Re-evolving a taste for blood: Climate change and blood-feeding behavior in pitcher plant mosquitoes

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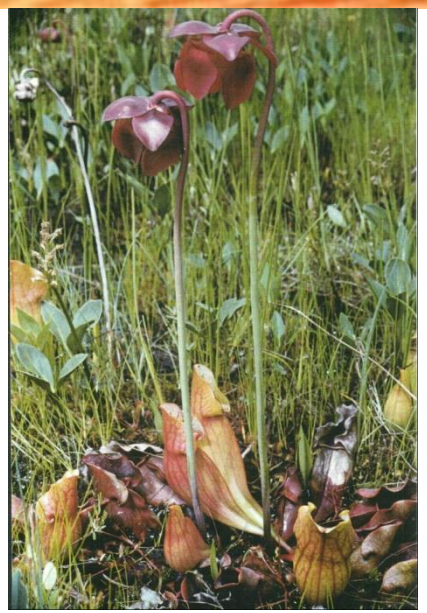


Wyeomyia smithii and *Sarracenia purpurea*: the pitcher plant mosquito and the purple pitcher plant

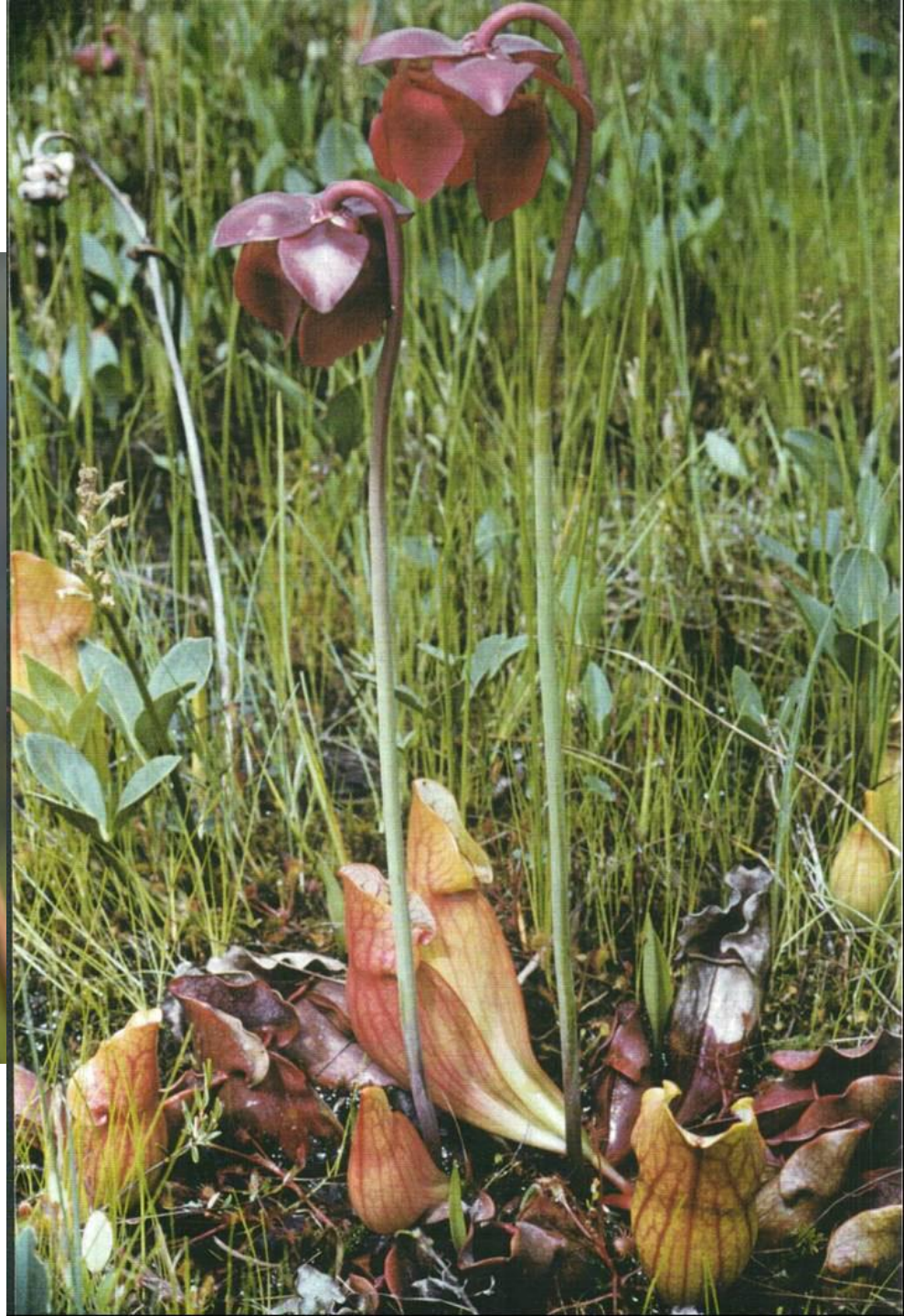
Distribution of *Sarracenia purpurea* and *Wyeomyia smithii*

