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# VARIATION AT THE MOLECULAR LEVEL OF RENIFORM NEMATODE POPULATIONS IN ALABAMA

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## Abstract

Cotton, a major crop in the world market, is grown mostly for fiber and is subject to many biotic stresses such as nematodes, particularly *Rotylenchulus reniformis*, the reniform nematode. The best method for combating reniform nematodes may be in developing genetic resistance in the crop; however, there are no cultivars of Upland cotton available which are resistant to reniform nematodes. Prior to focusing on biological resistance, it is important to assess molecular and physiological differences which may exist within the species, indicating a possible conflict with resistance strategies. Different populations of reniform nematodes were collected from 6 wide spread counties in Alabama. Nematodes were extracted from soil and DNA was isolated, subjected to PCR, cloning, and sequencing. PCR utilized primers spanning from a ribosomal RNA region – 18S rRNA region. Alignment of clonal sequences showed both inter- and intra-population differences. A phylogram of 18S sequences revealed 3 superclusters, with two being more closely related than the other. Each cluster could not be traced to a single population or region, indicating the genotypes were widespread. The variety of nucleotide base changes was unexpected, especially within a region (18S) considered highly conserved. This is the first report of genomic sequence information of reniform nematode.

## Introduction

Cultivated *Gossypium* spp., known collectively as "cotton," have many uses worldwide. Its seeds provide cottonseed oil used in culinary practices or oilcake residues for livestock as a protein-rich feed. Cotton is a major agricultural product of the U.S.A.; therefore, increasing yields for every acre of cotton planted is a major concern in the cotton industry (Ribera and Landivar, 1999).

Yield is determined by the action and interaction of three basic components – environment, management, and genetic traits. Among these, the environment, including weather, soil, and stresses, is minimally controlled by the grower. These stresses can be abiotic as well as biotic, including pathogens. Nematodes are such pathogens that cause serious harm to quality cotton production. The particular nematode of interest, *Rotylenchulus reniformis* Linford & Oliveira, commonly known as the reniform nematode, is found in 11 states in the southeast and mid-south regions of the U.S (Overstreet, 1999).

In order to combat the problem of reniform nematode infestation in upland cotton, in-depth studies are needed on the presence of different races within the morphologically identical species. If populations of reniform nematodes are uniform, then resistance strategies can be applied to have a widespread impact. However if they prove to be diverse, then resistance strategies developed may only be applicable to localized regions, with different regions being subjected to different strategies. Therefore, a preliminary study of reniform nematode diversity is required in order to proceed to effective management of this problem. This effort concentrates on developing tools and procedures to screen Alabama populations with the expectation that these studies can be expanded and followed by in-depth evaluations in this and other regions.

## Materials and Methods

Collections from Alabama cotton fields were made from nine locations in Alabama. The locations are shown in Table1.

The samples were obtained from both the Tennessee Valley Agricultural Experimental Station, Belle Mina, AL, under the direction of Mr. Charlie Burmester, and the Plant Diagnostics Laboratory through the Extension Services at Auburn University, under the direction of Ms. Kristie Siggers.

Cotton variety DPL-425-Bt/RR (Delta Pine Land Company, Tupelo, MS) plantlets were allowed to grow for two weeks in Pro-Mix soil (Premier Horticulture Inc., Red Hill, PA). A cotton plantlet, after two weeks of growth, was transplanted to nematode-infested soil inside styrofoam cups (12 ounce cups) which were labeled and dated and placed in a greenhouse at

~80°C (day and night). The populations were allowed to grow in the greenhouse for at least 70 days before nematode extraction from the soil. A portion of the extracted sample was used to reinitiate nematode cultures to maintain populations.

