Applications of Genetics to Conservation Biology

Molecular Taxonomy
Populations, Gene Flow, Phylogeography
Relatedness - Kinship, Paternity, Individual ID
Conservation Biology

- Population biology
- Physiology
- Island biogeography
- Hazard evaluation
- Veterinary medicine
- Environmental monitoring
- Social science
- Natural Resources
- Policy
- Management
- Genetics

Conservation Genetics

- Evolution (M,M,S,D)
- Systematics (Taxonomy)
- Small populations
- Population structure
- Inbreeding/Outbreeding
- Hybridization
- Genetic diversity
- Genetic management
- Reintroduction
- Species biology
- Forensics
How can genetics minimize extinction?

- **Understanding species biology**
  - Relatedness (kinship, paternity, individual ID)
  - Gene flow (migration, dispersal, movement patterns)

- **Molecular Systematics**
  - Resolve taxonomic uncertainty
  - Resolve population structure (phylogeography)
  - Define management units
  - Identify populations of concern
How can genetics minimize extinction?
(continued)

- Detect and minimize inbreeding and loss of genetic diversity
- Detect and minimize hybridization
- Non-intrusive (non-invasive) sampling
- Identify best population for reintroduction
- Forensics
Molecular Taxonomy

Using molecules (ie. DNA based techniques) for systematic study or to define taxonomic units (species, subspecies, ESUs and MUs)
Molecular Taxonomy: Molecules versus Morphology

- Cryptic species (sibling species)
- Morphological variation without genetic variation
Molecular Taxonomy: Conservation Relevance

- Unrecognized species may go extinct
- Incorrect species recognition
  - Non-optimal partition of management resources
  - Problems with hybridization
- Incorrect subspecies or population recognition
  - Not optimal partition of management resources
  - Problems with introgression
Populations, Gene Flow, Phylogeography

- Compare genetic traits among populations
- Resolve substructure among populations
- Infer movement patterns among individuals
- Infer historical events for species
Population, Gene Flow, Phylogeography: Conservation Relevance

• Determine units for management
• Heterozygosity estimates
  – Population bottlenecks
  – Hardy-Weinberg assumptions (mutation, migration, selection, drift, inbreeding)
• PVA (Population Viability Assessment)
• MVP (Minimum Viable Population Size)
• Effective population size
  – Number of breeding individuals
Relatedness (Kinship, Paternity and Individual ID)

Application of molecular genetic techniques, using hypervariable, repetitive DNA (ie. microsatellites, minisatellites) to questions of kinship, paternity or individual ID
Kinship, Paternity, Individual ID:

- Infer relatedness among individuals
  - First order, second order, etc.
- Infer paternity (maternity)
- Reproductive success (male, female)
- Interpret reproductive strategies
  - Monogamy, harem, female choice, etc.
- Interpret behaviors
  - Dispersal (male, female), care giving, others
- Individual ID
  - Populations size estimates
  - Forensics
Kinship, Paternity, Individual ID: Conservation Relevance

- Knowledge to aid management
  - Family structure
  - Reproductive strategy
  - Behavior
  - Dispersal
  - Inbreeding
  - Forensics/law enforcement
Important Techniques: Phylogenetic Analysis

• Resolve evolutionary relationships (species, subspecies, populations, individuals)
• Tool used to determine Evolutionarily Significant Units (ESUs)
  – Also resolves Management Units (MUs)
Phylogenetic tree with ESUs and MUs

ESU - 1

ESU - 2

MU
Phylogenetic Analysis: Phylogeography

• Combines phylogeny with geographical locations of populations
• Three likely outcomes for geographical populations
  – Equally different branches of tree
  – Shallow structure
  – Monophyletic group
Phylogenetic Analysis: Phylogeography

Equally Divergent

Shallow and Monophyletic
Important Techniques: Non-Invasive Sampling

Samples are collected without disturbing the individual
(includes scat, hair, feather etc.)
Non-Invasive Sampling

- Allows sampling without disturbance to individual
- Rare or hard to capture species
- Examples (hair, scat, feathers, saliva/cheek swab, regurgitated pellets, dried blood, biopsy dart, museum tissues)
Non-Invasive Sampling: Applications

- Species ID
- Individual ID
- Sex Determination (Sex Ratio)
- Gene Flow
- Previously described applications for genetics in conservation biology
Non-Invasive Sampling: Pros and Cons

