# Phenology of a relict population of *Wyeomyia smithii* (Diptera:Culicidae) in Tattnall Co., GA

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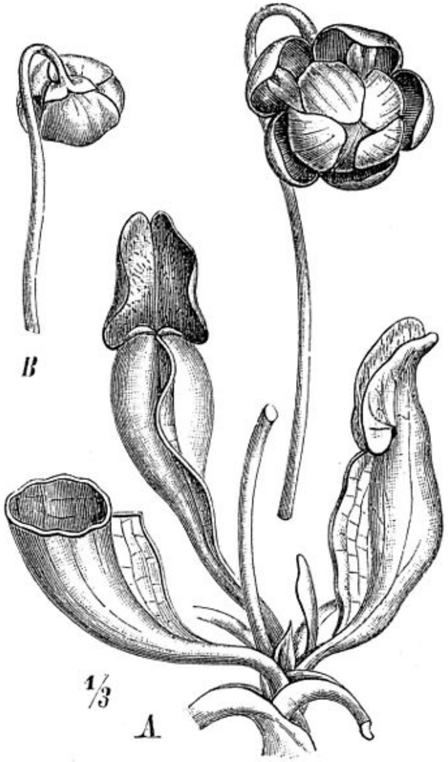
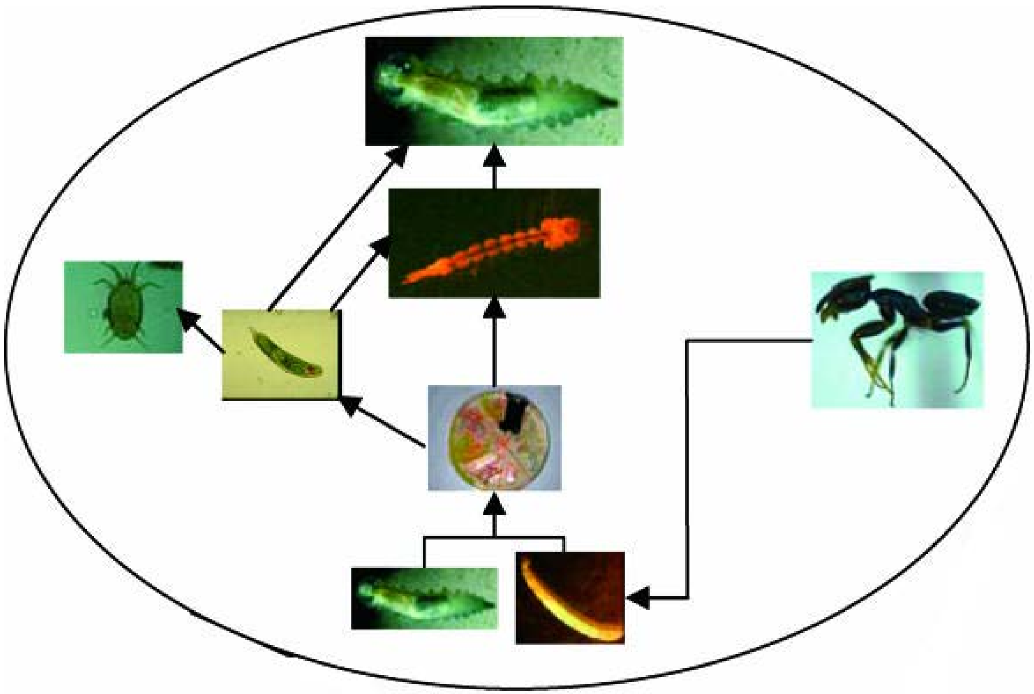
# Introduction to *Sarracenia purpurea*

- Range from Gulf of Mexico to Canada
- Grow in rosettes
- Hosts a diverse inquiline community
- Very rare in Georgia

# Introduction to *Wyeomyia smithii*

- Only found in *Sarracenia purpurea*
- All development occurs inside pitchers
- Nutrient processing chain commensalism



Gotelli and Ellison, 2006.

# Populations differ by latitude

Table 1. Comparison of attributes of Northern and Southern populations of the pitcher-plant mosquito, *Wyeomyia smithii*.

| Northern populations | Southern populations |
|---|---|
| Obligatory autogeny | Facultative autogeny |
| Univoltine | Multivoltine |
| Winter larval diapause | Summer and winter larval diapause |

