Spring 5-2013

Exploring Prairie Diversity: Plant Parasitic Nematodes of 9 Mile Prairie

Maggie Olson
University of Nebraska-Lincoln

Exploring Prairie Diversity: Plant Parasitic Nematodes of 9 Mile Prairie

Maggie Olson
May 1st, 2013
Environmental Studies Senior Thesis
Faculty Adviser: Tom Powers

Introduction

Nematodes are some of the most diverse and abundant animals on Earth. These microscopic invertebrates are found in virtually all terrestrial and aquatic habitats. While their abundance and diversity of form is well documented, their genetic diversity and distribution is largely unknown (Boag and Yeates, 1998). How this diversity is distributed across continents, ecoregions, and landscapes is still being discovered.

There are two primary objectives of this project. First, we wanted to determine how patterns of nematode diversity are distributed within and among tall grass prairies. Additionally, we wanted to generate new knowledge about belowground prairie diversity. One component to this was simply determining characteristics of the nematode communtities found within the prairie. Tall grass prairies are known to harbor high levels of plant diversity, so does the belowground nematode diversity reflect this aboveground diversity? These objectives were approached through an intensive nematode sampling survey of 9 Mile Prairie, followed by DNA analysis.

Four samples were collected from each of the seven management sections. Rhizosphere soil samples of three species of plants native to Nine-Mile Prairie, prairie dropseed, switchgrass, and lead plant, along with a 40 m x 40 m grid sample were collected. The nematodes found within soil samples were extracted, identified and analyzed morphologically and molecularly.

Approximately 4 out of every 5 animals on earth are nematodes. They are found in virtually all environments and it is estimated that between 1 and 5 million species exist, with 27,000 species having been described. Nematodes are slender, worm-like animals and are typically less than 1mm in length. They are structurally simple,

being made up of approximately 1,000 somatic cells and possessing reproductive, digestive, nervous, and excretory systems, but lacking discrete respiratory or circulatory systems. They may exist as parasites of plants, animals, or insects, or as free-living organisms, in which case they feed on bacteria, fungi or other nematodes (What is a Nematode?).

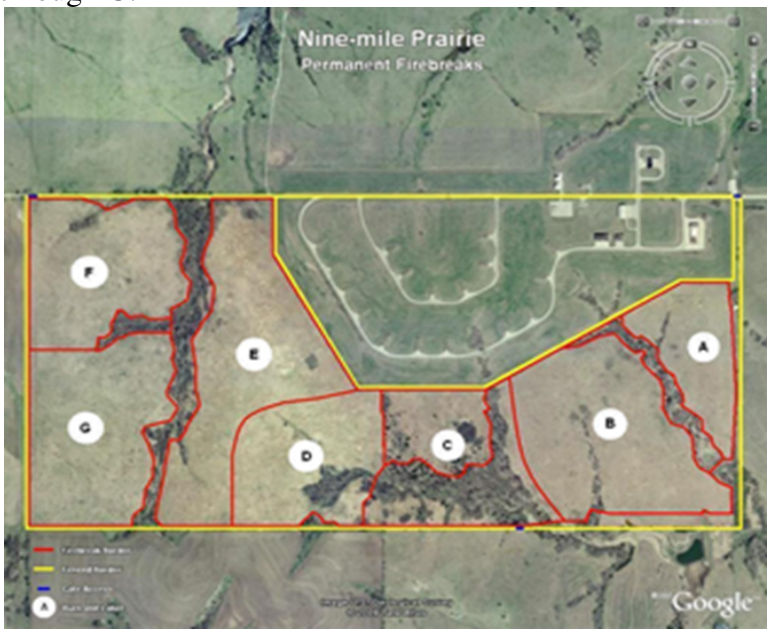
Within this project, plant parasitic nematodes of the family Criconematodae will be focused on. The group of nematodes, which in terms of size are some of the smaller known groups of nematodes, range in size from 0.3 mm to 1.5 mm. There are around 600 described species and several characteristics make the group good candidates for studies of biodiversity and biogeography. First, Criconematid nematodes are distributed worldwide and could be found in almost all environments. Many species are highly endemic and they are typically very sensitive to soil disturbances. Also, this group f nematodes lack specialized survival or dispersal stages or capabilities.