Extraction of nematodes was carried out followed by identification and collection of individual adult reniform nematodes (Figure 1). DNA was obtained from the individuals (8 individuals in 25 µL of $ddH_2O$) by rupturing the nematode under a microscope with a dental forcep. DNA was pooled together (250 µL aloquots) and portions were used for PCR amplification according to Harris (2001). After verification of amplified fragments via gel electrophoresis, samples were cloned and plasmid DNA was isolated through a Spin Miniprep Kit (Valencia, CA). Insert was verified to be present by both restriction enzyme digestion and PCR. Clones were then sequenced in forward and reverse directions on an ABI 3100.

## Results and Discussion

Sequences were BLASTed in GenBank to find homologous sequences (Figure 2). Vector sequence was removed and pairwise analysis was carried out after which consensus sequence was found (Table 2). Consensus sequences were aligned using ClustalW and phylograms generated using the embedded PHYLIP software (Figure 3).

Genomic differences were found, not only between different populations from different locations, but even within the populations from the same location. This high variability may be due to high genetic variability between individuals, a paradox since rRNA is considered to be "highly conserved." Another explanation may be that numerous genetically different introductions of reniform nematode were made by farm equipment, soil sampling, runoff, etc., throughout the region, resulting in established diverse populations.

## Conclusions

This is the first report of any sequence information from *Rotylenchulus reniformis*, the reniform nematode.

Reniform nematode population variation, not apparent from PCR bands, was only detected by sequencing and SNP analysis.

Reniform nematode populations, based on 18S rRNA, were highly variable within and between locations in Alabama.

Sequences from 18S rRNA fell within 3 superclusters, two of which were more closely related than with the third, possibly indicating rRNA "families" of sequence.

## Acknowledgements

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## References

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Overstreet, C. 1999. Reniform Nematode- An Introduction. In 1999 Proceedings of the Beltwide Conference. Vol. 1:42-42. National Cotton Council, Memphis, TN.

Ribera, L.A. and Landivar, J.A. 1999. Economic feasibility of variable rate application systems for cotton. In 1999 Proceedings of the Beltwide Cotton Conference 1:247-248. National Cotton Council, Memphis, TN.

Table 1. Nematode population sampling sites.

| County | Farm | Designation |
|---|---|---|
| Limestone | Unknown | LL |
| Limestone | Anderson | AL |
| Lawrence | Haney | LH |
| Lawrence | Posey | LP |
| Morgan | Unknown | M or LC |
| Morgan | Collins | MC |
| Escambia | Unknown | EC |
| Autauga | Unknown | AC |
| Colbert | Underwood | CU |

Table 2. Number of clones with sequence per nematode population sampling site.

| County | Farm | Designation | # of 18S Clones |
|---|---|---|---|
| Limestone | Unknown | LL | 1 |
| Limestone | Anderson | AL | 3 |
| Lawrence | Haney | LH | 11 |
| Lawrence | Posey | LP | 11 |
| Morgan | Unknown | M or LC | 9 |
| Morgan | Collins | MC | 6 |
| Escambia | Unknown | EC | 10 |
| Autauga | Unknown | AC | 10 |
| Colbert | Underwood | CU | 0 |

