Who's Your Daddy?

Molecular Markers for Mating and Kinship Studies in the Beaver (*Castor canadensis*).

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Introduction

Beavers are reported to be one of the few monogamous mammals. This aquatic furbearer exhibits several classic behaviors of a monogamous mating system, including bi-parental care, cooperative food acquisition, and territorial defense. Beaver colonies have been reported to consist solely of first-order relatives, housing a mated adult pair and their offspring from the past 2-3 breeding seasons. However, this social structure has only been inferred from observational data; DNA-based studies have yet to confirm relatedness among colony members.

To that end, we developed molecular markers to examine mating and kinship patterns in 2 Illinois beaver populations. Our specific objectives included:

1) Describe the mating pattern within colonies using genetic parentage analysis.
Do we see extra-pair mating or strict monogamy?

2) Investigate the occurrence of 2nd-order relatives in a colony.
Do we see a nuclear family or an extended family group?

To investigate these questions, we needed to isolate regions of highly variable DNA in the beaver. Microsatellite DNA is ideal and is characterized by:

1) Repeat DNA that shows a lot of variation among individuals.

2) Non-coding regions that are selectively neutral.

3) Scoring of alleles based on number of repeat units present:

5'…ATTACGAGAGAGAGAGAGCTCGTA…3' 6 dinucleotide repeats Allele 126

5'…ATTACGAGAGAGAGAGAGAGCTCGTA…3' 7 dinucleotide repeats Allele 128

Methods

Sample Collection:

- Tissue samples from live-trapped and trapper-harvested beavers were collected over 2 trapping seasons in central and southern Illinois populations (Fig.1).

Molecular Marker Development:

- Microsatellite DNA from the beaver was isolated following Glen and Schable (2005).
- Primer sets designed using Primer3 for 50 of 96 clones containing msats.
- Of these 50, 20 loci amplified by PCR.
- 30 individuals from each population were screened for polymorphism at 10 loci using fragment analysis on a CEQ8800 sequencer.
- Linkage Disequilibrium (LD) and Hardy-Weinberg Equilibrium tested using GENEPOP and CERVUS software.

Table 1 Characterization of 9 microsatellite loci isolated from the beaver.

H_o is the observed heterozygosity, H_E is expected heterozygosity, * indicates a significant deviation from HWE at $p < 0.001$. n = 60

Locus	Repeat	# Alleles	Size Range	H_o	H_E
Cca 4	$(AC)_{17}$	10	362-364	0.700	0.772
Cca 5	$(CT)_{21}$	11	157-185	0.317	0.621*
Cca 8	$(GATA)_{12}$	10	356-426	0.800	0.837
Cca 9	$(TG)_{21}$	10	136-156	0.767	0.753
Cca 10	$(TC)_{19}$	13	120-154	0.833	0.862
Cca 13	$(GT)_{11,}(GT)_{7}$	6	277-295	0.450	0.481
Cca 15	$(AG)_{6,}(AG)_{7}$	5	177-185	0.650	0.583
Cca 18	$(CT)_{10}$	5	205-220	0.500	0.513
Cca 19	$(TG)_{12,}(AG)_{10}$	12	220-266	0.867	0.815

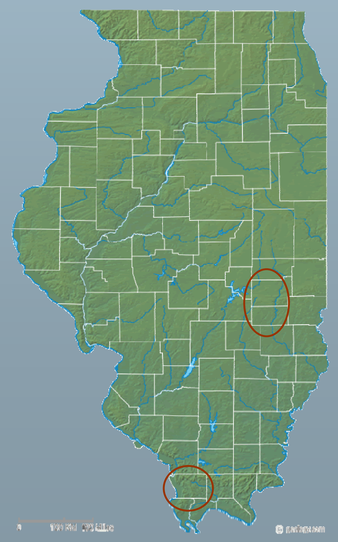

Figure 1. Population sampling from Coles, Cumberland, and Union Counties.

Results

- All loci showed polymorphism, having 5-13 alleles (Table 1).
- Locus pair Cca4/Cca5 failed LD tests in the S.IL population.
- Locus Cca5 deviated significantly from HWE.
- Locus 14 (not listed) was removed from analysis due to ambiguity in allele scoring.

Conclusions

- Several loci had moderate to high levels of variation among individuals and are appropriate for population-level studies.
- Linkage Disequilibrium in only 1 population indicates population structuring in S.IL, rather than a physical linkage between these loci.
- Most individuals had allele 281 at locus Cca13, thereby making this locus less useful.
- Cca15 and Cca18 also showed limited variation and will not be useful in parentage and kinship studies.

Still to come…

We are currently using these loci to conduct:

- Parentage Analysis using CERVUS software
- Kinship Analysis using *Relatedness* software
- Spatial Analysis of relatedness across colonies

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