Small mammal contact zones and evolutionary cascades: Methods for genomic investigations within complex host-parasite systems

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Regarding myself…

• Fundamentally: Biogeographer
  • Processes that influence distributions
    • Individuals → populations → lineages → species

• Functionally: Molecular Ecologist
  • Interpret evolutionary history to understand ecology
    • Phylogenetic trees

• Purpose: Conservation of functional wild systems
  • Inform policy and management
  • Reveal connections
    • Hosts and parasites

Hantavirus Mammal Crew
New Mexico
Ecology of Emerging Disease

“Satellite imagery characterizes local animal reservoir populations of Sin Nombre virus in the southwestern United States” – Glass et al. 2002, *PNAS*
Host-virus co-phylogeny

- Paradigm lost..

- Shrews, **not** rodents, as primordial reservoirs for hantavirus

- Tree-based methods reveal relationships

- Specimen archives are critical

Studying evolution within natural systems

Traditional molecular methods

TIME

Now

Phylogeography

Phylogenetics

Population Genetics

COMPLEXITY

Low

(Number of Loci)

High

Then

Low

High
Modern Methods…

Now

TIME

Population Genetics

Phylogeography

Phylogenetics

Landscape Genetics

Comparative Phylogeography

Phylogenomics

Eco-Gen

Functional Genomics/Speciation

??

COMPLEXITY

Then Low High
Impacts of environmental change

- Anthropocene trends
  - Increased rates of warming/development
  - More extreme events/disturbances
  - Elevated risk of extinction
  - Increased intensity of biological interactions

- Mitigation (from a phylogeographic perspective)
  - Resolve evolutionary complexity
    - Across landscapes
    - Across layers of biodiversity
Hope Lab Questions:
How are functional biodiversity connections influenced by changes in:
1) Ecological associations within local communities; and 2) Genetic backgrounds of species, across space and through time?
Most genomic assessments of wild organisms are specimen-based!

Genomic Contributions

Geo-referenced locality

Cataloged Host Voucher

Ecto & endo parasites

Virus isolates

Field notes, ecological data

Genomic data

Ultrafrozen tissues

Publications
Genomic Contributions

Evolutionary Rate Variation

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Geo-referenced locality

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Publications

Dicrostonyx groenlandicus, Lemmus trimucronatus, Microtus longicaudus, Microtus miurus, Microtus oeconomus, Microtus pennsylvanicus, Marmota broweri, Marmota caligata, Tamiasciurus hudsonicus (northern), Tamiasciurus hudsonicus (southern), Ochotona collaris, Gulo gulo, Mustela erminea, Sorex cinereus, Sorex hoyi, Sorex palustris, Sorex tundrensis, Sorex ugyunak

Figure 2.
Genomic Contributions

Evolutionary Rate Variation

Cataloged Host Voucher

Geo-referenced locality

Improved phylogenies

Field notes, ecological data

Genomic data

Host hybridization and speciation

Metagenomics

Ecto & endo parasites

Virus isolates
Genomic Contributions

Evolutionary Rate Variation

Selection (on metabolic genes)

Genus

Abundance

Candidatus

Genus

Abundance

Field notes, ecological data

Genomic data

Improved phylogeny

Geo-referenced locality

Parasite Metagenomics

Ecto & endo parasites

Host hybridization and speciation

Improved phylogenies
Resolving generalizable processes of change

Shrews, genus *Sorex* ~80 Holarctic species
- Tiny, abundant, ubiquitous
- High metabolism, turnover $\rightarrow$ rapid evolution
- Insectivorous $\rightarrow$ complex parasite life cycles
Masked shrew complex

- 13 described species
  - Closely related
  - Complex geographic distribution
- Diverse macro- and micro-parasite faunas
  - Communities within communities
  - Cascading change
- Multiple contact zones
  - Hybridization, competition, speciation
  - Naturally replicated experiment:
    - Evolutionary, geographic, ecological, temporal...
Masked shrew
“complex”
13 Species?

Present distributions
North America
Present distributions: Beringia
Interdependent communities

Shrew endoparasites - helminths
- Highly diverse with complex life cycles
- Variable levels of host specificity
- Woefully underexplored!