Recent research has suggested that endemic nematode species can be found across isolated North American remnant prairies. Remnant prairies are portions of undisturbed tallgrass prairies, which covered the central United States prior to land disturbances and conversions. Less than 1% of original tallgrass prairies remain intact and are typically isolated islands of prairie surrounded by converted land (Todd et al., 2006). These endemic nematode species found within islands of virgin tallgrass prairie bear no resemblance to nematode communities found in the surrounding agroecosystem.

Nine Mile Prairie is one of the largest tracts of virgin tallgrass prairie found in Nebraska and is the longest-studied natural area within the state. The 230-acre (97-hectare) prairie is located on the northwest edge of Lincoln in Lancaster County and is

home to more than 390 species of vascular plants and more than 80 species of birds. Nine Mile is owned by the University of Nebraska Foundation and managed by a committee of university and agency biologists. Management includes a springtime burning schedule, and periodic haying and herbicidal weed/brush control. The site has not been grazed since 1968, and with the exception of a small section in the 1940's, the site has never been plowed ("Nine-Mile Prairie" http://snr.unl.edu/aboutus/where/fieldsites/ninemileprairie.asp).

Figure 1. Aerial photo of 9 Mile Prairie depicting the seven management sections A through G.



http://snr.unl.edu/images/aboutus/where/fieldsites/ninemileprairie/9MPFirebreakspermanent3.jpg

This research is of significance for several reasons. First, nematodes can be used as environmental indicators. They can indicate quality or lack of quality of a habitat. Nematodes are highly sensitive to disturbances, both physical and chemical, natural and anthropogenic. Nematodes can also be used as climate change indicators due to their sensitivity to temperature change and freezing, and they can be found associated

with plants whose native range is altered due to climate change. Nematodes also act as indicators in that they may respond secondarily to invasive species. As environmental indicators, nematodes provide information about the evolutionary history of a habitat. Also, nematodes can be used as a baseline reference and belowground indicator of environmental restoration and the long-term impact of soil disturbances. From a conservation biology respect, unique nematode assemblage could identify a hotspot for diversity or identify an evolutionarily unique environment. The presence of specific nematodes may also indicate areas of high endemicity. Due to the endemic nature of nematodes and their specific niches within their habitat, once a prairie is disturbed, altered, or converted, the native nematode species associated with the prairie will be replaced (Todd et al. ,2006).

Throughout the course of this research project, the diversity of eight species of plant parasitic nematodes collected from samples associated with three species of native plants will be measured in Nine Mile Prairie. It was hypothesized that the nematodes in Nine-Mile Prairie and other remnant prairies have retained a high level of genetic variation compared to disturbed grassland habitats. This variation is documented by examining the nucleotide sequences of genes know to exhibit high levels of intraspecific variability. It was also hypothesized that if a remnant prairie has a long, distinct evolutionary history, then the genetic structure of the nematode species would reflect that history.

Materials and Methods

Sampling Strategy:

Nematodes were collected from Nine-Mile Prairie during the summer of 2012, over the course of approximately 7 days between May and August. Nine-Mile Prairie is composed of seven separate management sections. Three different plant species; switchgrass, leadplant, and dropseed were sampled from each section. A transect or 40mx40m grid sample was also taken in each section. Samples were taken in the form of soil cores, taken at a depth of approximately 10-20 cm. Two soil cores were taken from the edge of each plant being sampled. Samples were taken roughly 6 meters apart while walking across the center of sections. Soil cores for each target plant species within a section were combined to make one bulk sample for each species from each section. Approximately 1000cc of soil was collected per sample, which was stored in plastic collection bags. Bulk samples extracted from seven sections of three different plant species plus the transect/grid resulted in twenty-eight total samples. Bulk samples were stored in coolers for transportation and prior to arrival at the lab, where they were stored in refrigerators prior to nematode extraction.