The pitcher plant mosquito is found throughout the range of its host plant

Purple Pitcher Plant, *Sarracenia purpurea*

Sarracenia purpurea from Tattnall County, Georgia

Flowers of *Sarracenia purpurea*



Wyeomyia

Wyeomyia blood-feeding

Evolution & Ecology of Pitcher Plant Mosquitoes

- *Wyeomyia smithii* and *Sarracenia purpurea*
- Model system for:
  - Island biogeography
  - Nutrient processing chain commensalism
  - Evolution of autogeny: *Losing the blood feeding habit*
    - Autogeny: development of eggs without a blood meal
      - ***Obligatory autogeny***: never feed on blood
      - ***Facultative autogeny***: blood-feed after first egg batch laid
- Is blood-feeding behavior re-evolving due to climate change?

Distribution of *Sarracenia purpurea* and *Wyeomyia smithii*

The pitcher plant mosquito is found throughout the range of its host plant

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

1997:

- relict population of *Sarracenia purpurea* and *Wyeomyia smithii* located in Tattnall Co., GA (near Claxton) discovered

- studies begun on:

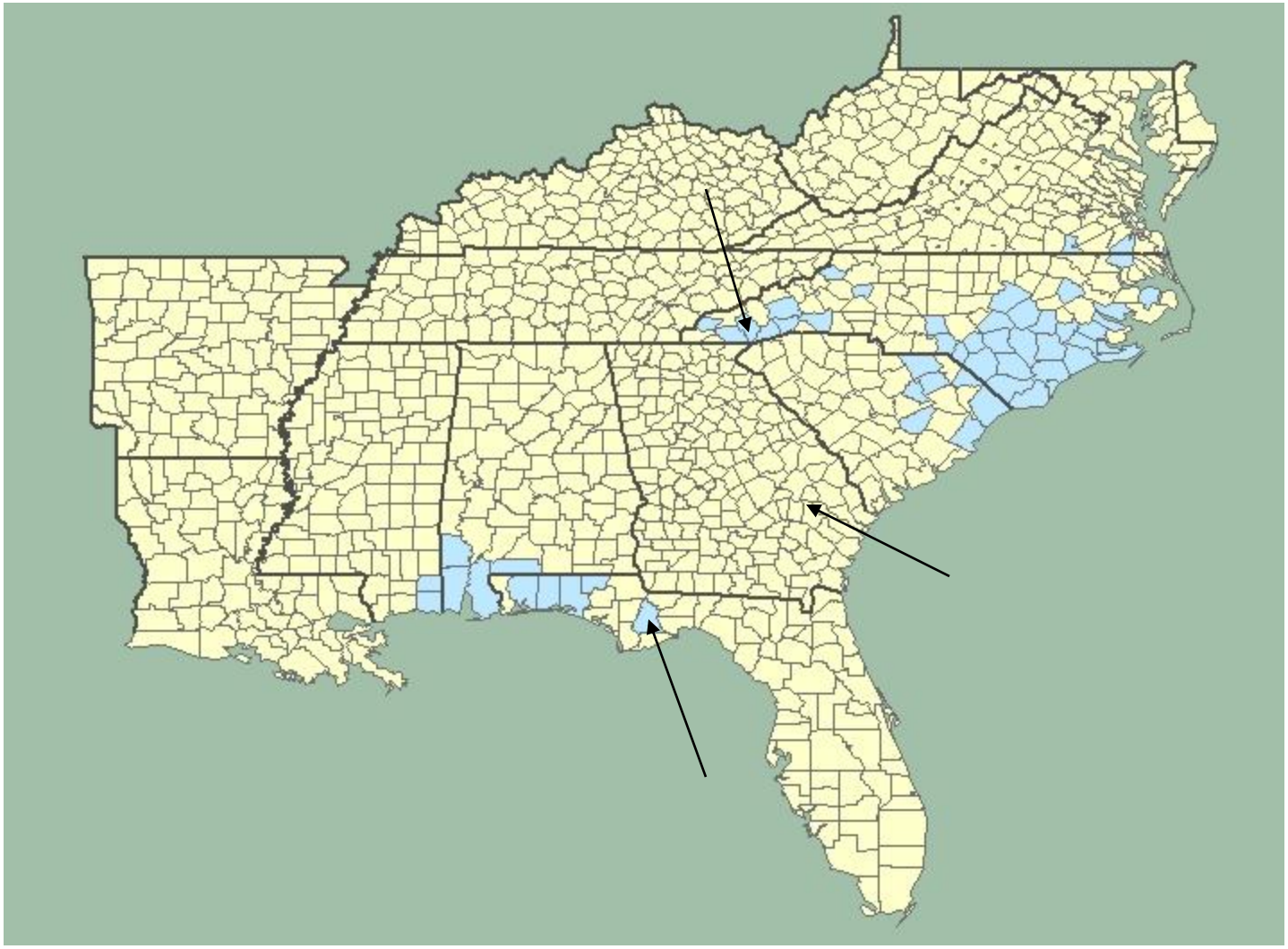
  - **feeding behavior**
  - **fecundity** (autogenous egg production)
  - population **genetic structure**
  - **hexamerin** (storage protein) accumulation and depletion