Tkach et al. *in prep* (x3)
Goals

1. Investigate species limits within *cinereus* group
   - Phylogeographic history
   - Phylogenomics
   - Selection/Drift

2. Characterize shrew hybridization
   - Are dynamics repeatable across multiple hybrid zones?
   - Function of conserved vs. introgressed genome regions
   - Role in continued diversification

3. Understand changes in associated parasite diversity
   - Evolutionary, geographic, ecological, temporal...
   - Relative host specificity across hybrid zones
   - Cascading impacts of change
     - Shrew $\rightarrow$ helminths $\rightarrow$ microbiome
1. Shrew species limits

- Shotgun sequencing of mitogenomes
  - 12 shrews on Illumina MiSeq
  - High coverage (10-350x /bp)

- Expanded mitogenome sequencing (in progress)
  - Long-range PCR of 190 shrews
  - Selection on metabolic genes across environmental gradients

- Nuclear reduced representation sequencing (in development)
  - Selection on nuclear genes (e.g. immune function)
  - Genomics of hybridization
Mitogenomes - phylogeny

- All mtDNA genes
- Bayesian analyses (BEAST)
- Strongly supported relationships
- Lineages coincident with geography
Present distributions
North America
Hypothetical ancestral distributions 20,000 yrs ago
Mitogenomes – adaptation to local environments

• Masked shrews - extremely high metabolism
• Two major clades - mesic or xeric habitats
• Expectations:
  • selection across environmental gradients
  • intensified in harsher environments
  • purifying or diversifying selection
  • reflects demographic history
Mitogenomes - selection

- REL (random effects likelihood) tests - HyPhy
- dN/dS ratios
- By site, by branch, by clade
- Only individual sites significant

![Box plots of dN/dS ratios across different regions](image)
Mitogenomes - selection

- Zoomed out...
- Overall very low dN/dS ratios (very low sample size)
- Positive selection in two genes

![Graph showing dN/dS ratios for various genes, with 'Diversifying Selection' highlighted.]
Mitogenomes to nuclear genomes...
Shrew Hybridization

- Complexity:
  - Evolutionary
  - Geographic
  - Ecological
  - Temporal
Shrew Hybridization

- Complexity:
  - Evolutionary
  - Geographic
  - Ecological
  - Temporal
2. Shrew Hybridization

- Shotgun sequencing (MiSeq) to develop >20 microsatellites
- Rigorous sampling across boreal-tundra contact zone
  - *S. cinereus* vs. *S. ugyunak*
  - ~400 archived specimens
3. Variation in Intestinal Biodiversity

• Metagenomic methods – 12S, 16S, 28S; MiSeq
  • Spatial gradients across host contact zones
  • In-situ temporal change using museum specimens

• Parasite (helminths)
  • Biodiversity discovery
  • Host-parasite co-evolution and phylogeography
  • Parasite hybridization, host switching

• Microbiomes
  • Fluctuating pathogens, commensals, symbionts
    • Temporal, spatial, host variability
Rarefaction curves
- Parasite OTUs from 25 shrew specimens

16S rDNA (300bp)

16S mtDNA (120bp)

Microbiome

Helminths

Greiman et al., in prep.
Major genera
- Parasites from old and new shrews

Microbiome

Genus
- Cyclophyllidea (unclassified)
- Hymenolepididae (unclassified)
- Lineolepis
- Lockerrauschia
- Mathevolepis
- Monocercus
- Skrjabinacanthus
- Soricinia
- Staphylocystis
- Staphylocystoides
- Urocystis

Abundance

Genera
- Acidothermus
- Anaeroplasma
- Brevinema
- Arthromitus*
- Hepatincola*
- Lariskella*
- Paenicardinium*
- Soleaferrea*
- Carnobacterium
- Chlamydia
- Chlamydompila
- Citrobacter
- Clostridium
- Ehrlichia
- Ewingella
- Gaiella
- Helicobacter
- Isosphaera
- Lactococcus
- Legionella
- Leuconostoc
- Mycobacterium
- Mycoplasma
- Nakamuraella
- Nocardoides
- Paenibacillus
- Patulibacter
- Pseudomonadica
- Rickettsia
- Rickettsiella
- Solirubrobacter
- Sphingobacterium
- Streptomycyes
- Tyzzerella
- Ureaplasma
- Verminephrobacter