Nematode Extraction:

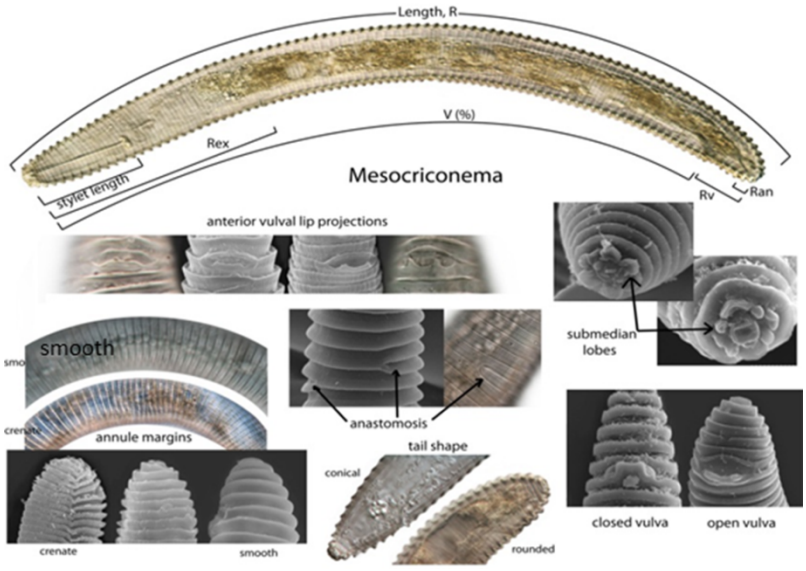
Nematodes were extracted from the soil samples through a floatation and sieving method, followed by sugar centrifugation. A sugar solution with the same density of nematodes facilitated separation of nematodes from other organic materials by centrifugation at 900g. Nematodes of the family Criconematidea were isolated to be measured, photographed, and stored for PCR/DNA sequencing and analysis. Select nematodes from each section that were not prepared for PCR/DNA were prepared for permanent slides or electron scanning microscopy.

Nematode Morphological Analysis:

Approximately 200cc of soil was washed out from the larger sample during the floatation and sieving method. The total number of nematodes collected from soil cores was recorded using a dissecting light microscope. Also recorded and isolated was the number of nematodes belonging to the criconematid family. Up to five individuals of those nematodes belonging to roughly eight species were measured and photographed using differential interference on a light microscope. Approximately fifteen different measurements were taken on each specimen, including total body length, length of stylet, and number of annules.

Figure 2 below depicts how nematodes are measured and identified morphologically. Roughly a dozen measurements are taken on each nematode, including the total length of the specimen and the length from the head to the esophagus. Along with measurements, morphological characteristics are noted. For example, it is typical for the “faces” of nematodes to vary greatly between species, so certain characteristics are important identifying species without the use of molecular tools.

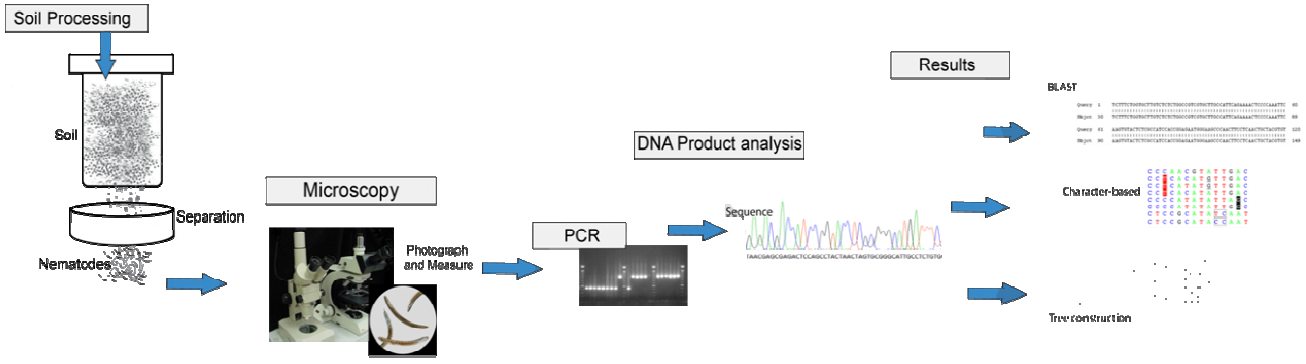
Figure 2. Measurements and important morphological features of a nematode in the family Criconematidae.



