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?

**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.

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

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat</th>
<th># Alleles</th>
<th>Size Range</th>
<th>( H_o )</th>
<th>( H_e )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cca4</td>
<td>(AC)(_{12})</td>
<td>10</td>
<td>362-364</td>
<td>0.700</td>
<td>0.772</td>
</tr>
<tr>
<td>Cca5</td>
<td>(CT)(_{11})</td>
<td>11</td>
<td>157-185</td>
<td>0.317</td>
<td>0.621*</td>
</tr>
<tr>
<td>Cca8</td>
<td>(GATA)(_{12})</td>
<td>10</td>
<td>356-426</td>
<td>0.800</td>
<td>0.837</td>
</tr>
<tr>
<td>Cca9</td>
<td>(TG)(_{12})</td>
<td>10</td>
<td>136-156</td>
<td>0.767</td>
<td>0.753</td>
</tr>
<tr>
<td>Cca10</td>
<td>(TC)(_{13})</td>
<td>13</td>
<td>120-154</td>
<td>0.833</td>
<td>0.862</td>
</tr>
<tr>
<td>Cca13</td>
<td>(GT)(_{11})</td>
<td>6</td>
<td>277-295</td>
<td>0.450</td>
<td>0.481</td>
</tr>
<tr>
<td>Cca15</td>
<td>(AG)(<em>{2k}) (AG)(</em>{2})</td>
<td>5</td>
<td>177-185</td>
<td>0.650</td>
<td>0.583</td>
</tr>
<tr>
<td>Cca18</td>
<td>(CT)(_{10})</td>
<td>5</td>
<td>205-220</td>
<td>0.500</td>
<td>0.513</td>
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<tr>
<td>Cca19</td>
<td>(TG)(<em>{12}) (AG)(</em>{10})</td>
<td>12</td>
<td>220-266</td>
<td>0.867</td>
<td>0.815</td>
</tr>
</tbody>
</table>

\( H_o \) is the observed heterozygosity, \( H_e \) is expected heterozygosity; * indicates a significant deviation from HWE at \( p < 0.001 \). n = 60

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