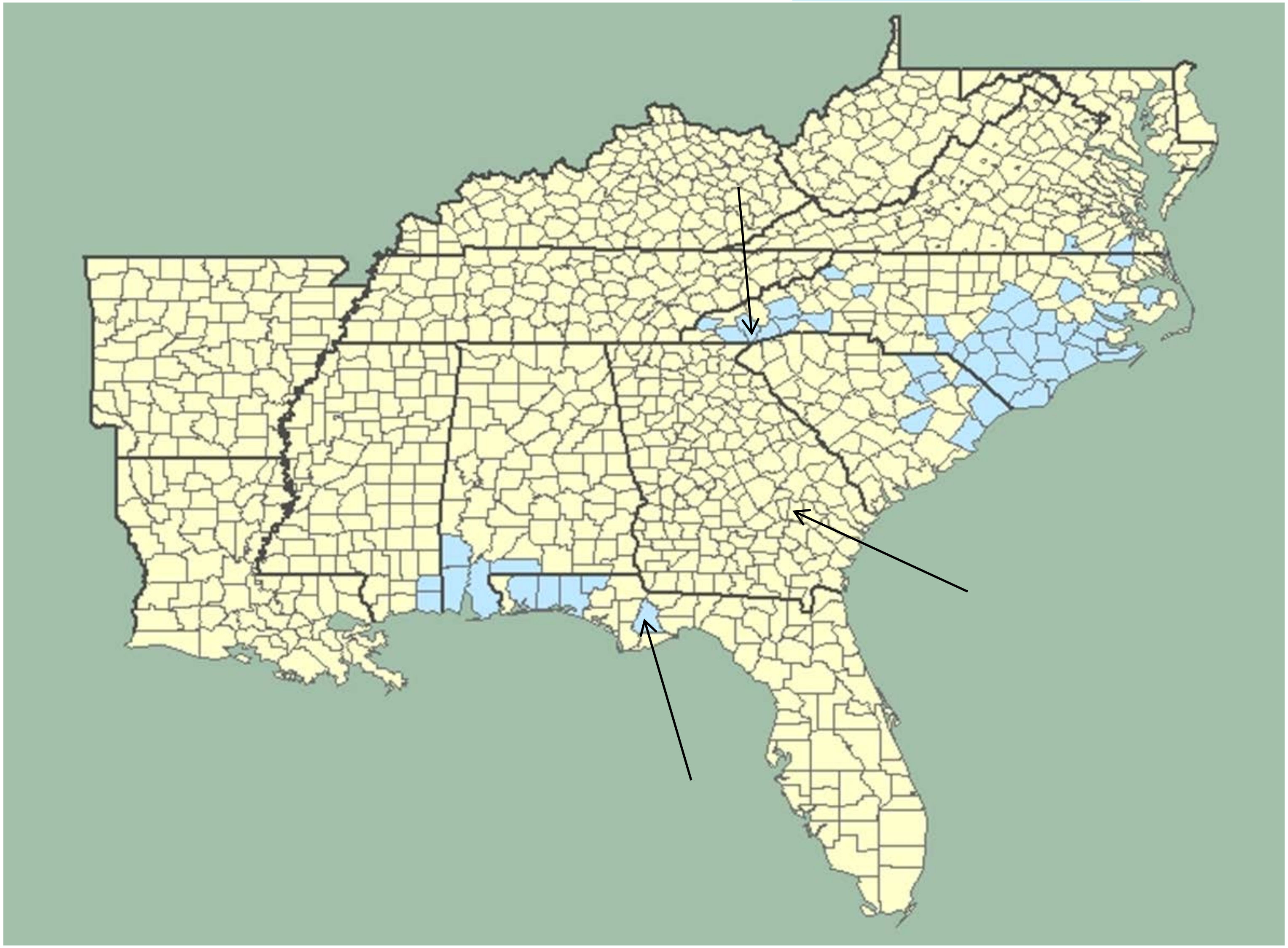
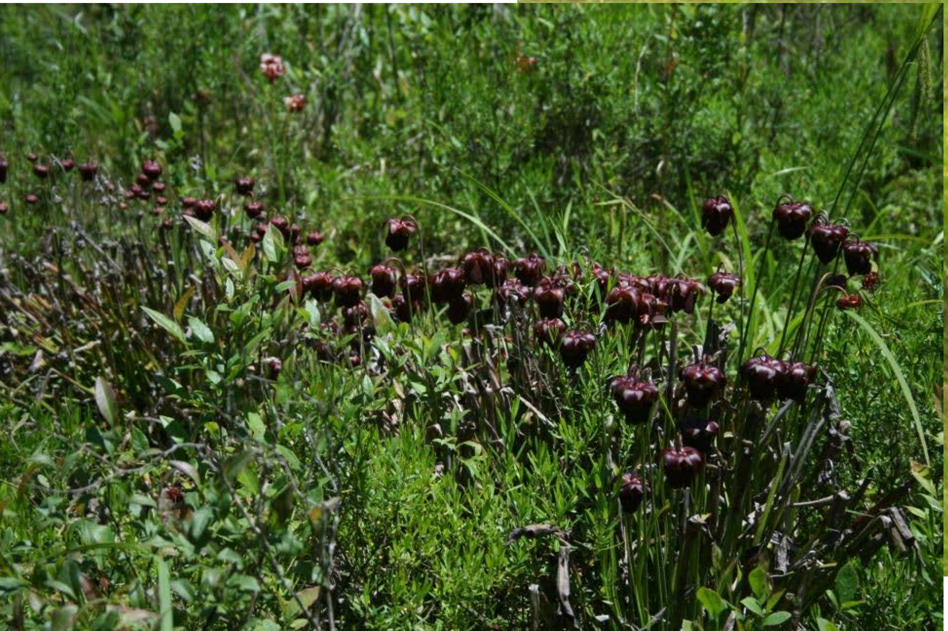
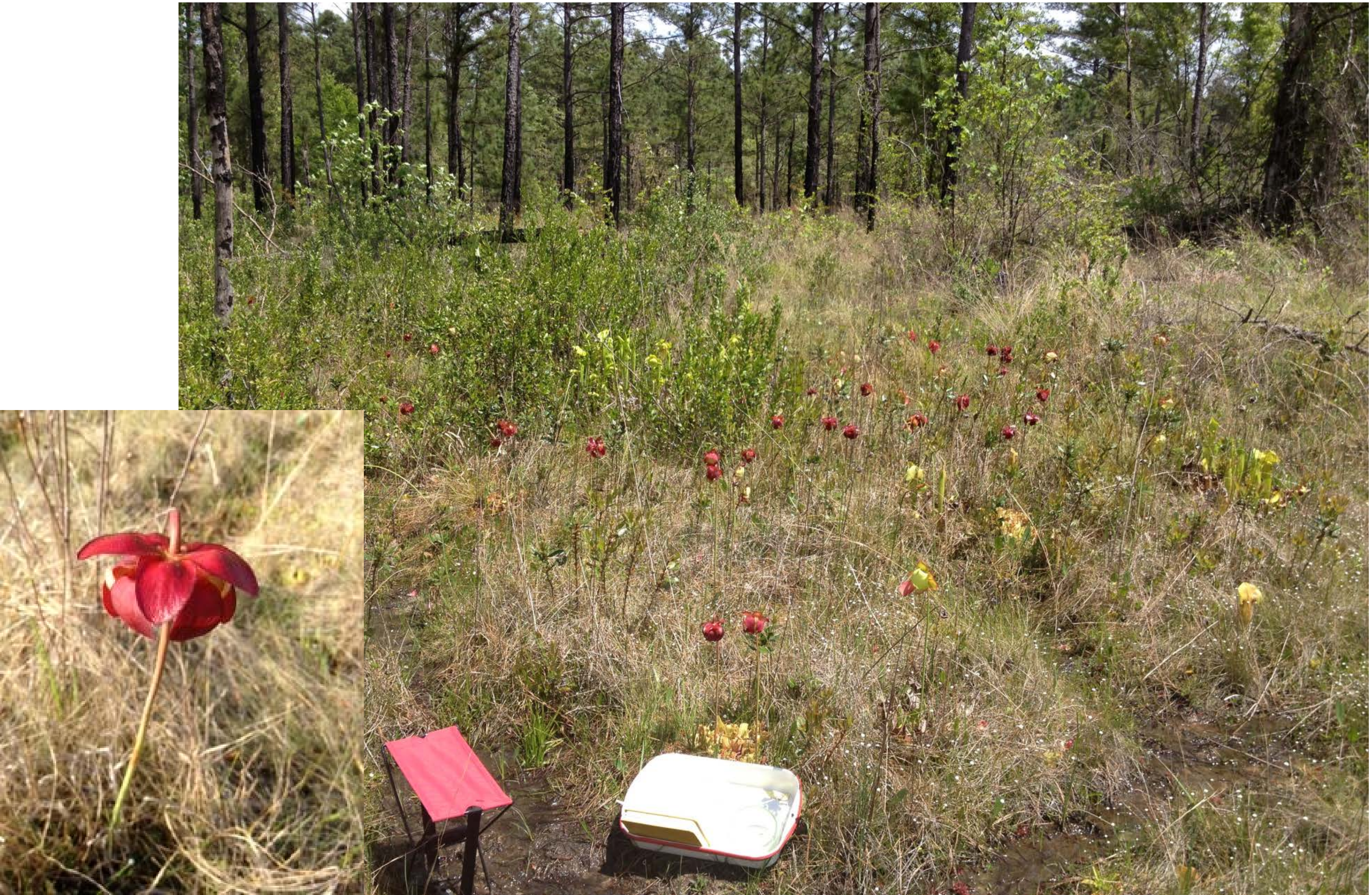


Figure 1. The distribution of S. purpurea in the southeastern United States. Arrows indicate field sites in Florida, Georgia, and North Carolina.

