Plant Virus Biodiversity and Ecology at Oklahoma's Tallgrass Prairie

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**Background: Virus Biodiversity**
- The ICTV recognizes 1900 species of viruses.
- The NCBI has about 1500 complete viral genome sequences in its databanks.
- Most of the species and sequences are hosted by species "important" to man and "disease-associated.

**Central hypothesis:**
The actual number of viral species in the world is much greater than the number currently recognized.

**Background: Virus Ecology**
- Viruses: *are obligate intracellular parasites.*
- May increase or decrease the fitness of their hosts or be benign,
- May protect a plant against infection by another virus,
- May synergize with another virus,
- And may depend on another virus for infectivity.

**Central hypothesis:**
Virus species distributions affect and are affected by the distributions of other virus species and of host and non-host species.

**Focus: Plant-associated viruses of the Tallgrass Prairie Preserve**
- Plants well suited as a model target
- Relatively immobile and accessible,
- Fewer regulatory and safety constraints
- The Tallgrass Prairie Preserve
  - Is owned and operated by the Nature Conservancy.
  - Contains 15,410 hectares in Osage Co., OK.
  - Includes more than 700 plant species and
    is managed by burning and bison grazing.

**Phase I: Biodiversity**
- Total Nucleic Acid
- Sequence ITS
- Sequence psbA
- Virtual Herbarium
- OSU herbarium
- Double-stranded RNA
- Microarray Hybridization
- Clone and Sequence
- Software
- Virus genera present

**Phase IIa: Ecology**
Questions that may be addressed:
- No. virus species = f (No. plant species, area, habitat)?
- How does the similarity in virus species composition between plots vary with distance between the plots?
- What are effects of burning and bison grazing on virus biodiversity?
- Does virus community structure map on to plant phylogeny?
- Are some habitats more conducive to high viral loads than others?
- Are exotic plant species more or less prone to infection than native species?
- How do virus communities vary seasonally?

**Phase IIb: Virology**
- The viruses that will be discovered through Phase I will be used for comparative virology to address questions in virology.
- What sequence features of capsid proteins account for differences in assembly and stability?
- How do viruses spread throughout the plant tissue?
- How have viruses evolved from common ancestors? When did divergences occur?
- Do viruses fall into clear taxonomic categories (species, genera, families, etc.) or are other classification schemes needed?

**Impacts**
- Basic research: we expect the work of the project to make significant contributions to thinking in community ecology and to understanding of structural, physiological, and evolutionary aspects of virology
- Agricultural biosecurity: we expect that knowledge of the existence and distribution of viruses to be essential for recognition of man-made epidemics.
- Biotechnology tools: viruses have long provided genetic parts for biotechnology; the viruses we uncover will likely contribute to the toolbox
- Research Infrastructure: we will become a global focal point for viral ecology and biodiversity.

**Abstract**
The International Committee for the Taxonomy of Viruses recognizes a global total of close to 2,000 species of viruses. DNA sequencing of marine environmental samples suggests that 2,000 is a gross underestimate of the number of viral species on earth. Estimation of the actual number can be approached using quantitative biodiversity theory. While these estimates have been made for viruses infecting single-celled organisms in an aqueous environment, studies assessing viruses infecting multicellular organisms lag behind. We have initiated a survey of the biodiversity of viruses infecting vascular plants in the Tallgrass Prairie Preserve of Oklahoma, home to over 700 species of vascular plants. In our first approach, we are screening the majority of preserve plant species for viruses. Vectoring and identification are by double-stranded RNA analysis, cloning, sequencing, and microarray analysis. The fraction of previously unknown viral species we identify in the survey will provide an indication of the extent of global underestimation of known viruses. The percentage of the plant species examined that contain each virus will distinguish generalist from specialist viruses. Subsequent surveying will collect data for individual plants to allow calculation of the percentage of all plants examined from one species that contain a particular virus. Another approach is the jackknife approach in which total plant material from a defined area constitutes an environmental sample that is used for sequence analysis. Data will be mined to address questions such as: does the ratio of number of viral species to the number of plant species in an area depend on plant species richness? Does the mix of viral species decay with distance along a transect? Does plant diversity lower the load of viral species? Our guiding hypotheses are that the majority of plants harbor some viruses and that most virus infections in plants do not cause disease. Since we are not limiting our search to economically important plants, already well studied, we expect to find many new viruses. These new viruses will feed into basic research on the mechanisms of virus replication, transcription, particle assembly and movement, as well as providing tools for biotechnology.

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