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Life cycle variation and the genetic structure of nematode populations

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Few data are available on population genetic structure in nematode species, and little of the available data allows direct comparison of the genetic structures of species having different life cycles. Here we use mtDNA sequence data to describe the genetic structure of a heterorhabditid nematode, and compare results to published data on other nematode species. *Heterorhabditis marelatus* is a parasite of soil-dwelling insects. Its life cycle and local ecology should result in small effective population sizes and restricted gene flow. As predicted, *H. marelatus* shows much lower mtDNA diversity within populations and over the species as a whole, and has a much more strongly subdivided population structure, than parasites of mobile vertebrate hosts. From data such as these we can begin to generalize about the effects of life cycle variation on genetic structure in different nematode species.

Keywords: effective size, gene flow, *Heterorhabditis*, mitochondrial DNA, *ND4*.

Introduction

We still know little about the population genetic structure of most parasite species, the exceptions being mostly species of medical or agricultural importance (e.g. Lymbery *et al*., 1990; Tibayrenc *et al*., 1991; Day *et al*., 1992; Anderson *et al*., 1995; Blouin *et al*., 1995; Dybdahl & Lively, 1996; Babiker & Walliker, 1997; Blair *et al*., 1997). This oversight is surprising because data on genetic structure are necessary for understanding important evolutionary processes such as adaptation to host defences, host-race formation, speciation, and the evolution of resistance to drugs or vaccines. Nematodes in particular are a grossly understudied taxon. Even though nematodes are one of the most species-rich, ecologically diverse and economically important taxa, we have information on genetic structure for only a handful of nematode species, and almost all of these are human or agricultural parasites (recently reviewed in Anderson *et al*., 1998). Virtually nothing is known of the genetic structure of any free-living nematode species (including *Caenorhabditis elegans*). Thus, more comparative studies on genetic structure in nematode species are clearly needed. Indeed, Hughes *et al*. (1997) specifically called for more data on nematodes in their recent review of patterns of population differentiation in different taxa.

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Parasitic nematodes display a wide variety of life cycles and life histories. For example, they parasitize almost all groups of plants and animals, and occur in virtually every marine, terrestrial and freshwater habitat. Their breeding system can be obligately or facultatively amphimictic (two distinct sexes), parthenogenetic or hermaphroditic. They range from highly host-specific to undiscriminating, and vary in the presence or absence of free-living stages and intermediate hosts. How this diversity of life cycles influences genetic structure in different nematode species is unknown. We currently have too few comparative data from which to make any but the simplest predictions.

What is needed are comparative studies of the genetic structure of nematode species that differ in key features of the life cycle, using similar sampling designs and the same molecular markers. For example, using mtDNA sequence data Blouin *et al*. (1995) showed that host mobility has a large effect on genetic structure in trichostrongylid species that parasitize different species of ruminants. We see the effect of differences in population size in comparisons between trichostrongylids and *Ascaris* species (Anderson *et al*., 1998). Both have similar life cycles (simple, one-host, obligately outcrossing, with a mobile vertebrate host), but differ by orders of magnitude in population sizes, and correspondingly in levels of both mtDNA and nuclear intron diversity. In contrast, plant parasitic nematodes having a predominantly parthenogenetic mode of reproduction show much lower overall mtDNA diversity than either

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Ascaris or the trichostrongylids (*Meloidogyne* spp.; Hugall *et al.*, 1994). Here we used mtDNA sequence data to describe the genetic structure of a species that parasitizes soil-dwelling insects.

Study species and predictions about genetic structure

Heterorhabditis marelatus is in the family Heterorhabditidae, one of two main families of entomopathogenic nematodes (Gaugler & Kaya, 1990). Entomopathogenic nematodes are obligate parasites of soil-dwelling insects. Infective juveniles (IJs) of these species actively seek insect hosts in soil. After penetrating a host, IJs release a symbiotic gut bacterium (*Photorhabdus* spp.) that rapidly kills the host, usually within 24–48 h. Nematodes reproduce within the cadaver, and large numbers of IJs escape into the soil to seek additional hosts.

