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# Forum article

## MOLECULAR SYSTEMATICS AND POPULATION BIOLOGY OF PHYTONEMATODES : SOME UNIFYING PRINCIPLES

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**Summary** – From the perspective of nematologists conducting their research in America, our discipline is receiving diminishing attention and a renewed commitment towards demonstrating the importance and impact of agricultural nematology to our society is urgently necessary. This forum article examines how molecular approaches to nematode systematics and population biology can strengthen the perception of our science through some simple coherence of focus.

**Résumé** – ***Systématique moléculaire et biologie des populations de nématodes phytoparasites : quelques principes unificateurs*** – Du point de vue des nématologistes conduisant leurs recherches en Amérique, notre discipline reçoit une attention décroissante et un engagement renouvelé pour démontrer l'importance et l'impact de la nématologie agricole pour notre société est instamment nécessaire. Cet article éditorial examine comment les approches moléculaires dans la systématique des nématodes et la biologie des populations peuvent consolider la perception de notre science à travers une simple cohérence des points de vue.

**Key-words :** Nematode systematics, population biology, diagnostics.

The assimilation of molecular data into investigation of nematode systematics and population dynamics has escalated dramatically in the last few years. It comes as no surprise that this trend has paralleled the increased accessibility of " user-friendly " DNA analytical techniques, which include molecular cloning, polymerase chain reaction (PCR) amplification methodologies, and DNA sequencing. The application of these techniques to nematological problems has propelled the publication of several useful review articles that specifically target the international nematology community (Hyman, 1990; Curran, 1991; Williamson, 1991; Hyman & Powers, 1991; Powers, 1992; Caswell-Chen *et al.*, 1993). These papers typically discuss the utility of DNA-based characters for diagnostics and phylogenetic studies at all taxonomic levels.

One such thoughtful and timely commentary that appeared in this Forum last year (Ferris, 1994) traveled beyond distilling the current status of biochemical approaches to nematode diagnostics, population biology and phylogenetics. Rather, Ferris alerted us to an alarming concern that confronts everyone involved in studying nematodes : the apparent decline in emphasis on nematode systematics. The problem is an enigmatic one. This decade represents an era of expanded interest in biodiversity and ecology (Wilson, 1992), replete with the understanding that rigorous taxonomic definitions are the foundation that supports the development of reliable diagnostics necessary to deduce relationships among organisms. Yet, the importance of nematodes to agriculture and the reliance of management regimes on sound identification and classification appears to be a lost concept, just at a most propitious time when the biotechnology necessary to address these problems has emerged.

The implicit message to be discovered within " The Future of Nematode Systematics " (Ferris, 1994) and echoed by the Committee on National Needs and Priorities in Agricultural Nematology (Barker *et al.*, 1994) is a renewed commitment towards demonstrating the importance and impact of our discipline on the needs of society. Admittedly, this is a perspective from nematologists conducting their research in the current funding climate of the United States, but evidence of a unified discipline is a worldwide responsibility, necessitating an ever increasing dialogue among nematologists with common interests but trained in different generations. This editorial perspective examines how nematode systematics and population genetics might be employed to strengthen the perception of our science through some simple coherence of focus.

### Nematode systematics : classical *versus* molecular data revisited

Hyman and Powers (1991) and Ferris (1994) briefly review what can be considered a two-tiered history of nematode systematics. Early on, the understanding of taxonomic affinities and the generation of rational, testable phylogenetic hypotheses were often based on morphological characters available for description by light, and occasionally, electron microscopy. These fundamental anatomical and cytological studies comprised an initial phase of nematode systematics and many of

our own academic pedigrees can be traced to the strong foundation provided by these pioneering nematologists.

The application of biochemical methodologies (Hussey, 1979) to taxonomic questions catalyzed a second era of investigation into nematode identification and systematics. In many cases, protein techniques including serology, isozymes, two-dimensional electrophoretic gel patterns, and more recently DNA analyses, have provided support for established taxonomic frameworks. However, in some instances, morphology and molecules do not agree, and neither approach can be used in isolation. The following example underscores this problem.