# Site location: Highlands, NC



# Site Location: Tattnall Co., GA



# Site Location: Apalachicola National Forest

# Previous Studies: 1997-2000

- **Behavioral**:
  - Feeding behavior of post-reproductive females assessed by HIC assay
- **Reproductive**:
  - Fecundity of autogenous females measured by dissection
- **Genetic**:
  - Variation in population genetic structure assessed by isozyme analysis
- **Biochemical**:
  - Variation in hexamerin expression assessed by SDS-PAGE

**Table 2**: Blood-feeding behavior of adult female *Wyeomyia smithii* after autogenous egg production during 1998.

| Population | Probing | Blood-feeding |
|---|---|---|
| Florida | + | + |
| Georgia | + | +/-* |
| North Carolina | - | - |

* one female probed, ingested minute quantity of blood;
10 trials, ~50 mosquitoes/trial

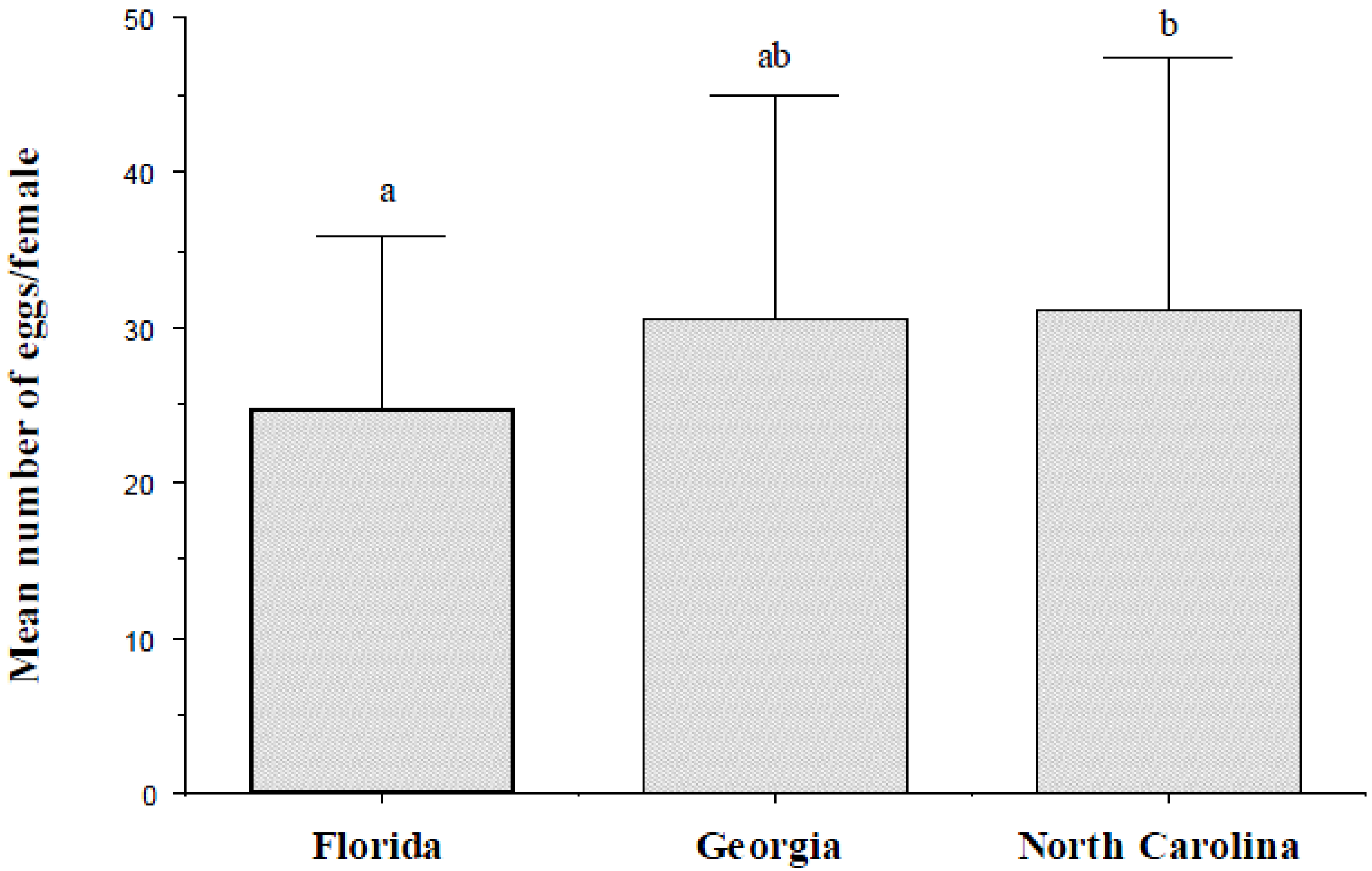



Figure 2. Clutch size for *W. smithii* collected from different locations

**Table 3:** Nei's Genetic Identity (above diagonal) and Genetic Distance (below diagonal) for three populations of *Wyeomyia smithii* (FL, GA, NC) and *Aedes aegypti* (AA) in 1998.

| Population | Populations | | | |
|---|---|---|---|---|
| | FL | GA | NC | AA |
| FL | - | 0.88 | 0.65 | 0.04 |
| GA | 0.13 | - | 0.72 | 0.02 |
| NC | 0.44 | 0.32 | - | 0.19 |
| AA | 3.11 | 3.75 | 1.67 | - |