Heterorhabditis marelatus occurs along the Pacific coast from the San Francisco Bay area (D. Strong, pers. comm.) to at least southern Washington (personal observation). Populations occur in sandy soils under vegetation, usually behind the dunes of sandy beaches, and up to a few hundred metres inland. On the Pacific coast their habitat is subdivided into what is essentially a linear series of habitat islands separated by stretches of rocky shoreline. Here we refer to the nematodes inhabiting a continuous stretch of suitable habitat (usually a discrete beach) as a population.

Like most nematodes, *Heterorhabditis* have minimal powers of dispersal on their own. Gene flow on a regional scale will depend on the opportunities for nematodes to be transported either in infected hosts, phoretically (by hitching a ride on nonparasitized hosts), or passively through the movement of wind and water. Infective juveniles are susceptible to UV light and to desiccation, so they cannot be exposed to the air for long (Downes & Griffin, 1996; Strong *et al.*, 1996). *Heterorhabditis* are tolerant of salt water, so movement along shore by ocean currents might occur in coastal species like *H. marelatus* (Griffin *et al.*, 1994). Transport in infected insects is possible, but heterorhabditids specialize on buried insects (as opposed to insects walking on the soil surface), and hosts are killed rapidly following infection. Thus, the first prediction is that gene flow is very restricted on a regional scale.

We also predict that *H. marelatus* populations will have small effective sizes, for two reasons. First, on a local scale (a few to tens of metres) the distribution of nematodes is very clumped and patchy, and patches go extinct and are recolonized at high rates (Stuart & Gaugler, 1994; Strong *et al.*, 1996). Secondly, it is likely that each patch consists of very closely related individuals descended from one or a few maternal founders. An infective juvenile that enters a host must reproduce hermaphroditically. Its offspring then mature into separate males and females who reproduce for one or more generations before producing infective juveniles that leave the host. A single infection can produce hundreds of thousands of IJs, and these tiny nematodes cannot move far on their own. Thus, patches probably contain the descendants of one or a few maternal founders. This sort of metapopulation patch structure should result in very small mitochondrial effective sizes within populations (McCauley, 1991; Caballero & Hill, 1992; Harrison & Hastings, 1996).

Therefore, *H. marelatus* should show lower overall genetic diversity, and a more strongly subdivided genetic structure, than obligately outcrossing parasites of mobile vertebrates, such as the trichostrongylids. To test this hypothesis, we used mtDNA sequence data to describe the genetic structure of *H. marelatus* populations along the Pacific coast of California and Oregon, and compared these data to the data from trichostrongylids (for which the same gene and sampling scheme were used, making the two datasets directly comparable).

Materials and methods

To describe population structure in *H. marelatus*, we sequenced 474 bp of the 3′ end of the mitochondrial *ND4* gene. *ND4* codes for a membrane spanning polypeptide of the hydrophobic subunit of NADH dehydrogenase complex I, and has been shown to be an excellent marker for population genetics studies in nematodes (Blouin *et al.*, 1998). We sequenced each of nine or 10 individuals per population, in six populations from coastal Oregon and California (Fig. 1). At each site we collected soil samples from an area spanning several hectares. We baited each soil sample with waxworms (*Galleria mellonella*), and isolated a single first generation hermaphroditic nematode from infected hosts. To avoid sampling related individuals from the same patch of soil, we made sure that no samples were taken any closer than several metres apart, and sequenced only one individual per soil sample. This region of the *ND4* gene was used so that the results could be directly compared to those of Blouin *et al.* (1995), who used the same gene and sample sizes to study the genetic structures of four species of trichostrongylid nematodes that parasitize ruminants in North America. Here the geographical scale over which we sampled *H. marelatus* populations (Oregon and northern California) is about the same as that over which populations of two of the trichostrongylids were sampled (south-eastern U.S. for *Mazamastrongylus odocoilei* and *Haemonchus placei*), and is smaller than