Nematode DNA Analysis:

Once the target species of nematodes were analyzed morphologically, they were prepared for DNA analysis and stored in a 0.5ml microfuge tube until analysis. PCR amplification of the mitochondrial COI gene followed by DNA sequencing allows for the genetic differentiation and relatedness among specimens was compared. Target species of nematodes from Nine-Mile Prairie were compared to the same species found in other remnant prairies in the central U.S. These DNA procedures are depicted the flow chart in Figure 3.

Figure 3. Flow of methods in nematode analysis.



Results

The table below shows the preliminary abundance data of this nematode survey. Columns of the table show management sections A through G of Nine Mile. Rows include the total number of nematodes estimated per sample, the percentage of criconematid nematodes found within each sample, and the total number of criconematid species counted in each section. The value "N" seen in section C and E represents that total number of plant species counted in a section. This number was determined by a professional botanist during a sampling day at Nine Mile Prairie (Dave Wedin, pers comm.).

Table 4. Nematode abundance per section

	Section A	Section B	Section C N = 74 *	Section D	Section E N = 68 *	Section F	Section G
Total # of Nematodes (per 100 cc)	490	178	211	382	506	1287	240
% Cricos	21	24	4	15	11	10	9
# of Species	7	5	2	5	6	3	7

*N = number of plant species counted in section, determined by professional botanist on day of nematode sampling

These data are based on one field season's collection, and will need to be duplicated in the future to make any statistically valid conclusions. However, there are a few observations worth noting.

First, section B presents a relatively high percentage of Criconematid nematodes. Almost 25% of the nematodes counted in the section were Criconematid, which considering there are hundreds of families is a significant value. It has been observed in past studies that Criconematid nematodes are often abundant in natural, undisturbed areas. This suggests the possibility that section B is a tract of relatively high quality prairie and has experienced little degradation.

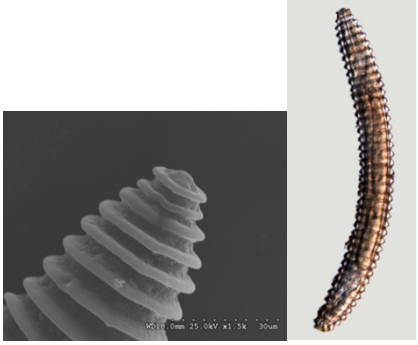
Another aspect that stands out in Table 4 is section C. It was noted earlier that section C is the only section of the prairie that has been plowed. The section was used to grow corn on and had been plowed as recently as the 1940's. While the abundance of total nematodes in this section doesn't stand out, the abundance and diversity of Criconematid nematodes does. Only 4% of nematodes found were Criconematids, making up two species in the section. While the nematode abundance and diversity for the section is relatively low, the aboveground plant diversity was found to be equal to, if not higher than surrounding sections. This suggests a "legacy" of soil disturbance. Even after the aboveground plant diversity has recovered, belowground nematode communities reflect a loss or decrease in diversity due to a historical disturbance.

Species Identified and Host Associations:

Within the seven sections of Nine Mile, eight species of Criconematid nematodes were identified. Some of these species were found to have specific plant host associations. The following are pictures and brief descriptions of the Criconemtid nematodes identified.

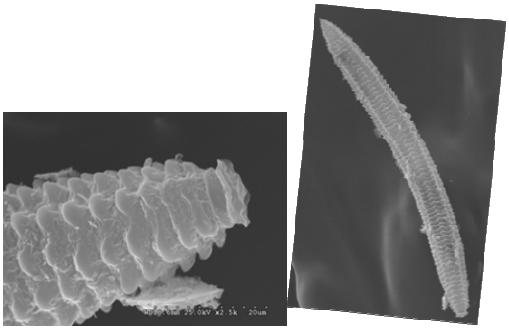
Mesocricnema xenoplax: This is a parasite of woody plant species, such as sumac found on the prairie. It was found in sections A, B, D, E, and F.