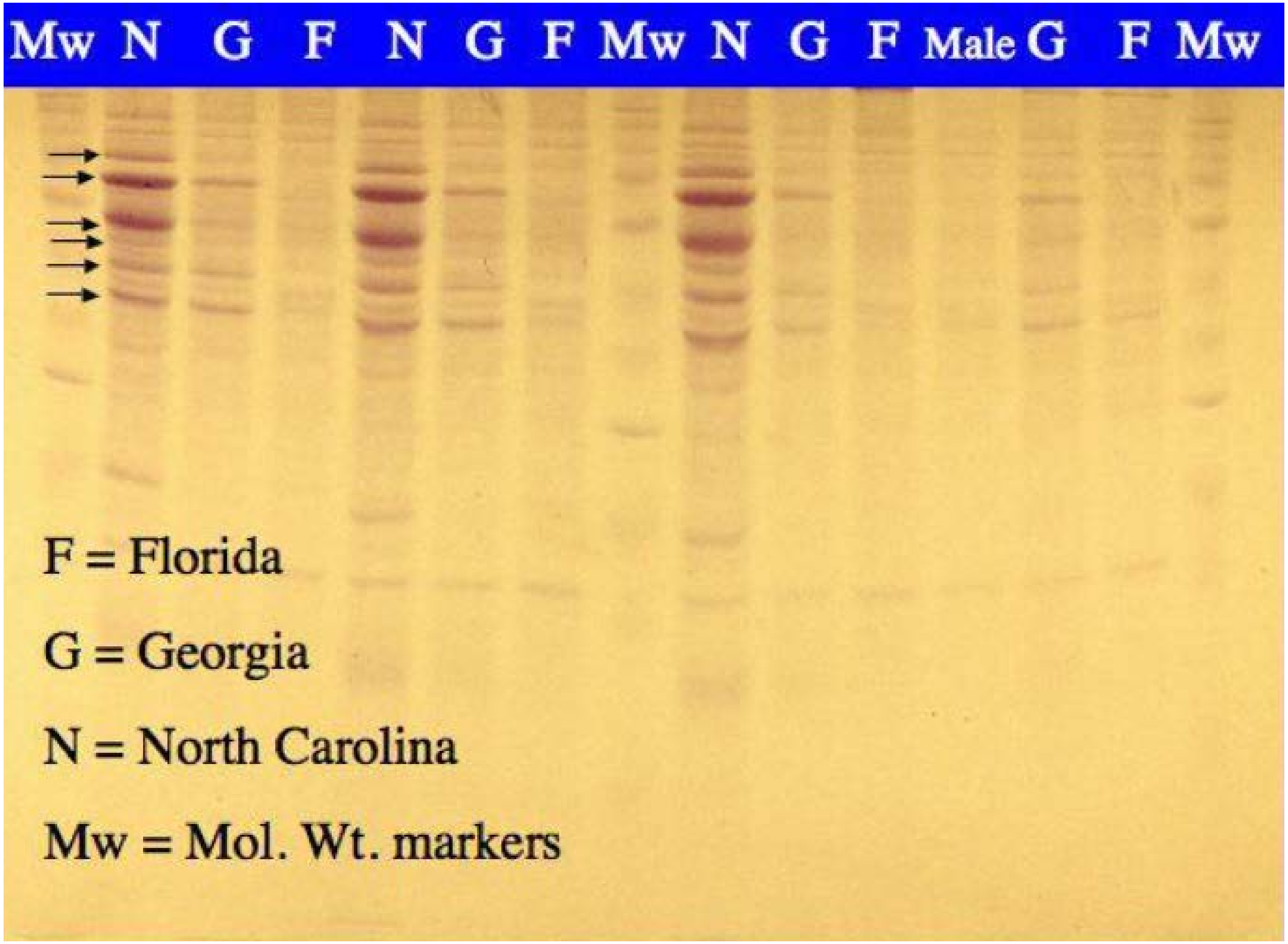
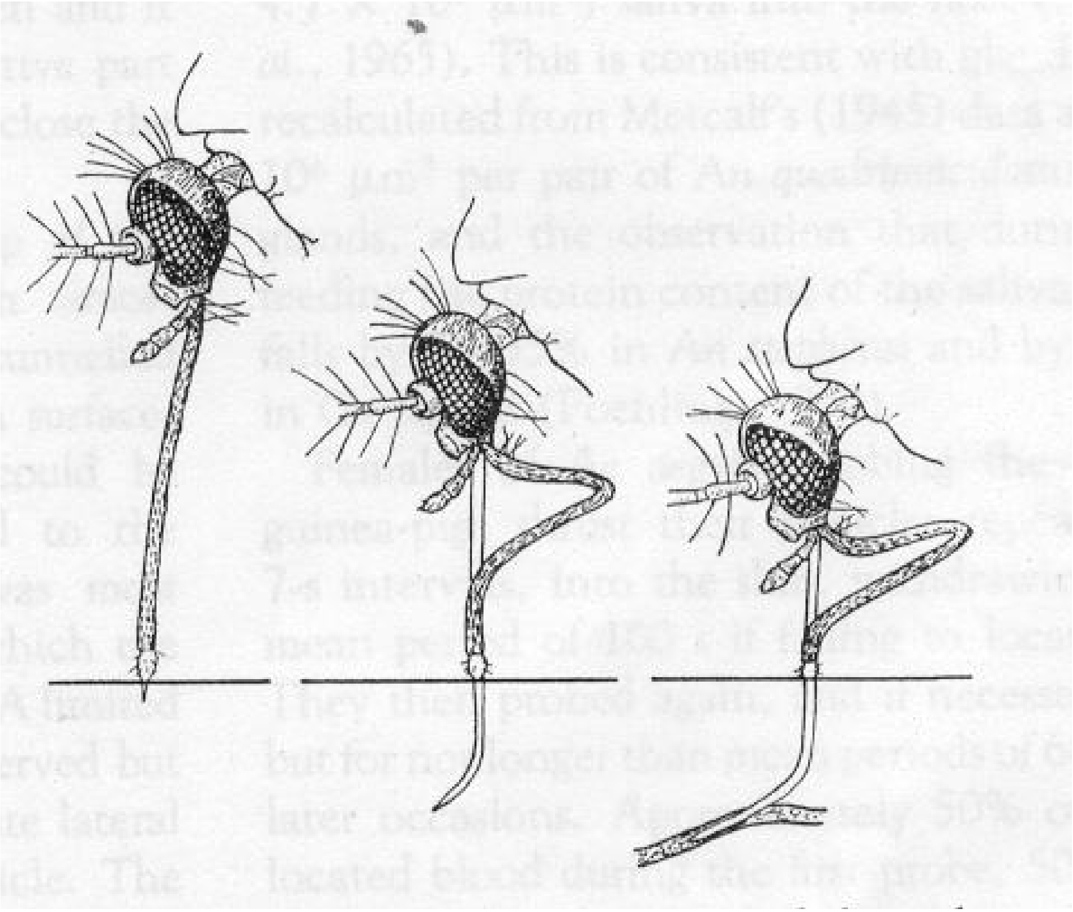



Figure 3. Differential hexamerin expression in adult female *W. smithii*

# Overall results of *W. smithii* studies done in 1997-1998

- Georgia population is **intermediate** in all measures between Florida and North Carolina populations
- Georgia population is more similar **genetically** to Florida population
  - isozymes
  - hexamerin use
- Georgia population is more similar **behaviorally** and **reproductively** to NC population
  - almost complete lack of blood-feeding
  - similar reproductive output