- Tattnall Co. population of mosquitoes compared to:

  - **Southern** population: Apalachicola NF, FL
  - **Northern** population: Highlands BRS, NC

- Is there ***clinal variation*** in these populations? *Yes!*

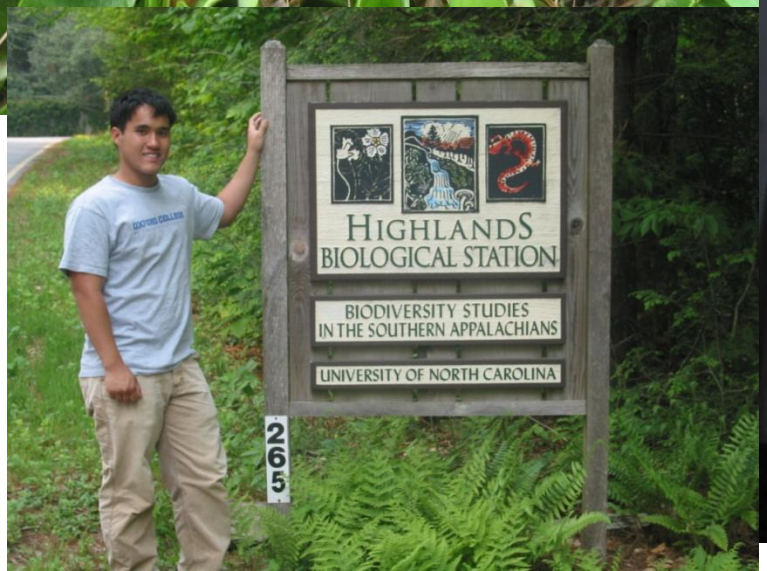
Distribution of *Sarracenia purpurea* and *Wyeomyia smithii*



Distribution of *Sarracenia purpurea* (and *Wyeomyia smithii*) in SEUS
Locations of FL, GA, and NC populations sampled are shown

Northern Population

Highlands, North Carolina
Highlands Biological Research Station
- Appalachian bog habitat



Southern Population: Apalachicola National Forest, FL

Longleaf pine forest habitat, Apalachicola NF, Florida

S. purpurea X *S. flava* hybrid

Tattnall County, Georgia
→ Sandhill habitat with isolated relict populations

Mosquito Collection & Rearing:

- 1000-2000 larvae and pupae/trip collected via pipette from each location
  - 1997, 1998, 2004, 2007, 2008, 2009