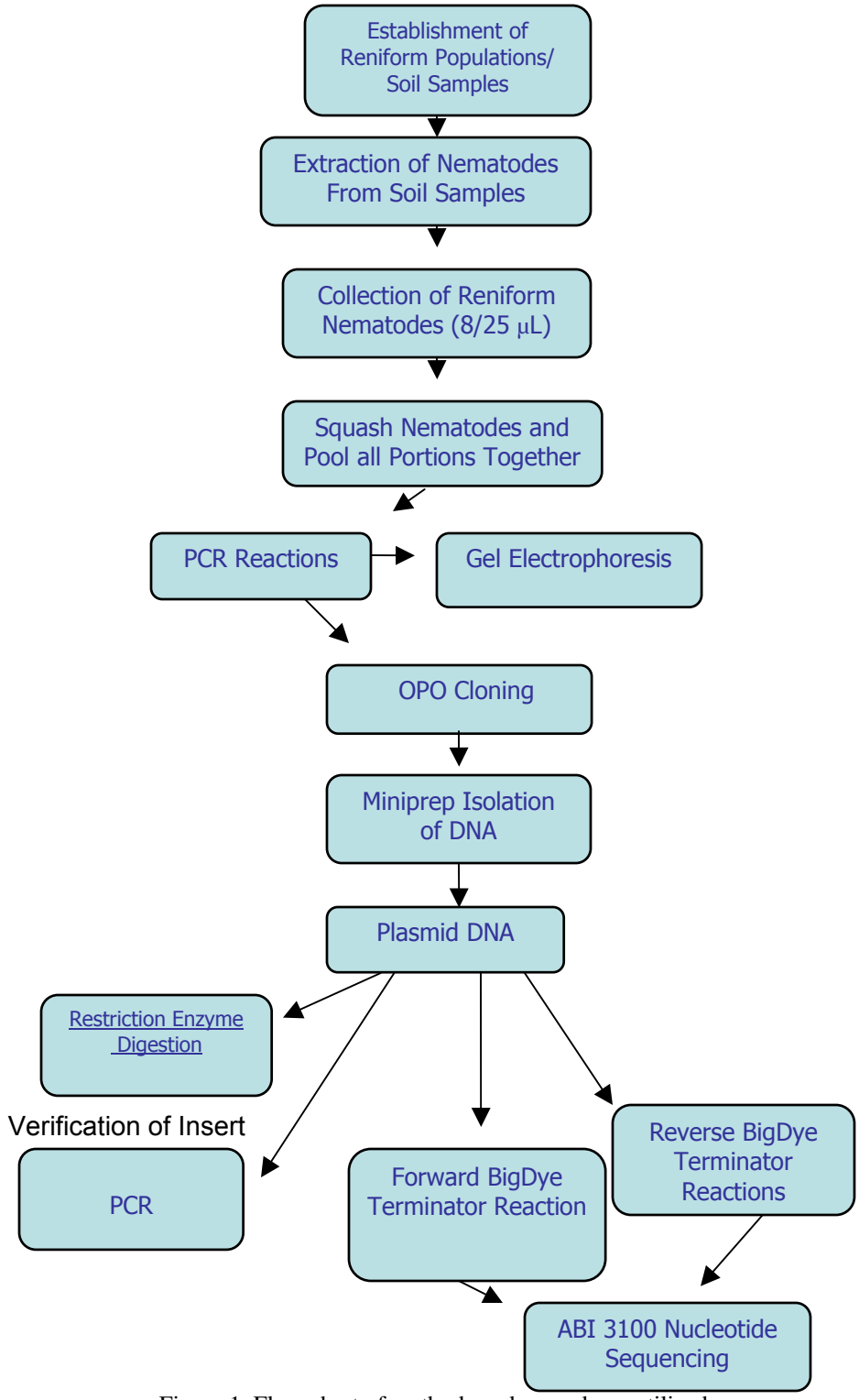
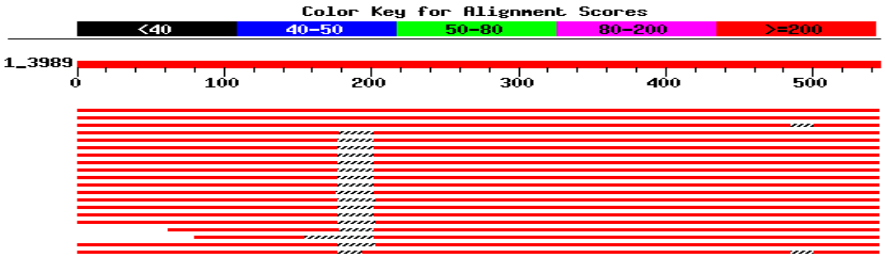



Figure 1. Flow chart of methods and procedures utilized.