*Ascaris suum* (an animal pathogen), *Caenorhabditis elegans* (a free-living nematode) and *Meloidogyne incognita* (a phytonematode) represent three different subclasses (Spiruria, Rhabditia and Diplogasteria, respectively) within the Secernentia, one of the two major nematode taxonomic classes. Mitochondrial DNA sequences were compared among these three nematodes and with *Romanomeris culicivorax,* a mermithid parasite of insects that represents the second major nematode class, the Adenophorea. Nucleotide sequence for the large mitochondrial ribosomal RNA (lg-rRNA) gene and both nucleotide and amino acid sequences for the genes encoding the mitochondrial proteins NADH dehydrogenase subunit 3 and cytochrome b derived from these four nematodes were aligned (Powers *et al.,* 1993).

Low similarity scores were generated among these four nematodes with any pairwise alignment involving *M. incognita* or *R. culicivorax*. This result was anticipated, given that the Nematoda is an evolutionarily ancient phylum; subclass divergence within the Secernentia has been depicted in a hypothetical evolutionary tree to have occurred over 550 million years ago (mya) (Poinar, 1983), and a split from the Adenophorea must have pre-dated that event. Unexpectedly, mtDNA comparisons between *C. elegans* and *A. suum* indicate a more recent separation about 65 million years, assuming a mammalian mtDNA molecular clock (Okimoto *et al.,* 1992); an updated estimation of nucleotide substitution rate within the mtDNA of these two lineages (Okimoto *et al.,* 1994) suggests divergence of the subclasses Rhabditia and Spiruria may be even more recent than originally measured.

Here, we are presented with conflicting morphological and molecular data. However, because gene sequences evolve at different rates (Avise, 1994) and the genes sampled in these studies represent but a small portion of the nematodes' DNA, the apparent incongruence between anatomical and biochemical characters may be overstated. Alternatively, the molecular results suggest the following questions : Is subclass divergence within the Secernentian nematodes unequal? Have the ascarids been taxonomically misplaced within the Spiruria? Clearly, a productive dialogue among the traditionally-trained nematode systematists and those adapting molecular tools to address problems in nematode phylogenetics will be required to resolve the different hypotheses supported by these data sets.

## Genetic variability of nematode populations : diagnostics or differentiation?

Microevolutionary patterning, or the genetic structure of nematode populations, remains the least understood aspect of nematode population biology (Caswell & Roberts, 1987). This realization is disappointing given the steady advancements reported for animal parasitic nematodes (Blouin *et al.,* 1992; Dame *et al.,* 1993; Anderson *et al.,* 1993; Nadler, 1996). These studies have revealed significant differences in how conspecific populations of two different nematode parasites of livestock, *Ascaris suum* and *Ostertagia ostertagi,* are structured. Movement with their cattle hosts provides the opportunity for extensive gene flow among populations of both nematodes, which is anticipated to diminish genetic variation between isolates. This appears to be the case for *Ostertagia,* where most of the genetic variability is apportioned within, but not between geographically separated populations. In contrast, *Ascaris* reveals significant, measurable differentiation among sampled isolates. These distinct patterns of genetic architecture structure may be a consequence of the relative effective population sizes estimated for these two nematodes, genotypic selection by varying environmental pressures, or other factors which may influence the extent of genetic drift within and between conspecific populations.

It is essential that the molecular population genetics of phytonematodes becomes elevated to the level of understanding that has been attained for their animal parasite counterparts. Quantitative description of genetic variability among plant pathogenic nematode populations will play a vital role in understanding critical operational phenotypes, and the underlying genotypes, defined by plant nematologists such as sibling species, sub-specific races of *Meloidogyne, Heterodera* and *Ditylenchus* spp., and pathotypes of *Globodera* isolates. Moreover, the selection of suitable management strategies, and the efficacy of these practices, will be directly impacted by the genetic variability within and between targeted nematode populations. It is necessary to understand the genetic substructure that underlies these isolates and address whether management regimes are capable of controlling only a portion of the lineages that contribute to the composition of inbreeding populations.