# Timed measures of blood-feeding stages: 2004-2008



Landing→ Exploration Probing Feeding

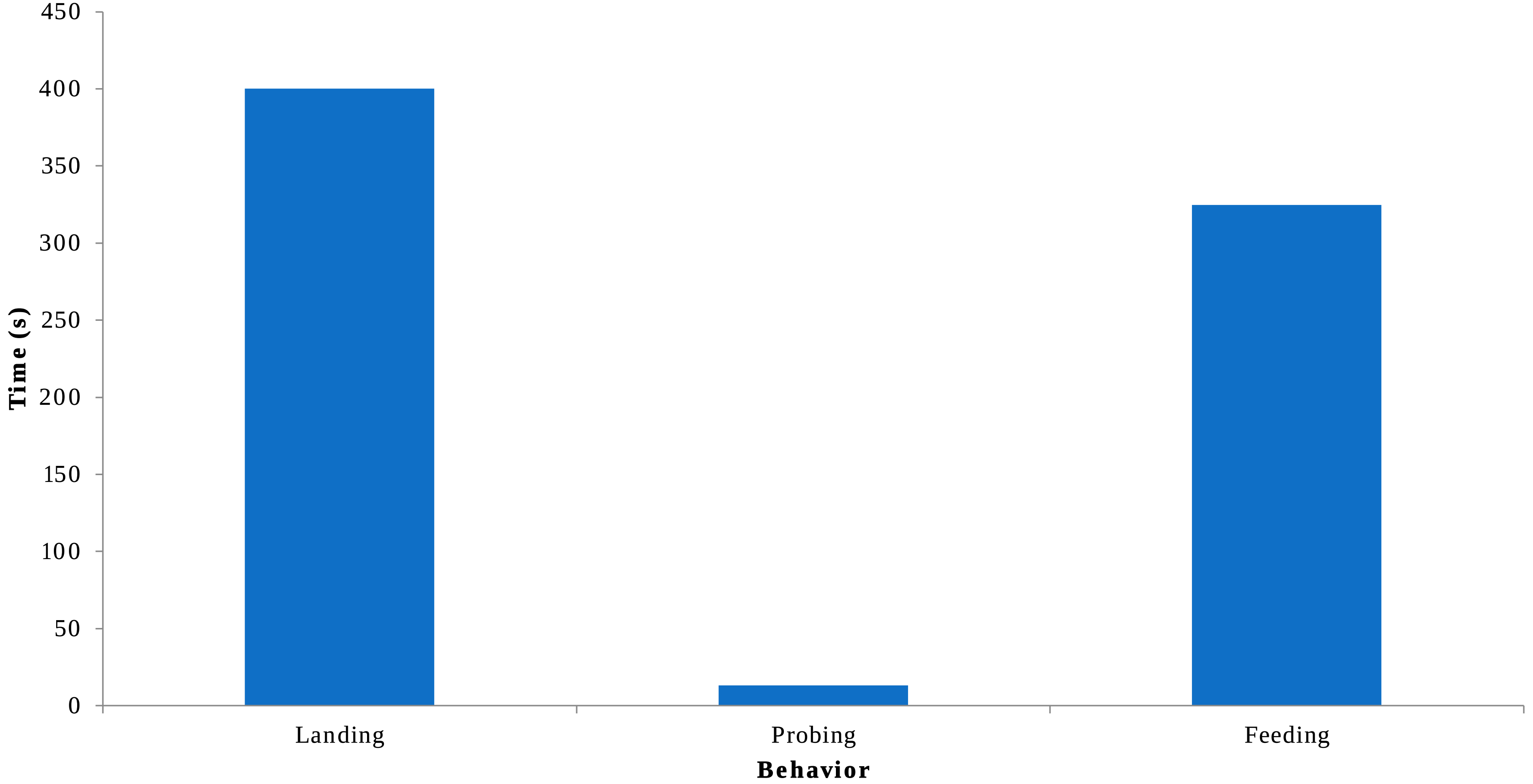



Figure 4. Mean duration of feeding behaviors of *W. smithii* in 2004

Table 4. Mean duration of blood feeding phases of W. smithii in 2004

| Species | Host | Mean Duration of Phase (seconds) | | | |
|---|---|---|---|---|---|
| | | Exploratory (Landing) | Probing | Feeding | |
| *Wy smithii* | Man | (400.1) | 13.32 | 324.7 | |
| *Ae aegypti* | Mouse | - | 42 | 213 | |
| | Guinea-pig | - | 56 | 132 | |
| | Man | 3 | 68 | 220 | |
| *Ae africanus* | Man | - | 32 | 80 | |
| *Ae cinereus* | Man | 11\| | 25 | 82 | |
| *Ae cantans* | Man | 8 | 28 | 150 | |
| *Cq richiardii* | Man | 16 | 92 | 162 | |
| *An plumbeus* | Man | 31 | 40 | 99 | |

Data other than for *W. smithii* from Clements, 1999.

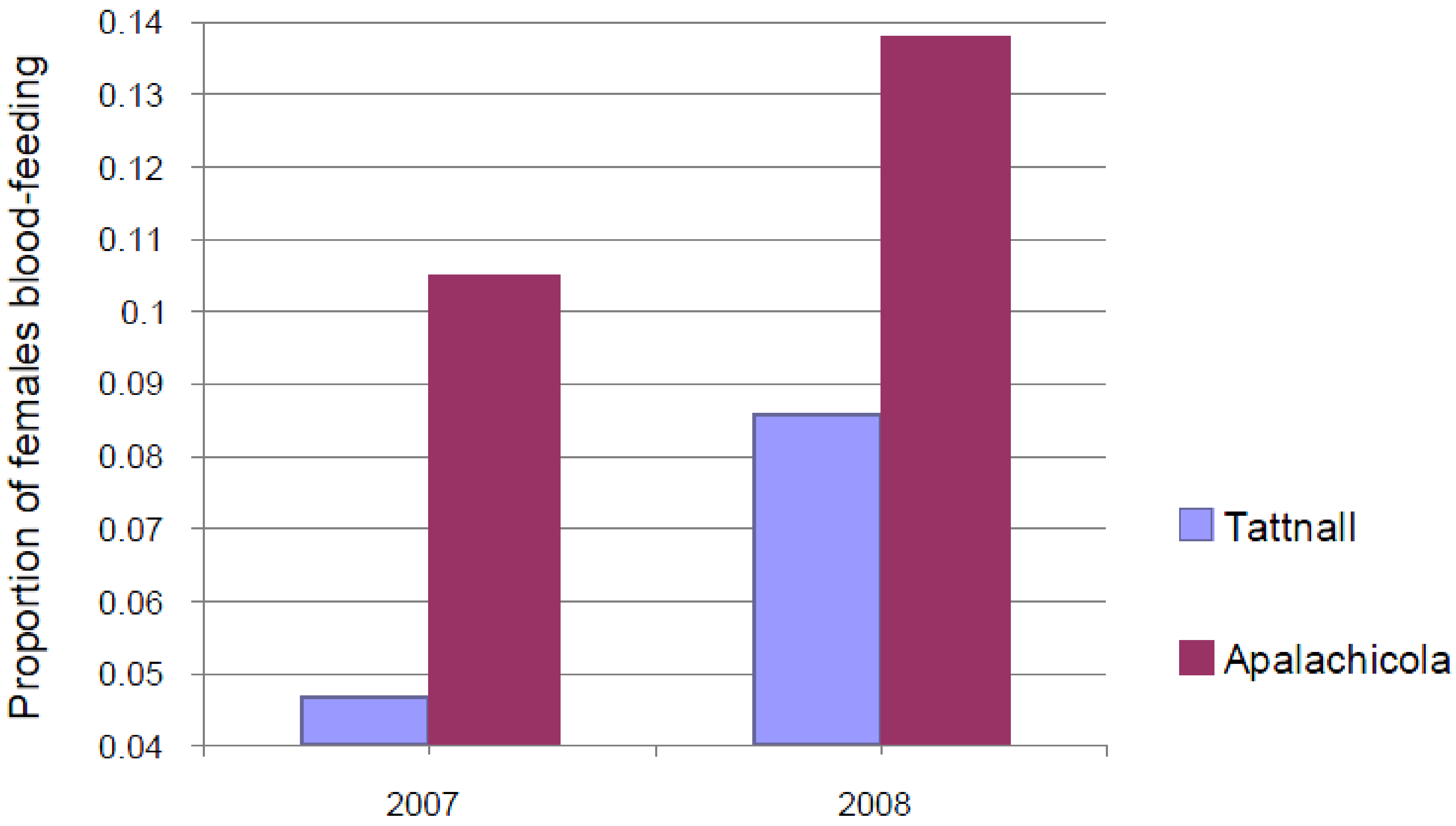



Figure 4. Results of cage blood feeding trials of *W. smithii* in 2007 and 2008

Table 5. Results of blood-feeding trials* for post-autogenous adult female *Wyeomyia smithii* from 2004-2011

| Population | Ave. % Feeding (± SD) by year | | | | |
|---|---|---|---|---|---|
| | 1998 | 2004 | 2007 | 2008 | 2011 |
| Apalachicola | +** | ND*** | 8.0 (7.3) | 13.8 (11.2) | 26.0 (15.1) |
| Tattnall | ± | 2.3 (1.3) | 3.8 (6.2) | 8.6 (5.7) | 20.8 (15.7) |
| Highlands | 0 | ND | 0 | 0 | 0 |

*6-12 15 minute feeding trials/population, 20-125 female mosquitoes/trial, all blood-fed specimens removed after each trial

**In feeding trials with mosquitoes from Apalachicola, FL, mosquitoes were observed to blood-feed; in trials with Tattnall Co., GA mosquitoes, one female probed but did not feed

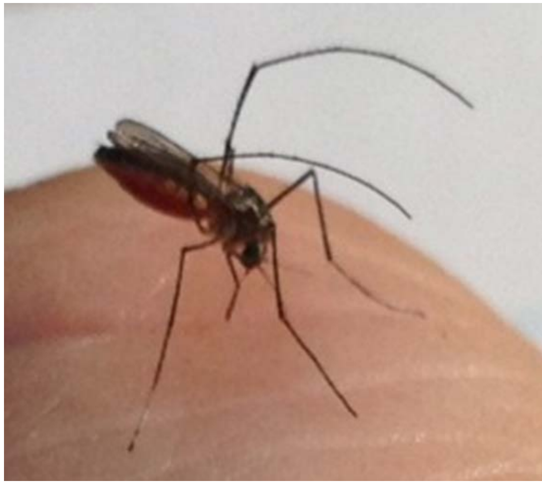
***not done

# Overall results, 2004-2011:

- Georgia mosquitoes are increasing:
  - frequency of blood-feeding (1.1 → 20.8%)
  - success at blood-feeding (albeit slowly):
    - 2004: partially engorged, ¼ contain clear fluid
    - 2007: fully engorged, 1/10 contain clear fluid
    - 2008: fully engorged, all contain blood
- Blood-feeding rate increasing in Florida as well