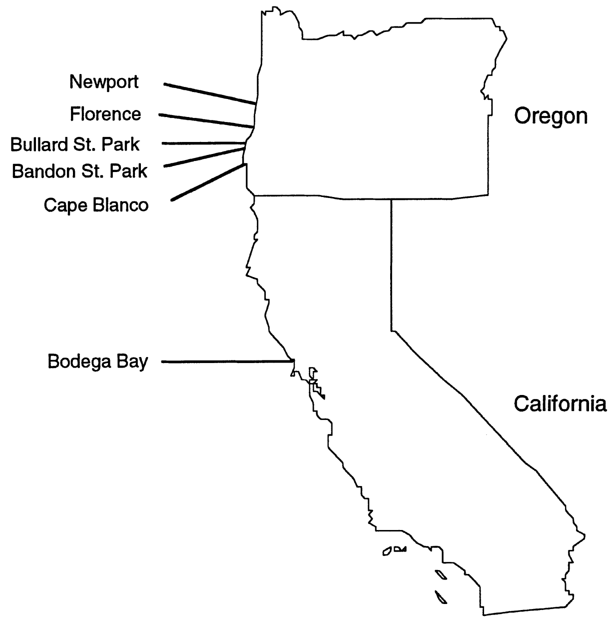

Fig. 1 Sampling sites. Partial *ND4* gene sequences in *Heterorhabditis marelatus* were obtained from nine or 10 nematodes from each site (Table 1).

Table 1 Genetic diversity statistics for the six *Heterorhabditis marelatus* populations sampled

Population	Haplotypes present	Haplotype diversity, H	Nucleotide diversity, π
Newport, OR	9 A, 1 B	0.20	0.0051
Florence, OR	1 A, 9 B	0.20	0.0051
Bullard St.Park, OR	8 B′, 2 B	0.36	0.0007
Bandon St. Park, OR	9 C	0	0
Cape Blanco St. Park, OR	9 B, 1 C	0.20	0.0038
Bodega Bay, CA	9 A	0	0
		F_{ST} = 0.78	N_{ST} = 0.86

'Haplotypes present' shows the numbers of each of the four haplotypes (A, B, B′ and C; see Figs 2 and 3) observed in each population. Haplotype diversity, $H = n(1 - \sum x_i^2)/(n - 1)$, where x_i is the frequency of the ith haplotype and n is the sample size in each population. Nucleotide diversity, π, is the average number of substitutions per site between pairs of haplotypes in each sample. N_{ST} was calculated following Lynch & Crease (1990). $F_{ST} = (H_T - H_S)/H_T$, where H_S is the average haplotype diversity in the populations, and H_T is the haplotype diversity for the sample as a whole (H_T and H_S calculated as for H above).

the scale over which populations of the other two were sampled (entire U.S. for *Haemonchus contortus* and *Teladorsagia circumcincta*).

Individual nematodes were crushed with a pestle in 20 μL of a 5% chelex solution, and incubated overnight at 55°C. Four μL of the supernatant was used to amplify the *ND4* region in a 25-μL PCR reaction (1.5 mM MgCl, 0.3 μM primers, GIBCO Taq and buffer) using a Perkin Elmer 9600 thermocycler (94°C denature for 3 min, then 35 cycles of 94°C for 45 s, 54°C for 1 min, 72°C for 1.5 min, then a 7-min extension at 72°C). The PCR product was then run on a 1% agarose gel, isolated using a Supelco GenElute spin column, and sequenced on an ABI 377 automated sequencer using the PCR primers as sequencing primers. Primers used were:
forward (mb5): 5′-GGC TGG CTT ATT ATT AAA ATT AG-3′
reverse (mb9): 5′-CAA AGA ATA ATA AAA AGA TAC CAA-3′.