Lobocriconema nsp: "NSP" refers to a new species. Only one other species of Lobocriconema has been found in North America, and based on DNA analysis, this is something different. It was found in sections A, D, and G. The host within the prairie is unknown, additional sampling, morphological analysis, and molecular analysis is necessary to describe the species.



Criconema permistim: This a plant parasite endemic to prairies. It's host is unknown and was found in sections A, E, and G.

Ogma decalineatum: This species was found in section A, C, D, E, F, and G and is associated with lead plant.



Mesocriconema spp. 1-2 nsp: This nematode, found throughout Nine Mile Prairie is either one or two new species. It was initially identified as *Mesocriconema curvatum* based on morphological features, but DNA analysis indicated that it is likely two new species. Additional sampling, morphological analysis, and molecular analysis are necessary to describe the species.

Mesocriconema inartatum: This species was found in sections A, B, C, E, F, and G. It is associated with prairie dropseed.

Criconemoides sp.: This is a species of plant parasitic nematodes found in section G which has been associated with cultivated plants.

Mesocriconema rusticum: This is another species found only in section G that has an association with cultivated plants. This species and the Criconemoides sp discovered are likely entering the prairie from bordering agricultural land.

Phylogenetic Diversity:

Figure 5. Evolutionary tree depicting phylogenetic diversity.

Pink highlights indicate specimen from Nine Mile Prairie, yellow highlights indicate specimen from Spring Creek Prairie.