*Candidatus

Greiman et al., 2018. J.Parasitol.
Pribilof Shrew
*Sorex pribilofensis*
St. Paul Island, Alaska.
This map illustrates the geography of Beringia, the land bridge that formed between North America and Asia during the last glaciation. Sea-level was lowered by ~120 m due to the growth of ice sheets and glaciers on the continents, which exposed the continental shelf. The Quaternary period (2.6 million years to present) is characterized by large fluctuations in global temperature and multiple glaciations occurred. Beringia forms during major glaciations and would have persisted for 1000s of years at a time. During warmer periods, such as our current interglacial, the land bridge is submerged. Exposure of the land bridge allowed plants and animals to be dispersed between the continents. Humans crossed Beringia from Asia to North America during the glacial period depicted on this map. Lakes and drainages have been reconstructed to provide a better perspective on the environment of the land mass according to bathymetric data having a grid spacing of 1 km (100 m for Norton Sound). Some of the lake basins are shallow and may have been wetlands rather than open water bodies. The Arctic Ocean captures much of the drainage from Beringia, including the Yukon River in its current course, which may have been diverted north during a previous glaciation on the Chukotka Peninsula. Holocene marine sedimentation has undoubtedly changed the topography of the continental shelf and was not accounted for in this reconstruction. The Arctic Ocean is depicted with sea-ice cover.

Paleodrainage map of Beringia
Last Glacial Maximum (~18,000 years ago)
Predictions for island biodiversity

1. Low species richness – faunal relaxation
   • Species interdependence $\rightarrow$ cascades (more extreme in north)
   • Low host richness $\rightarrow$ low parasite richness

2. Low genetic diversity
   • Small populations $\rightarrow$ periodic bottlenecks
   • Loss of diversity through rapid fixation of alleles (purging)
   • Lowered fitness?

3. High endemism
   • Divergence following fragmentation (high drift)

4. Host parasite evolutionary concordance?
Mammals of St. Paul Island
Mammals of St. Paul Island

Obtained 22 frozen shrews from Aleut Community on St. Paul

*Sorex pribilofensis*
Results – parasite diversity

• Very low Richness - 2 species of endoparasite
  • Cestode – *Lineolepis pribilofensis*
  • Trematode – *Maritrema* sp. → Normally in birds!!

• Very high parasite loads
  • Multiple hundreds of worms per shrew

*Karpenko and Dokuchaev 2012*
Results – shrew genetic diversity

- Cytochrome b gene (1140bp) for 22 shrews
- Virtually no genetic variability on St. Paul.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pi</th>
<th>Hd</th>
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<tr>
<td>Sorex pribilofensis</td>
<td>0.0001</td>
<td>0.22</td>
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<tr>
<td>Sorex cinereus (AK)</td>
<td>0.0039</td>
<td>0.95</td>
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<td>Sorex cinereus (Southwest)</td>
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<td>0.89</td>
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<td>Sorex portenkoi (Siberia)</td>
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<tr>
<td>Sorex jacksoni (St. Lawrence Is.)</td>
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<tr>
<td>Sorex haydeni (Mid-west)</td>
<td>0.0031</td>
<td>0.86</td>
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<tr>
<td>Sorex preblei (West)</td>
<td>0.0014</td>
<td>0.85</td>
</tr>
</tbody>
</table>
Shrew morphology – PCA

S. pribilofensis

Other sibling species

Hope et al. *in prep*
Results – co-evolution?

• Shrew cestode with North American origin.
Results – co-evolution?

- Shrew trematode normally parasitizes birds!
Natural History

1. Definitive hosts
   - shorebirds
   - shrews

2. Primary intermediate host
   - molluscs

3. Secondary intermediate host
   - crustaceans

Hope et al. 2016,
NOAA Arctic Report Card
Implications

1. Host switching and emerging disease
   - Bird to mammalian
   - Marine to terrestrial
   - Intercontinental movement

2. Low parasite diversity
   - High parasite loads
   - “Missing biodiversity” dynamics

3. Low shrew genetic diversity
   - Loss of adaptive capacity
   - Future environmental impacts (raised sea levels!)
Summary

• Genomic methods allow for highly integrated eco-evolutionary framework
  • Hybridization, speciation, co-evolution, community assembly

• Combined host-parasite analyses provide greater resolution of generalizable landscape processes

• Understanding interdependency among species...
  • Requires extensive diagnosis of existing biodiversity
  • Requires temporally deep, spatially broad, site intensive, and taxonomically comprehensive sampling
Thanks!

USGS, Alaska Science Center
National Science Foundation
- Beringian Co-evolution Project
Numerous Field Crews!!