# Current work:Bionomic Analysis

- Weekly surveys of larvae in pitchers
  - Six pitchers from six rosettes
- Measured volume in each pitcher
- Counts of larvae at each instar
- Blood-feeding behavior recorded





# Bionomic Analysis Results

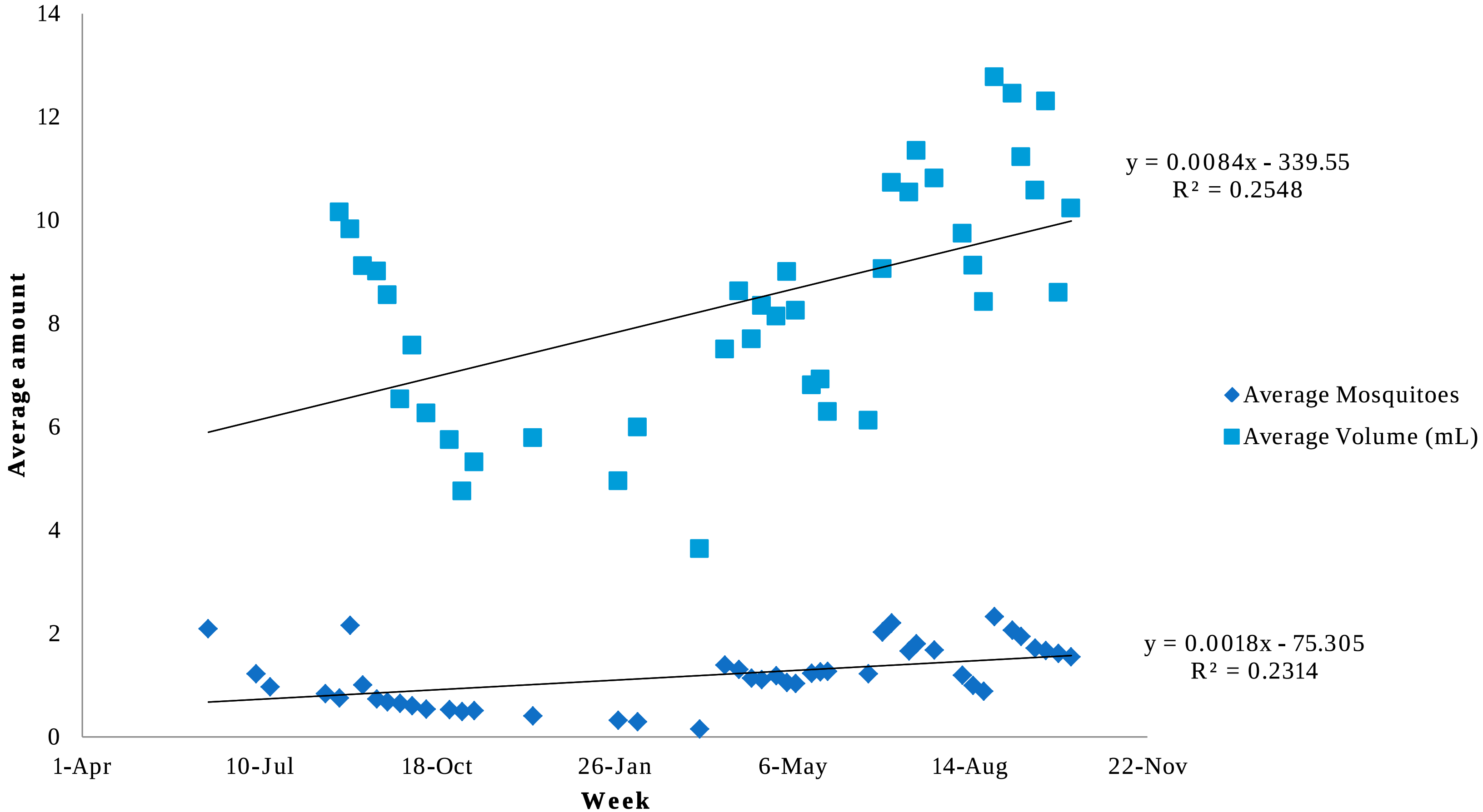



Figure 5. Regression of the average amounts of *W. smithii* larvae with the average volume in pitchers over time