Query= AL18S1 (545 letters)
Distribution of 39 Blast Hits on the Query Sequence




| Sequences producing significant alignments | Score (bits) | E Value |
|---|---|---|
| gi\|2707748\| Globodera pallida small sub18S | 890 | 0.0 |
| gi\|6983966\| Subanguina radicicola 18S... | 837 | 0.0 |
| gi\|1147729\| Meloidogyne arenaria 5S, 18S.. | 731 | 0.0 |
| gi\|6983959\| Pratylenchoides magnicaud.18S | 617 | e-175 |

Figure 2. Representative BLAST result for 18S clone sequence. GenBank query restricted to taxon Nematoda.

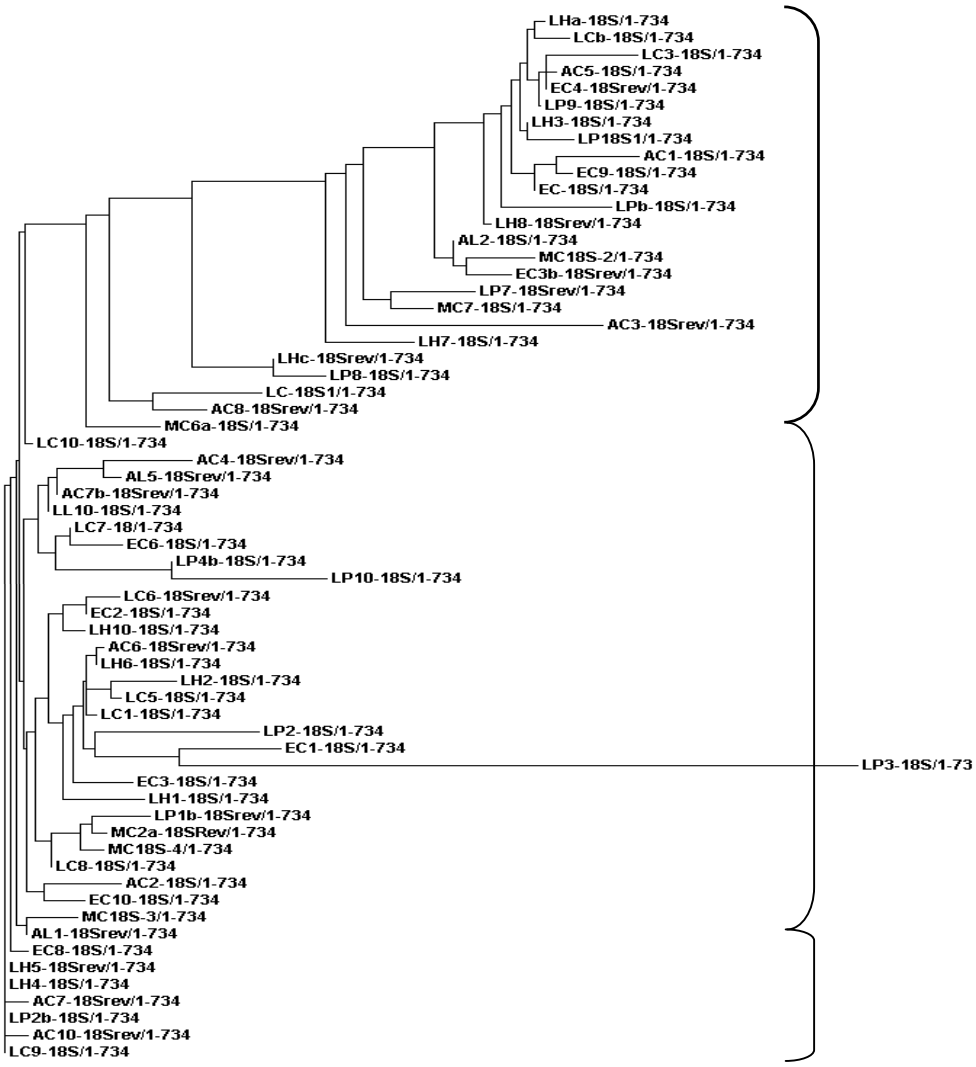



Figure 3. **Phylogram of 18S clone sequences.** First two letters of each clone correspond to location of reniform infested soil samples as per Table 1.