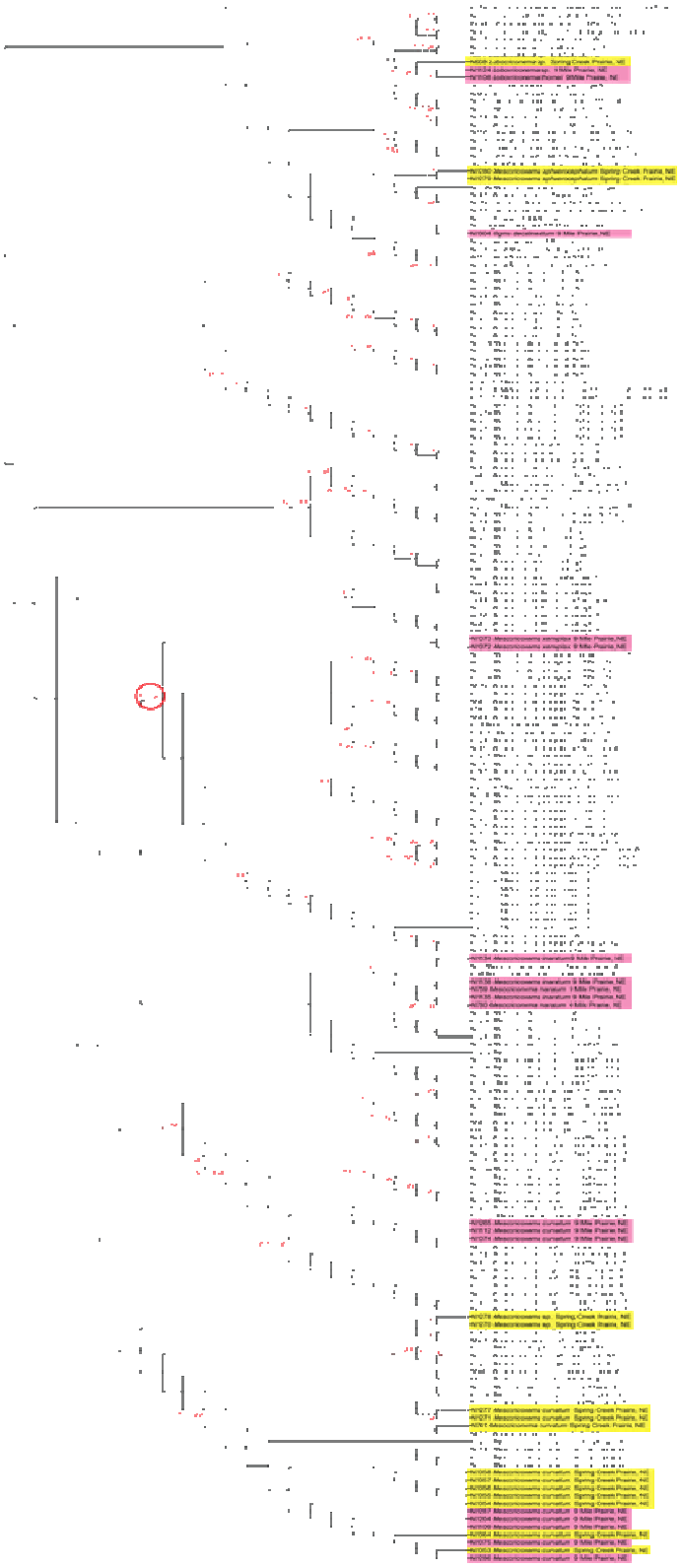


Figure 5 on the previous page is an evolutionary tree which depicts the phylogenetic diversity between individual nematodes. The tree was created by amplifying the CO1 gene of the mitochondrial DNA using PCR. The lines of the tree are "branches," which connect to individual nematodes which have been sequenced. This tree contains all nematodes belonging to the family Criconematidae from all over North America. The individuals highlighted in pink are from Nine Mile Prairie, and those highlighted in yellow are from Spring Creek Prairie. Spring Creek is a small remnant prairie outside of Lincoln, Nebraska in which a similar nematode survey was conducted. It is known to be more degraded than Nine Mile, and individuals from this prairie have been highlighted to act as a comparison for Nine Mile.

The evolutionary tree is significant because it shows the distribution of individual genotypes. The nematodes from Nine Mile are distributed across the tree on different branches, which indicates that the species and individuals identified are different and diverse.

Conclusions:

Four primary conclusions have been made from this survey of the plant parasitic nematodes of Nine Mile Prairie. First, the distribution of genotypes from 9 Mile Prairie show broad phylogenetic representation on the evolutionary tree. Individual nematodes are spread out into distinct groups across the tree, which indicate that there is great diversity between the species of nematodes that were identified. Second, some species of Criconematid nematodes were commonly associated with a specific plant host. It makes sense that plant parasitic nematodes would be closely associated with specific species of plants, which was seen between *Ogma decalineatum* and lead plant, for example. Next, it

was suggested that historic soil disturbances leave a belowground legacy of reduced diversity well after the aboveground plant diversity has recovered. This was seen in section C, where even after roughly seventy years since the site had been plowed, the nematode community was significantly less diverse than surrounding sections. Lastly, the high plant diversity of Nine Mile Prairie may support many species of plant parasitic nematodes, resulting in high nematode diversity, but historical soil disturbances (like in section C) may disrupt those relationships and the overall diversity of the prairie.

References

Boag B, Yeates GW (1998) Soil nematode biodiversity in terrestrial ecosystems. *Biodiversity and Conservation* 7:617-630.

Graham, Pam. "The Tallgrass Prairie: An Endangered Landscape." CSA. Web. 23 Apr 2013. <http://www.csa.com/discoveryguides/prairie/review.pdf>.

"Nine-Mile Prairie." University of Nebraska School of Natural Resources. N.p.. Web. 22 Apr 2013. <http://snr.unl.edu/aboutus/where/fieldsites/ninemileprairie.asp>.

Powers, T.O., Harris, T.S., Higgins, R.S., Sutton, L.A., and K. S. Powers. 2010. Morphological and molecular characterization of Discocriconemella inarata, an endemic nematode from North American native tallgrass prairies. Journal of Nematology 42:35-45.

Shah, Anup. "Biodiversity." Global Issues. N.p., 06 Apr 2011. Web. 23 Apr 2013. <http://www.globalissues.org/article/170/why-is-biodiversity-important-who-cares>.

Todd, T.C., T.O.Powers, and P.G.Mullin. 2006. Sentinel nematodes of land-use change and restoration. Journal of Nematology 38:20-27.

"What is a Nematode?." Plant and Parasitic Nematodes. University of Nebraska-Lincoln Nematology, n.d. Web. 11 Apr 2012. http://nematode.unl.edu/what-is-a-nematode.htm