# Bionomic Analysis Results

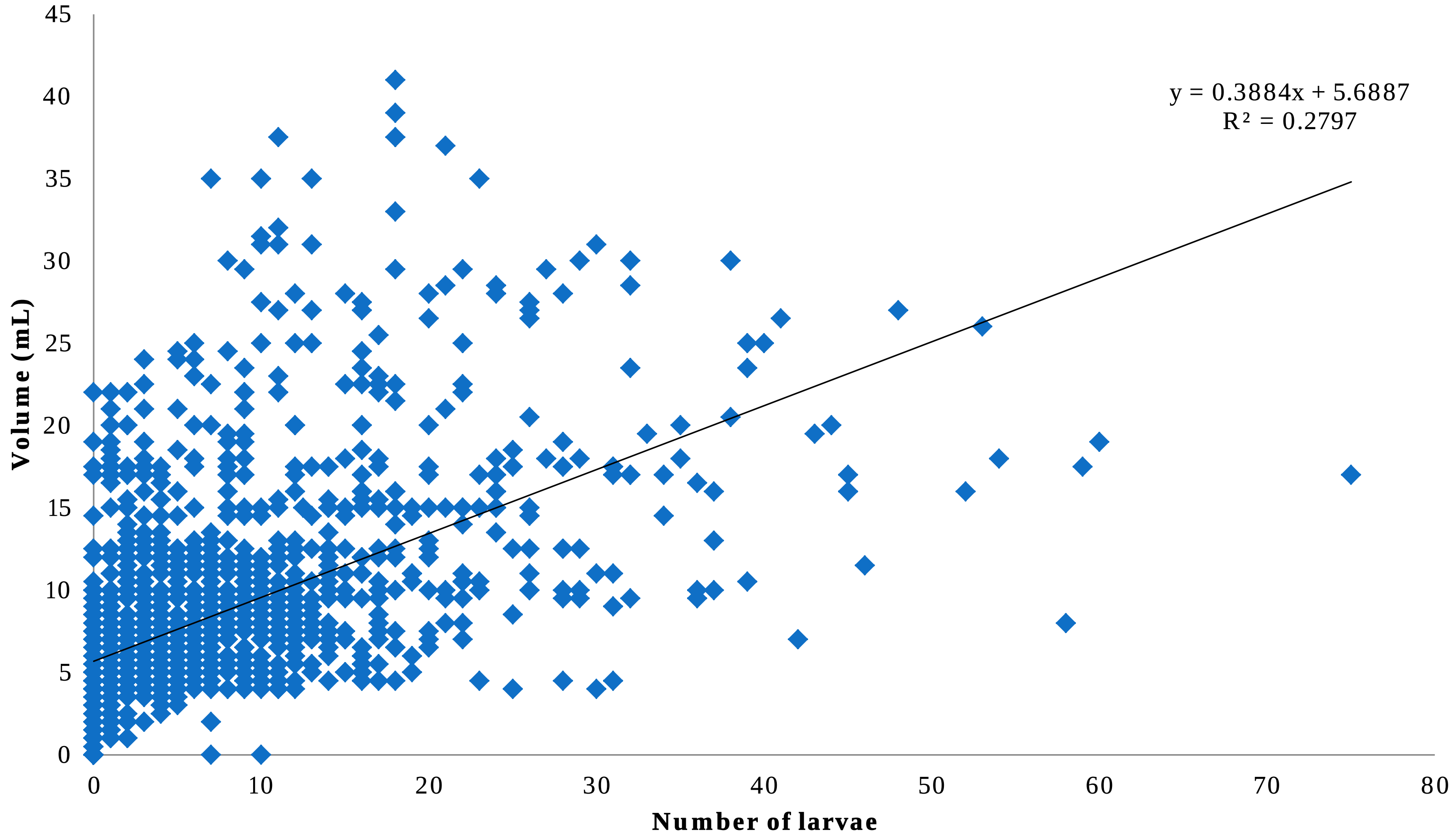



Figure 6. Correlation of the amount of *W. smithii* larvae with the volume of the pitcher

# Blood Feeding Behavior

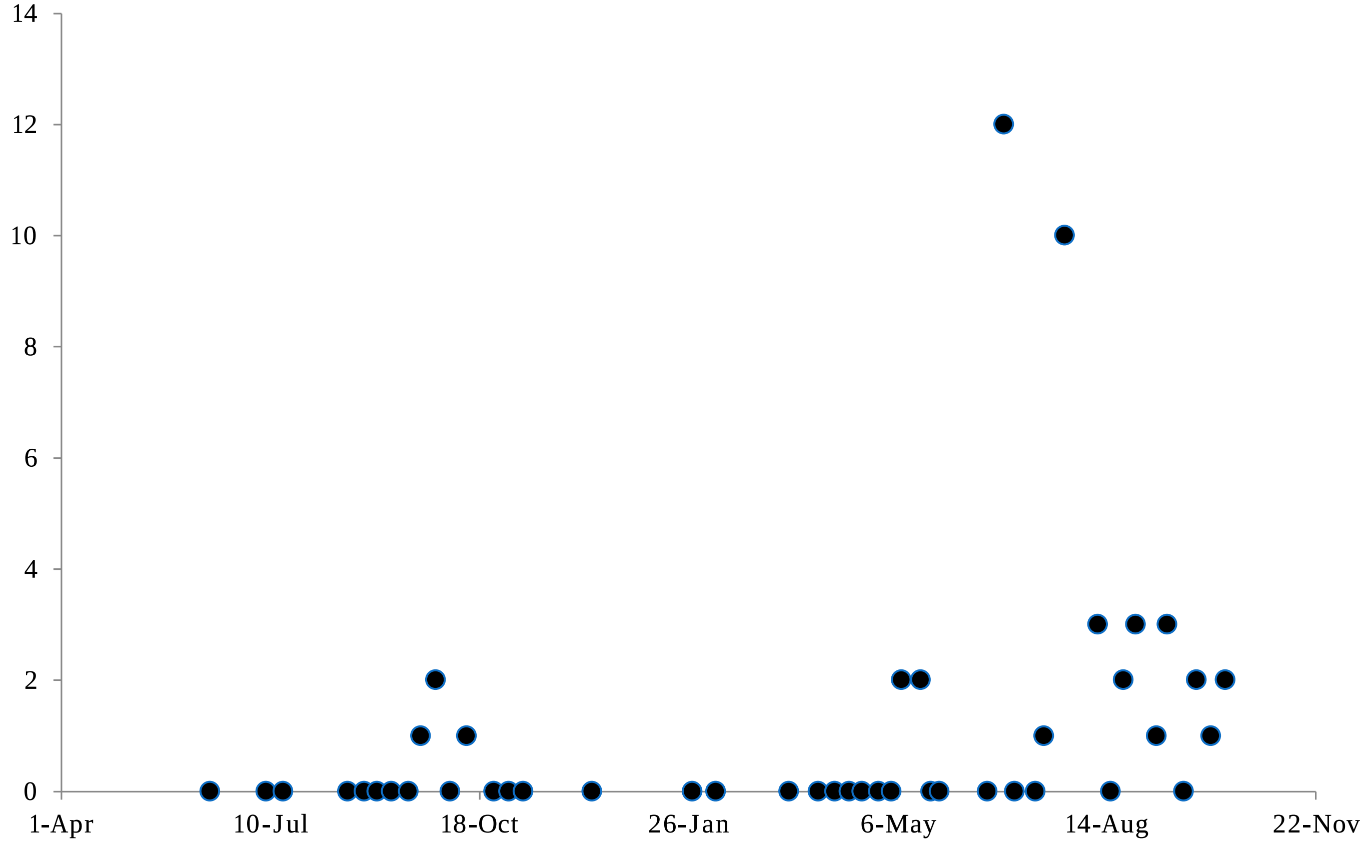
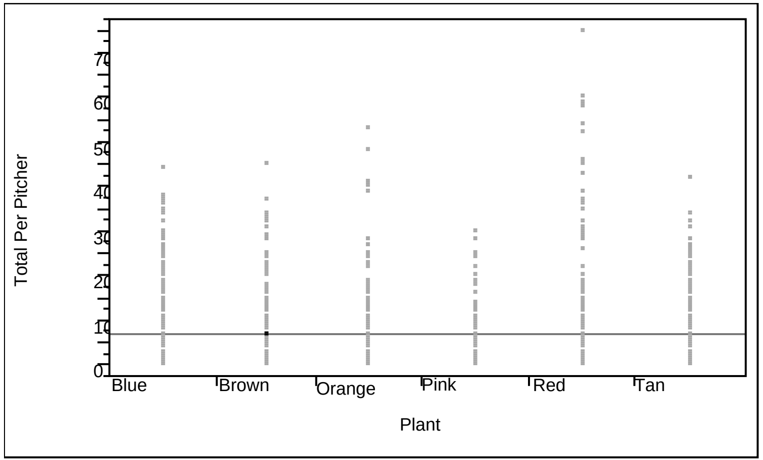



Figure 7. Number of times blood meals were taken by female W. smithii per week

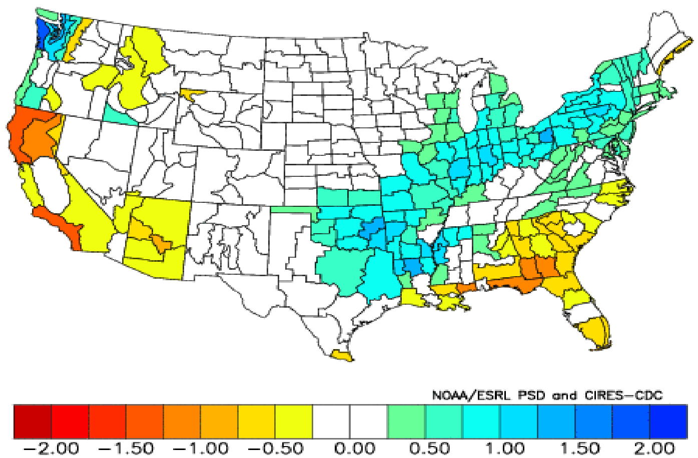
# Statistical Analyses

- Spearman's Correlation
  - Spearman ρ 0.7841, Prob>|ρ| <0.0001
  - Strong correlation between volume of fluid in pitchers and number of mosquitoes present
- Kruskal-Wallis Test
  - H- 84.3877, DF- 5, Prob>H- <0.0001
  - Distributions of larvae have different locations by rosettes.