Results

Heterorhabditis marelatus shows strong differentiation among populations and low genetic diversity, both within populations and in the species as a whole (Table 1). We found only four distinct haplotypes (labelled A, B, B′ and C) out of 58 sequences in the entire sample (Figs 2 and 3), and at most two haplotypes in any population (Table 1). This diversity was strongly structured, with 86% of the total sequence diversity (N_{ST}; Lynch & Crease, 1990) and 78% of the haplotype diversity (F_{ST}) distributed among populations (Table 1). Even the two closest populations (Bandon, OR, and Bullard, OR, 8 km apart; Fig. 1) had no haplotypes in common, and the only private allele (Slatkin, 1985) in the sample had a frequency of 8/10 in its population. Note also that this most geographically restricted allele (allele B′) also appears to be the most recently derived of the four haplotypes (Fig. 3a), and that it occurs in a population with its parent allele (allele B; Table 1). This pattern is exactly what one expects under restricted gene flow, because the geographical range of a haplotype should be strongly correlated with its age (Templeton *et al.*, 1995). Finally, the distribution of pairwise sequence differences in *H. marelatus* clearly differs from that expected in a single population under drift–mutation equilibrium, there being too few haplotypes, given the distances among them (Fig. 3b; Tajima's $D = 3.12$, $P < 0.01$; Tajima, 1989). Assuming neutrality, this pattern is again consistent with historical subdivision of the species into isolated units.

Discussion

Drift and gene flow in H. marelatus

The above results are all consistent with the small effective population sizes and low rates of gene flow predicted by the life cycle of *H. marelatus*. That there are too few haplotypes given the large genetic distances between them is interesting. A selective sweep cannot be

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A     ACTGTGGGCT TTTTGCGTAT TTTAGGGAGT ATAAATTTTA TTCATAATAA TATCTGGATT
B     .......... .......... ......A... .......... .......... ..........
B'    .......... .......... ......A... .......... .......... ..........
C     .......... .......... ......A... .......... .......... ..........

A     ATTATTGCTT TTTTTGGTAT AATTTTAGGG TCTTTTTGTT GTGTTTTTCA GAGAGATTCT
B     .......... .......... .........A .......... .......... ..........
B'    .......... .......... .........A .......... .......... ..........
C     .......... .......... .......... .......... .......... ..........

A     AAGTCTTTAG CAGCATATTC TTCTGTTACA CACATAAGAT TTTTGTTATT ATCCTTAGTG
B     .......... .......... .......... .......... .......... ..........
B'    .......... .......... .......... .......... .......... ..........
C     .......... .......... .......... .......... .......... ..........

A     TTTATTAGAA TAAGGGGAAA AGTTAGAAGT TTAATACTAA TATTAGCCCA CGGTTATACA
B     .......... .......... .......... ......T... .......T.. .........T
B'    .......... .......... .......... ......T... .......T.. .........T
C     .......... .......... .......... .......... .......... ..........

A     TCTACATTAA TATTTTATTT TATCGGTGAG TATTATCATA CTACATCTAC ACGTATAATT
B     .....G.... .......... ...T...... .......... ....T..... ..........
B'    .....G.... .......... ...T...A.. .......... ....T..... ..........
C     .....G.... .......... ...T...... .......... .......... ..........

A     TATTTTATAA ATAGATTTTT TAGGTCAAGA ATAATTATAG GGATTTTATT TTCTGTAGTA
B     .......... ....G..... .......... .......... .A........ ..........
B'    .......... ....G..... .......... .......... .A........ ..........
C     .......... ....G..... ......G... .......... .......... ..........

A     TTTTTGTCTA ATAGTGGTGT TCCACCATCT TTATCTTTTA TTTCTGAGTT TTTGGTTATT
B     .......... .......... C..G...... .......... .......... ..........
B'    .......... .......... C..G...... .......... .......... ..........
C     .......... .......... C......... .......... .......... ..........

A     AGAAATGGTT TTATTTTGTT CAAGTCTATG TTTTTTTTAT TATTTTTGTA TTTT
B     .......... .......... .......... .......... .......... ....
B'    .......... .......... .......... .......... .......... ....
C     .....C.... .......... .......... .......... .......... ....
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Fig. 2 Alignment of the four *ND4* haplotypes observed in *Heterorhabditis marelatus*. All polymorphisms are in silent sites, except for the substitution in position 268 that separates haplotype B′ from haplotype B.

ruled out, but rapid drift within populations, combined with occasional long-distance gene flow, could produce the same pattern. That two common alleles (A and B) are widespread throughout the species' range, whereas even adjacent populations can be fixed for different alleles, is consistent with this scenario. Perhaps migration occurs in an isolation-by-distance fashion on land, and occasionally over long distances via ocean currents. More intensive sampling of populations throughout the range of the species might well reveal more haplotypes, but the overall pattern of strongly restricted gene flow on a local scale, with widespread common alleles, is unlikely to change.