- reared at 26^{o}C, 16:8 L:D; fed excess Tetramin fish flakes

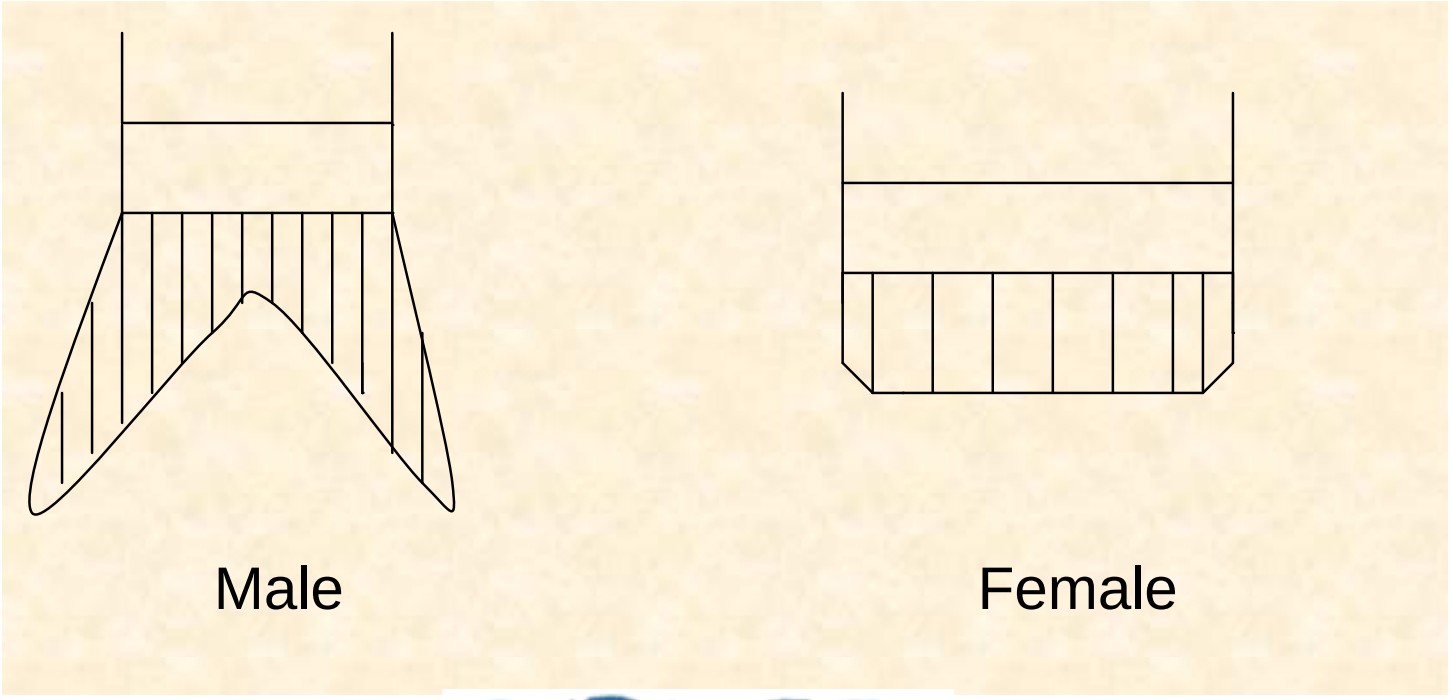
- upon pupation, transferred to cages, kept under same regime at 80% RH; provided with 10% sugar water
  - some pupae sexed for hexamerin studies

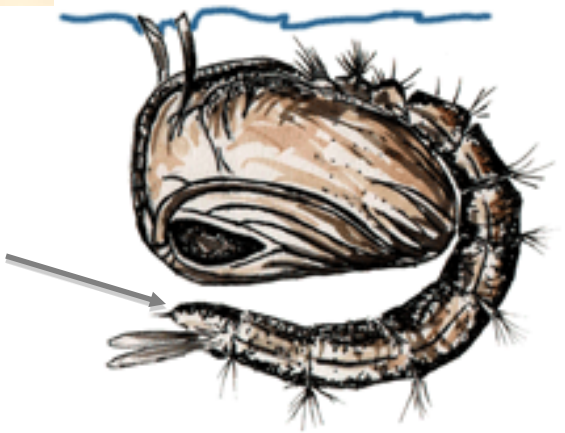
- for feeding studies:
  - observations of feeding behavior begun after autogenous egg production observed (7-10 days after adult emergence)
  - 50 – 250 mosquitoes in each cage during trials

Rearing *Wyeomyia* larvae in the incubator

Adult cages: 80% RH via saturated NaCl solution

Distinguishing between male and female pupae of *Wyeomyia smithii* via genital tubercle morphology





Studies conducted during 1997-1999

- **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

Hand-In-Cage (HIC) Assay

HIC Assay

Feeding trials were conducted for 15 minutes .

