Using genetics to understand Behavior

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Behavioral Observations

Photo: Jen Smith

What about species that are difficult to observe?

- Nocturnal
- “Skittish”
- Mating takes place out of view (underwater)

Ways to use genetics as a tool

- Migration
  - Movements
  - Geneflow
- Population size
  - Molecular Mark Recapture
    - Non invasive sampling
- Identify relationships
  - Genetic structuring
    - Family groups
Ways to use genetics as a tool

- Life history
  - Age of reproduction
  - Length of reproduction (iteroparous)
  - Males!
- Mating
  - Assortative mating?
  - Direct fitness
  - Inclusive fitness
  - Identification of individuals

An example: My dissertation research

- Mating systems of freshwater turtles

The problem:

Despite many researchers studying turtles, very little is actually known about the mating systems of turtles. As in many species, we know (comparatively) a lot about females, but not much about males.

The problem:

We cannot observe matings. So how do we find out:
At what age do males start/stop reproducing?
Who is mating with whom?
Quantify reproductive success
For both Males and Females
RS = the number of offspring + quality of offspring

However, the way reproductive success (RS) is attained is different
RS of females is primarily constrained by resources: egg size, egg number, potentially number and quality of mates

Bateman 1948, Trivers 1972

For both Males and Females
RS = the number of offspring + quality of offspring

However, the way reproductive success (RS) is attained is different
RS of males is primarily constrained by the number of females mated and the quality of those females

Bateman 1948, Trivers 1972

Multiple paternity and RS

Potential benefits for females include increased genetic variability of offspring, increased probability of having offspring sired by a high quality male, and genetic complementarities

Potential benefits for males include increased number of offspring sired.

(The double-edged sword: Multiple paternity may increase or decrease an individuals reproductive success)
Study Site:
The University of Michigan’s
Edwin S. George Reserve
Microsatellites

- Short tandem repeats
- Mendelian inheritance pattern
- Highly variable
Tail clip or Blood  DNA

DNA → PCR

PCR: Polymerase Chain Reaction

30 - 40 cycles of 3 steps:

Step 1: denaturation
1 min at 94 °C

Step 2: annealing
45 seconds at 54 °C
forward and reverse primers

Step 3: extension
2 minutes at 72 °C
only dNTPs

PCR → Gel and DATA!

Gel Image!
So what have we done so far?

- We have examined multiple paternity in snapping turtles and painted turtles
- Analyzed incidence of multiple paternity as a function of characteristics of the female (age, body size)
- Tested hypotheses of the benefits of multiple paternity
- Looked at potential use of stored sperm

Snapping turtle: *Chelydra serpentina*

- Long-lived (>55 years)
- Delayed sexual maturity of females (11-17 yrs on ESGR)
- Sexual size dimorphism with males larger than females
- Females store sperm
- Multiple paternity of clutches (Galbraith et al. 1992)

Snapping Turtle Mating System

- Sexual size dimorphism, forced insemination (?) and male-male combat combine to make an interesting mating system compared to many other species of turtles
Female attributes related to RS

Among adults there is no correlation between body size and age

• Larger females have larger clutches and slightly larger eggs than do smaller females

• Older females do not have larger clutches, but have larger eggs than do younger females

Congdon et al., 1987, Iverson, 1990, Congdon unpublished data

Because older and larger females have increased RS, males of all phenotypes would benefit from mating with those females

Predictions:

P1: Larger and/or older females will have a higher incidence of multiple paternity than will smaller females

P2: Larger and/or older females will have a lower incidence of multiple paternity than will smaller females

(However, older but small females may be less able to fend off males)

Preliminary Results

- 1999-2004 Overall incidence of multiple paternity 42% of 40 clutches
- Incidence of multiple paternity was highly variable among years
  - 2002: 20% (n=15)
  - 2003: 60% (n=15)
  - 2004: 50% (n=8)

Sample sizes will increase when unobserved females can be genetically identified
Generalized linear mixed effect model: Logistic Regression Analysis

Dependent Variable: Paternity
0 = single paternity
1 = multiple paternity

Independent Variables
- Random effect: Year
- Fixed effects
  - Clutch size (p = 0.032)*
  - Body size of female (carapace length) (NS, p = 0.33)
  - Age of female (NS, p = 0.07)

Statistics performed using “R”

Preliminary Conclusions

Predictions:
P1: Larger and/or older females will have a higher incidence of multiple paternity than will smaller females

- Combined analyses suggest the potential for a positive correlation between age and multiple paternity

P2: Larger and/or older females will have a lower incidence of multiple paternity than will smaller females
(However, older but small females may be less able to fend off males)

- No significant relationship was observed between body size and incidence of multiple paternity. However, the slight positive relationship between multiple paternity and body size provides potential support for prediction 1.
Ongoing Work