Until recently, allozyme analysis as a measure of allele frequencies has been the method of choice for geneticists who studied the genetic structure of populations and diversity among the individuals that comprise them. Adoption of this approach to describe the architecture of phytonematode populations has been impeded by the small size of these organisms. In most cases, allozyme analysis proves too insensitive to genotype individual

nematodes. However, in studies that permit analysis of pooled individuals, this approach has proven useful in studies at higher taxonomic levels such as species identification in the genus *Meloidogyne* (Fargette & Braaksma, 1990), when conservation of morphological characters does not permit differentiation.

Contemporary DNA technology, including molecular cloning, polymerase chain reaction (PCR) amplification, and nucleic acid sequencing have largely surmounted the earlier methodological limitations that hindered measurement of genetic variability among nematode isolates (Hyman, 1990; Curran, 1991; Hyman & Powers, 1991; Williamson, 1991; Caswell-Chen *et al.,* 1993). Moreover, DNA analysis has the potential to provide a broader census of genetic variability because protein phenotypes survey only a portion of the genome and because nucleotide substitutions that do not result in allozyme electrophoretic variants would remain undetected.

Although phytonematologists have enthusiastically adapted new molecular techniques to their studies of population and evolutionary genetics, an unfortunate confusion has arisen as to how we define our studies in the context of the data that molecular tools can generate. Often, molecularly-oriented work on plant pathogenic nematodes emphasizes the identification of DNA markers that correlate with species within genera, or with subspecific phenotypes such as races and biotypes. *R*estriction *f*ragment *l*ength *p*olymorphisms (RFLP) and modified PCR procedures that generate *r*andom *a*mplified *p*olymorphic *D*NA (RAPD) markers have become increasingly popular for identifying different nematode isolates. However, the characterization of molecular markers that may discriminate among nematode populations (Powers, 1992) does not necessarily provide a rigorous definition of population sub-structure nor offer a stringent foundation to formulate phylogenetic affinities. In her Forum Article, Ferris (1994) explains that " proper understanding of such [phylogenetic] relationships is... the basis for sound classification and diagnostic procedures ". The opposite circumstance, when diagnostic RFLP-or RAPD-generated molecular markers are employed to infer taxonomic relationships, requires a clear understanding of the available methodological and analytical procedures (Hadrys *et al.,* 1992). One common simplification is the immediate assumption that DNA bands of the same electrophoretic mobility are " shared " characters between taxa. Care must be taken to demonstrate that these bands represent homologous portions of the genomes under comparison so that false affinities are not derived. This practice will ensure that the accumulated data is representative of the organismal genealogies. In addition, it is essential to employ a genetic analysis most appropriate for the form of data under evaluation. For example, algorithms designed to interpret independent nucleotide substitution events (Nei & Li, 1979) may not be directly applicable to deducing phylogenetic affinities from RAPD markers (Clark & Lanigan, 1993).

Unfortunately, the important distinction between " diagnostics " and " genetic differentiation " has become less defined as molecular techniques have been used to develop phytonematode species identifiers. We must continue to define our studies that employ molecular markers with explicitly stated objectives; diagnostics and population genetics are interdependent, but they are not " seamless ».