Table 3: 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

Fig. 4: Clutch sizes for pitcher plant mosquitoes

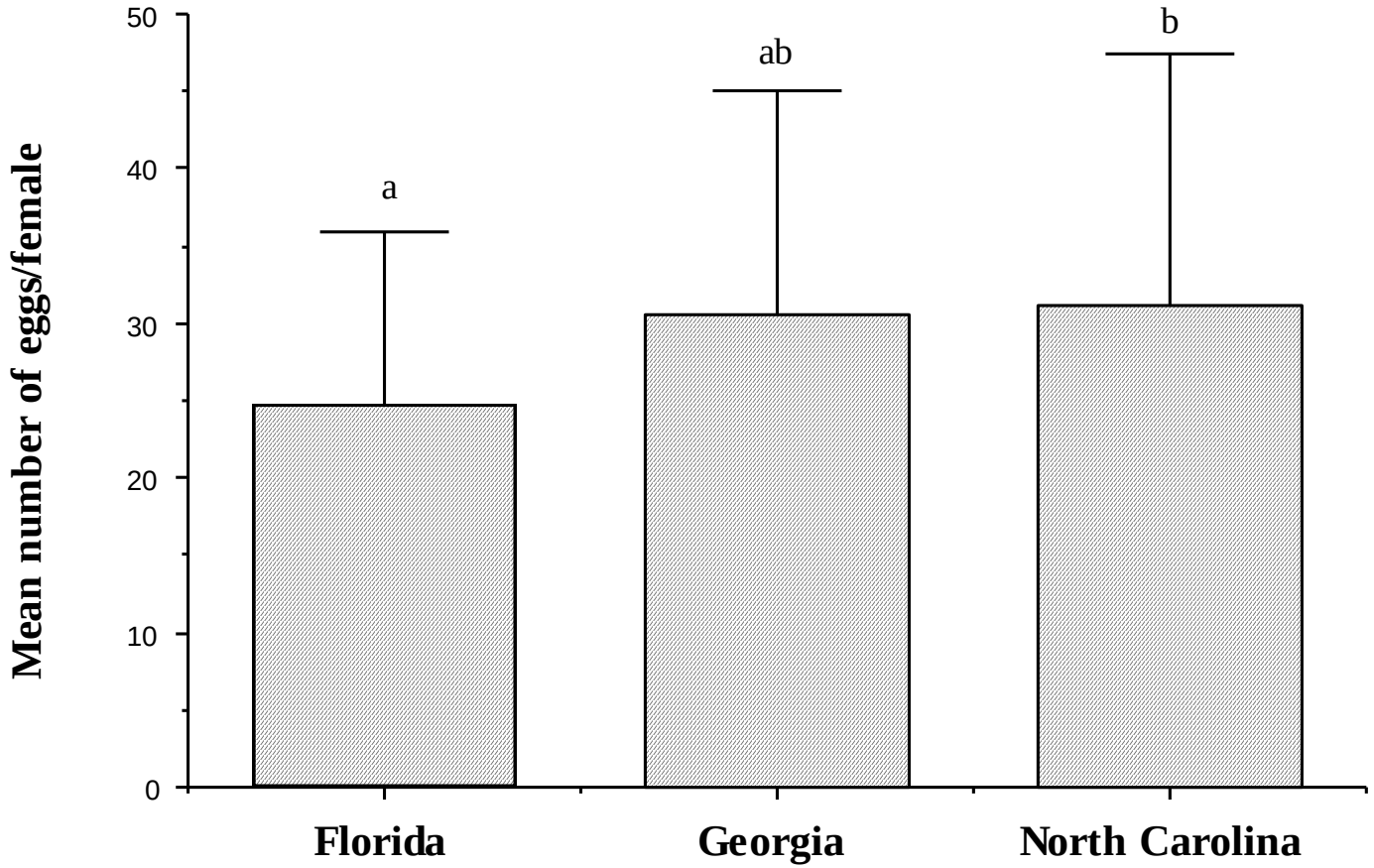


Table 2: Enzymes resolved for adult *Wyeomyia smithii*.