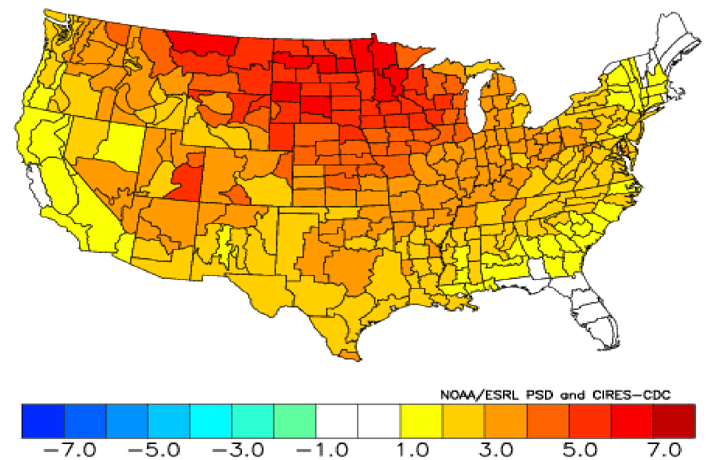


# Climatic Data

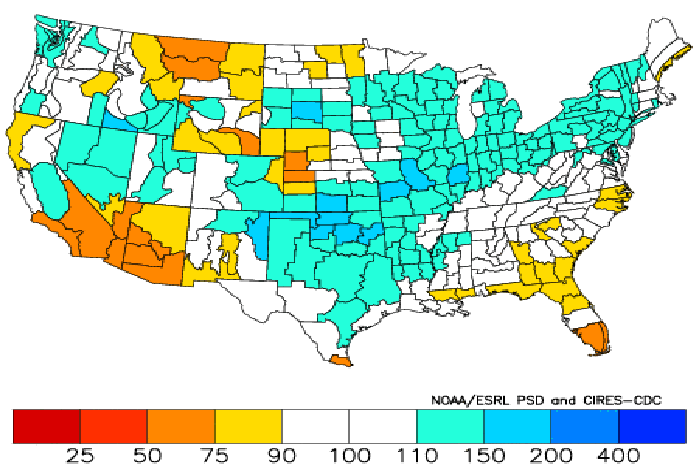
Composite Precipitation Anomalies (inches)
Jan 1998 to 2007
Versus 1950–1995 Longterm Average



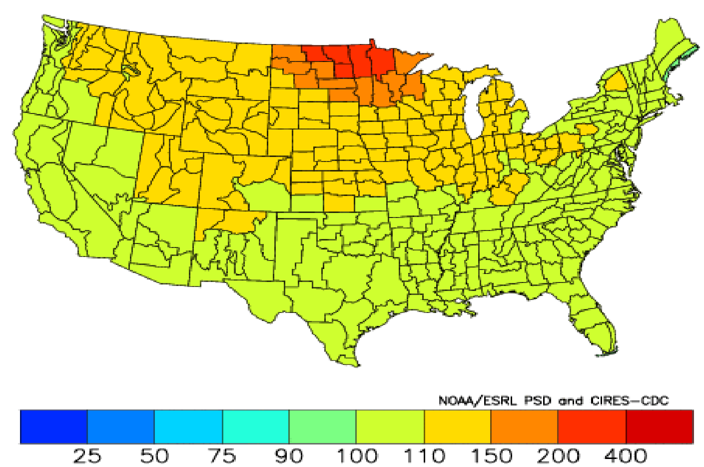

Composite Temperature Anomalies (F)
Jan 1998 to 2007
Versus 1950–1995 Longterm Average




Composite Percent of Normal Precipitation 1950–1995
Jan 1997 to 2007




Composite Percent of Normal Temperature 1950–1995
Jan 1997 to 2007



# Population Genetics Analysis

- Assessed via isozyme analysis of isocitrate dehydrogenase (IDH) using polyacrylamide gel electrophoresis (PAGE)
- Evaluations between 6-12 from each of 9 plants
  - 4th instar larvae
  - Collected from field and reared at 25°C, 16L:8D

# Population Genetics Results

Table 6. Observed heterozygotic frequencies and expected heterozygotic frequencies of W. smithii

| Subpopulation (S) | Samples (N) | Observed Heterozygotic Frequencies | Expected Heterozygotic Frequencies |
|---|---|---|---|
| **S1** | 6 | 0 | 0 |
| **S2** | 12 | 0.08333 | 0.21875 |
| **S3** | 9 | 0.33333 | 0.27777 |
| **S4** | 9 | 0.44444 | 0.34567 |
| **S5** | 9 | 0.11111 | 0.10492 |
| **S6** | 12 | 0.16666 | 0.15277 |
| **S7** | 11 | 0.36666 | 0.29752 |
| **S8** | 9 | 0 | 0 |
| **S9** | 11 | 0.18888 | 0.16528 |

# Population Genetics Results

Figure 8. Resolution of IDH -1 (monomorphic) and IDH -2 (polymorphic) alleles using PAGE

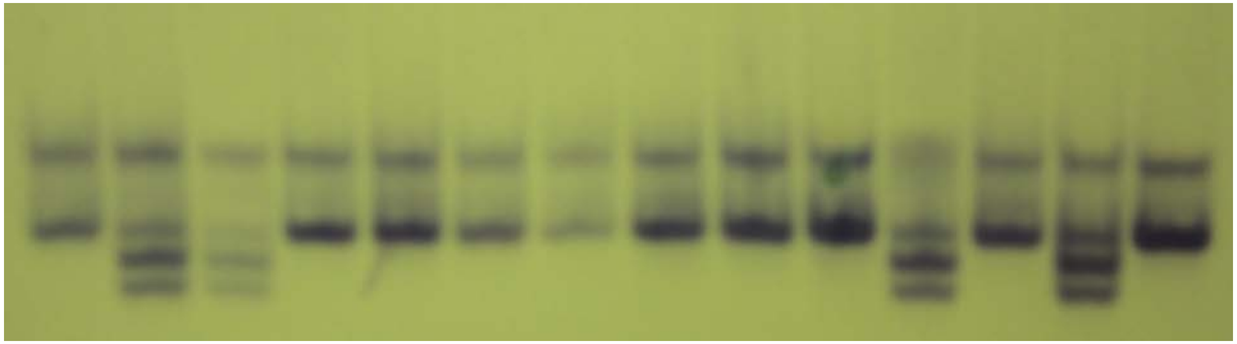


Table 7. F-statistics for comparison of population genetic structure for pitcher plant larvae within subpopulations (in pitchers within rosettes) and between rosettes

| Site | $F_{IS}$ | $F_{ST}$ | $F_{IT}$ |
|---|---|---|---|
| Tattnall, GA | -0.057 | 0.049 | -0.005 |

# Conclusions

- Low heterozygote frequencies for IDH
  - F-statistics indicate high level of inbreeding
- Bionomics shows coincidental declines in larvae and pitcher volume
  - Reflective of low volume
  - Volume strongly correlated to total larvae
  - Distributions of larvae differ by plant
- Blood feeding behavior is increasing in the field
- Population of *W. smithii* is at risk
  - Long term drought and increasing temperatures
  - Reduction of *S. purpurea*

# Acknowledgements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