Practical applications

Heterorhabditis spp. are extensively studied for their biocontrol abilities, and there is great interest in finding new strains that differ in characters such as host-seeking behaviour, environmental tolerance and ability to control different pests (Bedding *et al*., 1983; Kung & Gaugler, 1991; Gaugler *et al*., 1997). Their symbiotic bacteria are equal partners in killing insects, and trait variation in the bacteria may be as or more important than in the nematodes. For example, the toxins produced by *Photorhabdus* spp. are some of the most potent insect killers known, rivalling the well-known *Bacillus thuringensis* toxin, and different species and strains of *Photorhabdus* carry different toxins (Bowen *et al*., 1998). Consequently, there is also a major impetus to search for and characterize new strains of symbiotic bacteria, particularly those adapted to unusual hosts or habitats. Nevertheless, surprisingly little work has been carried out on the basic ecology and genetics of *Heterorhabditis* or their bacteria in nature (Gaugler & Kaya, 1990; Strong *et al*., 1996; Gaugler *et al*., 1997). Ours are the first data on genetic structure in a heterorhabditid, and there has been no population genetic work on *Photorhabdus*. Because the symbiotic bacteria can presumably only disperse in association with their nematode, their population genetic structure should mirror that of the nematode. As part of an unrelated study we recently sequenced 616 bp of the bacterial 16S gene of bacterial isolates from each of five nematodes from Florence, OR, and from five nematodes from Newport, OR (≈80 km apart; Fig. 1) (unpubl. data). Isolates from the two populations were fixed for different 16S rRNA haplotypes. Although these data are anecdotal, they suggest that the nematode and their symbiotic bacterium may both have population genetic structures that promote genetic drift and the opportunity for local adaptation over short distances. Thus it may be fruitful to search for useful new strains of nematode and bacteria (i.e. those adapted to unique hosts or environmental conditions) over very small geographical scales.

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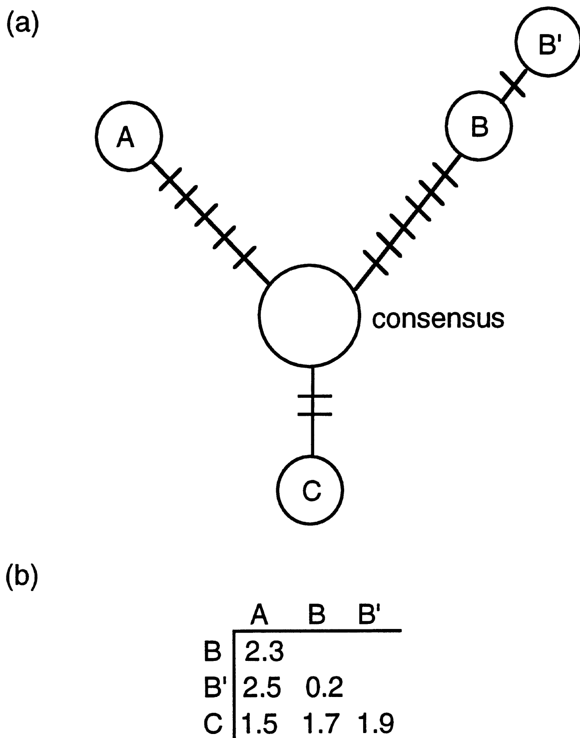

Fig. 3 (a) Network tree showing number of substitutions separating each *Heterorhabditis marelatus* haplotype from the consensus sequence. All substitutions in each branch are unique to that branch. (b) Percentage of sequence difference between haplotypes.

