Quantitative Consulting Center Memorandum

Topic: Tadpole Development Analysis       Date: February 4, 2003
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Background and Experiment Statement:
Male Frogs have two distinctive types of mating call distinguished by their duration. The frog can be classified as ‘long-call’ or ‘short-call’. The client is interested in the effect of call length on two areas of tadpole development – body mass and length at metamorphosis. In addition to call length, several other factors were considered and controlled if possible. The client tested for effects of maternal half-sibship, father’s call duration, and density of tadpoles within field enclosures using a three-way fully factorial design.

The performances of half-sibling offspring sired by males with long versus short calls were compared. Tadpoles from twelve such maternal half-sibships were raised in enclosures in their natal pond. The twenty-four full sibships represented twelve individual mothers, twenty-four fathers, and two levels of call duration. Genetic benefits in two realistic natural environments were tested. Tadpoles from each of these twenty-four families were reared in field enclosures at low and high densities, for a total of 48 treatments. Treatments were randomly assigned to enclosures within three randomized blocks. Each block consisted of two adjacent rows of enclosures. The three blocks were positioned in three areas of the pond from which the parent frogs had been collected. Because of insufficient numbers of tadpoles, there was no low-density enclosure for short-call family 8 in block 3. Thus, the experiment included 143 independent experimental units (enclosures) and 1435 tadpoles. (Number of experimental units = 12 mothers * 2 fathers * 2 densities * 3 blocks – 1 missing short-call = 143 units. See diagram below.) Please see the following diagram for the arrangement of the treatment enclosures.

To assess offspring quality, the client used three response variables that are important determinants of fitness: larval period, mass at metamorphosis, and survival to metamorphosis. Larval period was defined as the number of days from the beginning of the experiment until metamorphosis. Because the enclosure, rather than the individual, was the experimental unit, the mean larval period and mean mass at metamorphosis was calculated for each enclosure. Survival to metamorphosis for each enclosure was calculated as the proportion of individuals that survived to be collected upon crawling out of the water.
Data:

The data set consists of calling type (Long, Short), half sibship (1~12), density (High, Low), block (1~3), larval period (age at metamorphosis), mass at metamorphosis and survival rate of 143 enclosures.
Analyses:

1) Generalized $T^2$ test.
To show that the high-density population is significantly different than the low-density population, first assuming a bivariate normal distribution for larval period (age) and mass at metamorphosis (mass), a difference in means test was performed. Since the presence of unequal covariances was indicated a generalized paired $T^2$ test was used for the paired high-low density groups. (Table 1). Since the p-value is less than 0.01, the conclusion is that there is a significantly different in mean mass and length for the two densities.

To test for a significant difference in the mean mass and length at metamorphosis between the long and short call populations, the same $T^2$ test was performed within each of the high and low populations. The p-values were 0.086 and 0.06 for the high and low densities, respectively. This indicates that there exists a somewhat significant difference in the two call patterns while considering the population densities. In order to conduct the test, normality was assumed. See Figure 1. for an empirical distribution plot.

2) Nonparametric signed rank test
A difference in means of high and low density populations was tested using Wilcoxon’s signed rank test. For both age and mass, the differences are very significant.(p-values < 0.0001)
Secondly, the same test for differences of two calling type in each density groups was performed. Surprisingly, not all of the p-values were significant. Thus, a third exploration was considered that could deal with the two variables as one vector. (See Analysis 3: MANOVA.)

<table>
<thead>
<tr>
<th>p-values</th>
<th>Generalized $T^2$</th>
<th>Signed rank for age</th>
<th>Signed rank for mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diff. Of H-L density</td>
<td>&lt;0.01</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diff. Of L-S call in H density</td>
<td>0.086</td>
<td>0.0886</td>
<td>0.4302</td>
</tr>
<tr>
<td>Diff. Of L-S call in L density</td>
<td>0.06</td>
<td>0.4714</td>
<td>0.1586</td>
</tr>
</tbody>
</table>

Table 1

3) Multivariate Analysis of Variance (MANOVA)
Multivariate Analysis of Variance is used to investigate the main and interaction effects of one or more independent variables on multiple dependent variables. This enables a researcher to examine whether two population mean vectors are significantly different and if so, which component(s) differ significantly. In this case the population mean vectors are the vector of observed mass at metamorphosis and the vector of observed length at metamorphosis. The independent variables are call length, density level, sibships, etc. The magnitude and direction of the overall mean vector is examined to determine the strength of the effect each level of the dependent variable (call length) is having on mass and length at development.

Since MANOVA requires the assumption of equal variance across the treatments (homoscedasticity), to use MANOVA, log transformation were performed on both age and mass to ensure the responses were homoscedastic. The MANOVA result for call effect is shown below.

The GLM Procedure

Multivariate Analysis of Variance

Characteristic Roots and Vectors of: $E^{-1} \cdot H$, where

$H$ = Type III SSCP Matrix for call

$E$ = Error SSCP Matrix

Characteristic   Characteristic Vector  $V'EV=1$
Root  Percent  logmass  logdate
0.07680548  100.00  0.86089887  0.80940124
MANOVA Test Criteria and Exact F Statistics for the Hypothesis of No Overall call Effect

\[ H = \text{Type III SSCP Matrix for call} \]
\[ E = \text{Error SSCP Matrix} \]

\[ S=1 \quad M=0 \quad N=47 \]

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Value</th>
<th>F Value</th>
<th>Num DF</th>
<th>Den DF</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilks' Lambda</td>
<td>0.92867284</td>
<td>3.69</td>
<td>2</td>
<td>96</td>
<td>0.0287</td>
</tr>
<tr>
<td>Pillai's Trace</td>
<td>0.07132716</td>
<td>3.69</td>
<td>2</td>
<td>96</td>
<td>0.0287</td>
</tr>
<tr>
<td>Hotelling-Lawley Trace</td>
<td>0.07680548</td>
<td>3.69</td>
<td>2</td>
<td>96</td>
<td>0.0287</td>
</tr>
<tr>
<td>Roy's Greatest Root</td>
<td>0.07680548</td>
<td>3.69</td>
<td>2</td>
<td>96</td>
<td>0.0287</td>
</tr>
</tbody>
</table>

By looking at the characteristic vector \((0.86, 0.81)'\) for first characteristic root \((0.0768)\), it can be seen that the calling pattern has an effect on age and mass via a vector approximately 45 degrees (slope=1). The p-value of 0.0287 for Wilks’ lambda tells indicates that this effect is significant.

**Conclusion:**
Both the Nonparametric Signed Rank Test and the Generalized \(T^2\) test indicate that population density has a significant effect on mass and length at development. The Generalized \(T^2\) test also indicates that the effect of call length on mass and length at development is relatively significant. Multivariate Analysis of Variance indicates that call length, when its effect is considered for mass and length simultaneously, has a significant effect on mass and length at development.

**References:**