- Allow studies that are not possible by other methods
- Error/failure rates associated with having very minimal amount of DNA
- Use fresh tissues when possible to avoid potential errors
Examples

• Taxonomy, Population Subdivision, Gene Flow, Phylogeography
  – Puma (cougar, mountain lion)

• Kinship and Paternity
  – Madagascar Fish-Eagle
Subspecies Taxonomy, Phylogeography, Gene Flow: Puma (cougar, mountain lion)
32 Puma subspecies, as of the early 1900s
Objectives

• Does current population differentiation reflect
  – Trinomial descriptions?
  – Physical or ecological barriers?
  – Isolation by distance?

• Are current levels of genetic variation the same within each population?

• Does population structure and genetic variation reflect
  – Historic migrations?
  – Historic dispersals?
  – Historic bottlenecks?
Modern and museum puma samples collected, total of 315
Molecular Methods Used

• Mitochondrial gene sequencing
  – 16SrRNA
  – NADH-5
  – ATPase8

• Nuclear microsatellite length determination
  – 10 domestic cat microsatellite loci
Mitochondrial DNA Haplotypes (in a geographical cline)

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- Ancestral haplotypes
- 2 historical radiations
- NA is most recently founded population
### Microsatellite Alleles at FCA008

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</table>
- Geographic clustering of individuals

~ Six groups identified

2 distance methods agree
- Subspecies associate into same 6 groups
- Statistical support from bootstrap values
- 2 distance methods agree
- 6 groups identified using microsatellites

- mtDNA haplotypes overlayed onto map, supports 6 groups

- Location of 2 ancestral haplotypes
## Wright’s Fst Estimates and Slatkin’s Migration Estimates

### mtDNA

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### microsatellites

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Major restrictions to gene flow:

- Amazon River
- Rio Parana
- Rio Negro
- Andes?
Fossil Record versus Molecular Divergence Estimates

- Oldest fossils in North and South America date to 0.2-0.3 Mya
- From mtDNA mutation rate of 1.15%/My, divergence for extant puma lineages is 390,000 years ago
- From mutation rate of $5 \times 10^{-9}$/yr for microsatellite flanking regions, pumas are less than 230,000 years old
Historical Inferences

• Extant pumas originated in Brazillian Highlands (ancestral haplotypes)
• Fossil record suggests dispersal to NA soon after the common origin in Brazil
• 2 historical radiation events occurred
- Ancestor to puma crosses land-bridge ~2-3 Mya

- Puma origin in Brazilian Highlands ~300,000 ya
2 Major historical radiations

- One locally distributed
- One broad ranging
Puma Bottlenecks

• Subspecies-level
  – North America low overall genetic variation

• Population-level
  – Florida monomorphomic at 8/10 microsatellite loci
  – Olympic Peninsula and Vancouver Island, monomorphomic at 5/10 microsatellite loci
Puma Conclusions

• Pumas originated in Brazil approximately 300,000 years ago
• Possible extirpation and recolonization in North America (Pleistocene age?)
• Molecular data does not support 32 subdivisions, instead 6 groups
• Pumas are fairly panmictic within 6 groups
Conservation Implications

- Maintain habitat connectivity within 6 large groups
- Management should consider effects of bottlenecked populations
- Eastern cougar, Florida panther and Yuma puma management take into account revised subspecies
Paternity Application: Madagascar Fish-Eagle

Ruth Tingay, PhD candidate
U of Nottingham,
Antsalova wetland region of western Madagascar
Background and Methods

• Endangered eagle in Madagascar
• 2-3 males, and one female, attend each nest (cooperative breeding)
• Dominance hierarchy among males at nest
• Multi-locus DNA fingerprinting used to infer potential fathers, and estimate adult relatedness among and between nests
DNA Fingerprinting Alleles at 4 nests
(6 representative bands out of 34)

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Conclusions

• At all nests with young (n=3), subordinate males fathered all offspring
• Dominant males have higher energy investment
• Dominant male may be first-order relative to adult female
• One dominant male may have full-sib within nest
Conservation Implications

• Preliminary results, more samples needed
• Advantageous to raise young of full-sib
• Conservation management may consider
  – Adult relatedness in area
  – Number of males that successfully breed