable for the cloning of dominant genes, or the transgenic complementation of recessive mutations. Strategies based on transposon tagging can be conceived but, although we have isolated several candidate transposons from *H. glycines* (S. Hogarth, D. Bird, and C. Opperman, unpubl.), a viable mutagenesis system remains to be developed. However, one tool that might readily be exploited is that of conserved synteny.

Conserved synteny describes the co-linearity 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, it is not surprising that *C. elegans* and *C. briggsae* exhibit a high degree of conserved synteny, and this has been exploited in gene-cloning experiments (Bird et al., 1997). However, synteny also is strikingly conserved across wider evolutionary distances. Rice, maize, and wheat, species believed to share a monophyletic origin more than 70 million years ago, exhibit sufficient co-linearity to permit the reconstruction of a hypothetical, ancestral grass genome (Bennetzen and Freeling, 1997), and significant blocks of synteny have been conserved during the 400 million years since vertebrate diversification (Elgar et al., 1996). Unlike vertebrates and plants, the fossil record for nematodes is poor (Poinar, 1983), but it would not be surprising if terrestrial plant-parasitic nematodes co-evolved with their terrestrial hosts, which were well established as early as 400 million years ago. Based on molecular phylogenetic analysis of small subunit ribosomal RNA genes, Blaxter et al. (1998) placed tylenchids and rhabditids as sister taxa in a well-supported "rhabditid" radiation. If one assumes the same evolutionary rate of globins in nematodes as in mammals (Vanfleteren et al., 1994; M. Blaxter, pers. comm.), then divergence of the rhabditids from the tylenchids is estimated at between 500 and 350 million years ago, a value consistent with that proposed by Poinar (1983). Although the dates should not be overemphasized, it seems that the vertebrates shared a common ancestor at approximately the same time as did *H. glycines* 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 (Elgar et al., 1996).

Exploiting conserved synteny: 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 we are developing for *H. glycines* will permit these types of analyses to be performed. However, our primary 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 valuable in identifying 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 gene in the dauer pathway). 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 *H. glycines;* if synteny is conserved in that region, then sequencing the region around the *H. glycines* metabolic enzyme gene should reveal the *H. glycines daf* gene.

Other types of predictive mapping, chromosome walking, and gene discovery can be based on exploiting conserved synteny, even if the degree of conservation is low. For nematologists studying parasitic forms, the major utility of the *C. elegans* physical map and genome project might not be the gene sequences per se but rather information about gene order.

Conclusion

In establishing the field of *C. elegans* research, Brenner (1988) emphasized the

need for model systems, and indeed *C. elegans* has become the preeminent model for understanding many aspects of metazoan biology, development, and behavior (Riddle et al., 1997). However, *C. elegans* is not a parasite. Although, as we have discussed here, *C. elegans* can serve as a tool to study the biology and evolution of parasitism, ultimately the research needs to focus on parasites per se. As we have endeavored to argue, we anticipate that the basic mechanisms of parasitism will be largely conserved, even between parasites with seemingly very different hosts (e.g., plants and animals). If this assumption is true, then there will be great utility in studying a model, particularly one that is amenable to genetic analysis. We have developed *H. glycines* as a model parasitic nematode, and will continue to emphasize a genetic approach toward understanding parasitism.

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