Comparison with other nematode species

Table 2 confirms the prediction that *H. marelatus* has lower overall diversity (both species-wide and within individual populations) and a more strongly subdivided genetic structure than the trichostrongylids. Only four unique haplotypes were found in 58 sequences from *H. marelatus*, whereas samples of 40 trichostrongylid sequences yielded 31–39 unique haplotypes. Within populations the haplotype and nucleotide diversity is almost an order of magnitude greater in the trichostrongylids than in *H. marelatus*, and a much greater proportion of the total sequence diversity is distributed among populations in *H. marelatus*. Because the geographical scale over which *H. marelatus* populations were sampled is smaller than that over which some of the trichostrongylids were sampled, the higher F_{ST} in *H. marelatus* is even more striking. Within each of the four trichostrongylid species Tajima's D is not significantly different from zero, indicating tree topologies that are not significantly different from that expected under neutrality in a single population (see figs 2,3,4,5 in Blouin *et al*., 1995, for haplotype trees). So in these species we do not see the signature of historical subdivision into isolated populations that is apparent in the tree of *H. marelatus* haplotypes.

In the trichostrongylids vs. *H. marelatus* we see two extremes in a spectrum of genetic structures. Trichostrongylids show levels of mtDNA variation that are greater than those typically seen in other taxa, and the species that infect livestock show exceptionally high rates of gene flow over vast geographical areas (Blouin *et al*., 1995; also M. Blouin, S. Richter and E. Hoberg, unpubl. data on *Teladorsagia circumcincta* from Iceland vs. North America; C. Constant, unpubl. data on *Ostertagia ostertagi* from Australia vs. North America). In contrast, *H. marelatus* shows very low variation within populations and in the species as a whole, and very restricted gene flow on a small scale. In these respects the genetic structure of *H. marelatus* may be more similar to that of parthenogens such as *Meloidogyne* spp. than to that of outcrossing parasites

Table 2 Within- and between-population genetic diversity statistics compared between trichostrongylid species and *Heterorhabditis marelatus*. Both studies used the same region of the *ND4* gene and sample sizes of 9–11 individuals per population

			Within-pop. diversity		
Taxon	Total indiv. sampled	Number unique haplotype	Average haplotype diversity	Average nucleotide diversity	N_{ST}
Trichostrongylidae					
Haemonchus contortus	40	37	1.00	0.023	0.01
Haemonchus placei	40	31	0.98	0.019	0.04
Teladorsagia circumcincta	40	39	1.00	0.024	0.02
Mazamastrongylus odocoilei	51	49	0.99	0.028	0.31
Heterorhabditidae					
Heterorhabditis marelatus	58	4	0.16	0.002	0.86

Diversity measures calculated as in Table 1.

of vertebrates. For example, only six unique mtDNA haplotypes were found in 48 *Meloidogyne* individuals sampled from throughout the eastern half of Australia (data from RFLP of entire mtDNA; Hugall *et al.*, 1994). Individual *Meloidogyne* samples were spread over a wide geographical area in that study, so we cannot directly compare levels of within- and between-population diversity in *Meloidogyne* spp. to that in *H. marelatus* or the trichostrongylids. However, a testable prediction is that the distribution of mtDNA diversity within and among populations in *Meloidogyne* will be most similar to that in *Heterorhabditis*.

Here we designed a study to compare the genetic structures of two groups of nematodes, by using the same molecular marker and similar sampling schemes. Obviously more comparative data such as these are needed before we can generalize about the effects of life cycle on genetic structure in nematodes. In particular, we need data on species in 'natural' habitats (i.e. species that are not human associates). To our knowledge, there are no data on genetic structure in any free-living species, and of the parasitic species, only three are not parasites of humans or their domesticated plants or livestock (these include the present data on *H. marelatus*, mtDNA data on *Mazamastrongylus odocoilei*, which is a parasite of deer [Blouin *et al.*, 1995], and allozyme data on Anisakid nematodes of fish and cetaceans [e.g. Paggi *et al.*, 1991; Nascetti *et al.*, 1993;]). Clearly this is a wide-open field of study that deserves more attention.

Acknowledgements

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