- Adding data from 31 clutches from 2005, 23 clutches from 2006
- Adding more clutches through genetic identification of unobserved females that produced nests
- Total final sample size will be 127 nests
- Adding more loci for genetic identification of individuals
- Identification of the males siring clutches
  - Male phenotypes

Painted turtle life history: ESGR

- Long-lived (>54 years)
- Delayed sexual maturity (females 7-13, males ?)
- Adult sex ratio 2.3:1 (M:F)
- Sexual size dimorphism (females larger)
- Females store sperm
- Multiple paternity

Congdon et al. 2003

Painted turtles: Reproduction

- Clutch frequency is highly variable:
  - Maximum of 2 clutches each year (Schwarzkopf and Brooks, 1985, Congdon et al., 2003)
  - Some not every year (Tinkle et al. 1981)
- Clutch size
  - Average 7.6
  - Range (2-13)
Painted turtles: summary

- Much is known
  - Information about life history
    - Females
      - Female reproduction
    - Sex ratio
    - Population size
    - Long lived
    - Highly variable in quality

Female Attributes related to RS

- Clutch size increases with body size
- Egg size increases with body size
- Clutch frequency increases with age

Because older and larger females have increased RS, males of all phenotypes would benefit from mating with those females

Predictions

1. Incidence of multiple paternity will be the same between first and second clutches
2. Incidence of multiple paternity among years will vary
3. Larger females will have higher incidence of multiple paternity than smaller females
4. By comparing only those females with high reproductive frequency, we will not see an age-based effect in incidence of multiple paternity

Preliminary findings: Painted turtles

- Preliminarily, we used five polymorphic loci to determine single or multiple Paternity of clutch
- Analyzed 35 pairs (70 clutches) from 28 females
Preliminary Results

Multiple paternity between First and Second Clutches

<table>
<thead>
<tr>
<th>Year</th>
<th>% MP</th>
<th>Ave Sample Size per nest</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>0</td>
<td>6.42</td>
<td>12</td>
</tr>
<tr>
<td>2004</td>
<td>25</td>
<td>6.55</td>
<td>20</td>
</tr>
<tr>
<td>2005</td>
<td>13.3</td>
<td>6.03</td>
<td>30</td>
</tr>
<tr>
<td>2006</td>
<td>0</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Incidence of multiple paternity by female age

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Multiple Paternity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-15 years</td>
<td>9</td>
</tr>
<tr>
<td>16-20 years</td>
<td>9</td>
</tr>
<tr>
<td>21-25 years</td>
<td>6</td>
</tr>
<tr>
<td>25+ years</td>
<td>20</td>
</tr>
</tbody>
</table>

Incidence of multiple paternity by female body size

<table>
<thead>
<tr>
<th>Carapace length (mm)</th>
<th>Multiple Paternity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>126-132mm</td>
<td>12</td>
</tr>
<tr>
<td>133-137mm</td>
<td>9</td>
</tr>
<tr>
<td>138-144mm</td>
<td>10</td>
</tr>
<tr>
<td>145+ mm</td>
<td>20</td>
</tr>
</tbody>
</table>

Preliminary Results: Generalized Linear mixed effects model: Logistic regression

- Random effects: Year and Female
- Fixed effects:
  - Clutch size (p<.04)
  - Female age (p=.12)
  - Female Carapace (p=.65)

Preliminary Conclusions

- P1: Incidence of multiple paternity will be the same between first and second clutches
  - Some difference between first and second clutches.
- P2: Incidence of multiple paternity among years will vary
  - In the years we have tested to date, there is variability among years, however this is not linked with sample size variation as we would expect.
Preliminary Conclusions

• P3: Larger females will have higher incidence of multiple paternity than smaller females
• P4: By comparing only those females with high reproductive frequency, we will not see an age-based effect in incidence of multiple paternity
  ➢ Marginal significance with age, but not body size however not statistically significant

So what?

• Even among the most fecund females:
  – Highest reproductive frequency
  – Large clutch sizes
• Preliminarily, we find marginally significant variation in incidence of multiple paternity with age
• Not as strong as in snapping turtles?
  – Subsample using only most fecund females
  – What about the entire population??

The importance of individuals

• 3 out of the 35 pairs of nests (8.5%) one nest in the pair was single paternity while the other nest was multiple paternity.
• Two of the three nests the first nest was single paternity and the second was multiple paternity
• Not exclusively stored sperm

Future Work

• Adding more loci
• Adding more clutches
• Identification of males
  – Test hypothesis of male phenotypes
  – Assortative mating
• Comparing clutches from females that lay only one nest in a year
Conclusions

- Preliminary evidence for males cueing in to some aspect of female quality in both Snapping turtles and Painted turtles
- Possible age effects which
  - Evolution of longevity
- We are finding a lot of neat things about turtle mating systems using genetics
- More work needs to be done!!!