The genetic structure of populations is typically expressed in quantitative terms by measuring the frequencies of different alleles at targeted loci. Genetic differentiation can be identified by electrophoretic variants of allozymes, RFLP and RAPD DNA bands, or by scoring nucleotide substitutions using DNA sequencing methods. Several statistical methods, first pioneered by the F-statistics of Wright (1922), partition measured allele frequencies within and among populations, thereby revealing population substructure. Such quantitative descriptions have been used to describe the decidely different genetic architectures in the animal parasitic nematode *Ascaris* and *Ostertagia.*

We have been studying a highly polymorphic locus within the *Meloidogyne* (root-knot nematode) mitochondrial genome. The genetic variability of this mitochondrial DNA (mtDNA) segment is not due to nucleotide substitution, the typical " measure " of genetic variability. Rather, small portions of the mtDNA have become duplicated to generate short, repeated regions (Okimoto *et al.,* 1991). Nematodes maintain mtDNA molecules that carry alleles with different copy numbers of these short repeats, termed *v*ariable *n*umber *t*andem *r*epeats (VNTRs). We have employed the computational logic developed by Birky *et al.* (1989) to characterize variability within and among samples of organelle genomes, and have applied this methodology to analyze a size-polymorphic 63 base pair (bp) VNTR represented at different copy numbers within root-knot nematode mtDNA molecules. By employing this approach, mtDNA variation can be apportioned among different levels of population hierarchy, providing a quantitative signature of genetic structure among nematode isolates : variation of *Meloidogyne* mitochondrial genomes can be quantitated within individual nematodes, between individuals within a population and importantly, among conspecific populations.

Based on our studies to date (Whipple & Hyman, 1995), an emergent trend has appeared. Among the populations that we have analyzed, most of the genetic variability at this size-variable mtDNA locus resides within individuals, not within or among populations. Such genetic structuring is similar to what has been observed for *Ostertagia* (Blouin *et al.,* 1992). This observation can be interpreted in several different contexts. In population studies, observed allele frequencies are a consequence of balance between mutation rate (in our

system the changes in VNTR copy number within a mtDNA molecule) and genetic drift. Mutations that change the size of VNTRs will generate new alleles (different 63 bp repeat copy number in the root-knot nematode mitochondrial genome) and increase diversity whereas genetic drift (sorting of mtDNA molecules during germ line cell divisions) will decrease variability. When the mutation rate greatly exceeds fixation of alleles by drift, the spectrum of mtDNA molecules will appear similar because the same collection of size-variable alleles will be present in populations under comparison; this is precisely what we have observed among the *Meloidogyne* isolates currently examined. Interestingly, when Hugall *et al.* (1994) used RFLP analysis as a measure of nucleotide substitution among Australian root-knot nematode isolates, minimal nucleotide sequence divergence among mitochondrial genome haplotypes was also observed among conspecific *Meloidogyne* populations. Together, it appears size-variable mutation rate within the mitochondrial genome may be excessively rapid, while mtDNA nucleotide divergence may be too minimal to provide quantitative signatures for reproductively isolated *Meloidogyne* populations. Beyond that of species diagnostics that typifies much of the current nematode " molecular marker " literature, an expanded quantitative treatment of phytonematode nuclear DNA markers, such as those employed in studies of *Bursaphelenchus* (Beckenbach *et al.*, 1992); *Heterodera* (Caswell-Chen *et al.*, 1992) and *Xiphinema* (Vrain *et al.*, 1992) isolates, will be an essential component of our collective efforts to address the genetic structuring of nematode populations.

## Conclusions

With this brief commentary, I have identified molecular approaches to nematode systematics and population genetics as examples where focusing our priorities is both scientifically sound and strategically essential if we are to present ourselves as a unified, contemporary discipline. When viewed in isolation, molecules alone may not generate a true representation of phylogenetic affinities among nematodes at any taxonomic level. We must overlay the current molecular work upon a rich, descriptive literature provided by traditionally trained nematologists so that rigorous, explicit hypotheses that describe relationships among nematodes can be deduced. Though essential in the development of rational control strategies, molecular markers must go beyond the descriptive, diagnostic stage of development and be exploited in a more quantitative fashion to unravel the genetic structure of nematode populations, including effective population sizes, incidence and extent of gene flow, complex lineage structure within and between isolates, and host-race definitions. Coherence of thought and expression in these and other areas of contemporary nematology can only improve our science, justify continued extramural support, and provide attractive career choices for the coming generations of biologists interested in studying these intriguing organisms.

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