Enzyme	Abbreviation
Malic enzyme	ME
Malate dehydrogenase	MDH
Hexokinase	HK-1, -2
Glucose phosphate isomerase	GPI
Glucose-6-phosphate dehydrogenase	G-6-PDH
Phosphoglucose mutase	PGM
Isocitrate dehydrogenase	IDH-1, -2

30-40 specimens/population analyzed/enzyme

Glucose phosphate isomerase polymorphism in pitcher plant mosquitoes (*Wyeomyia smithii*)

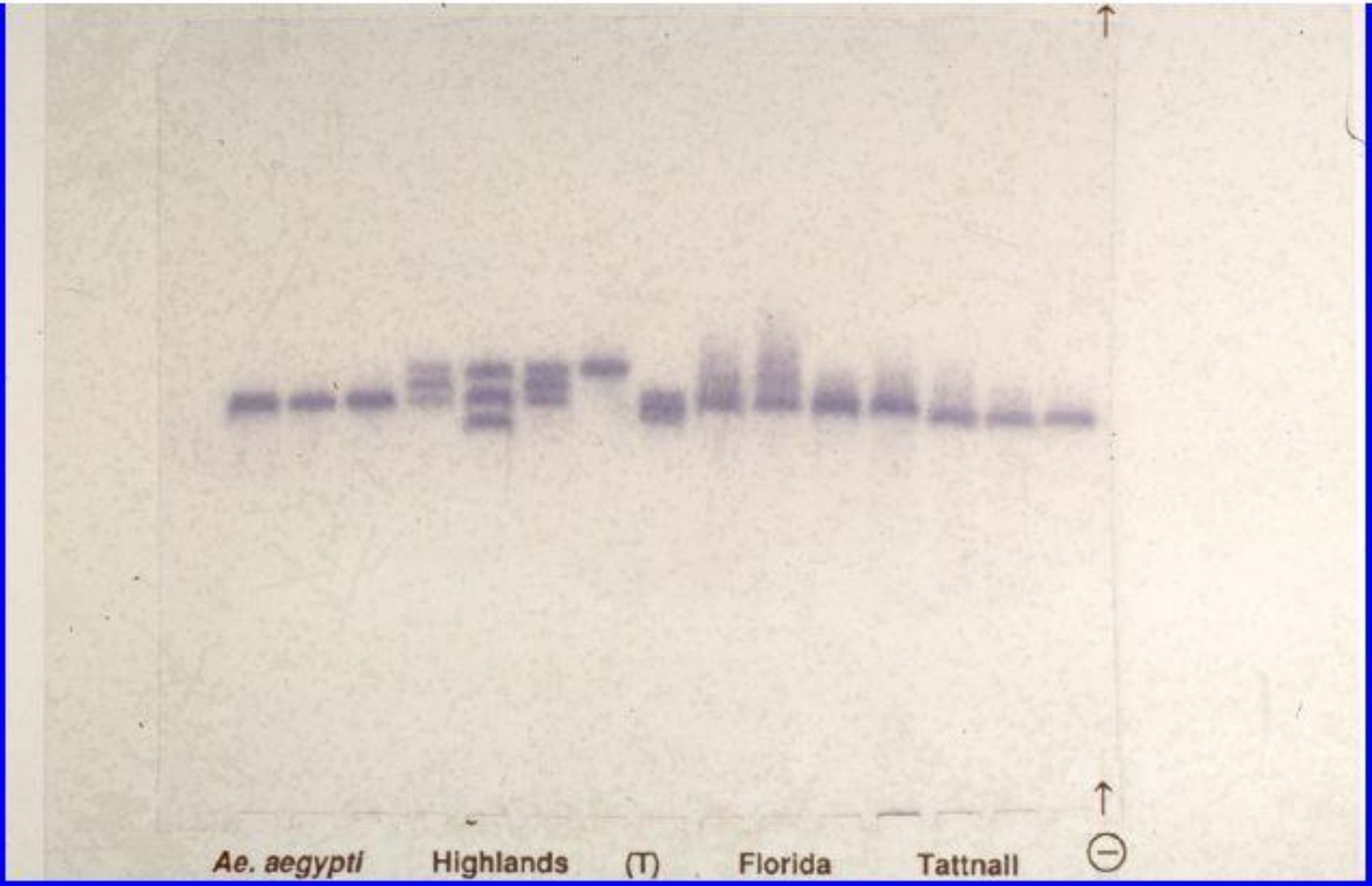


Table 5: Nei's Genetic Identity (above diagonal) and Genetic Distance (below diagonal) for three populations of *Wyeomyia smithii* (FL, GA, NC) and *Aedes aegypti* (AA) in 1998.

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	-

Differential hexamerin expression in adult female *Wyeomyia smithii*

Mw N G F N G F Mw N G F Male G F Mw

Overall results of *Wyeomyia 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

Studies conducted 2004-2009:

- More extensive evaluation of hexamerin use conducted

- Larval feeding behavior examined: inconclusive

- Feeding studies more extensively conducted:

    - 2004: Tattnall population reevaluated
    - 2007-2008: all 3 populations compared

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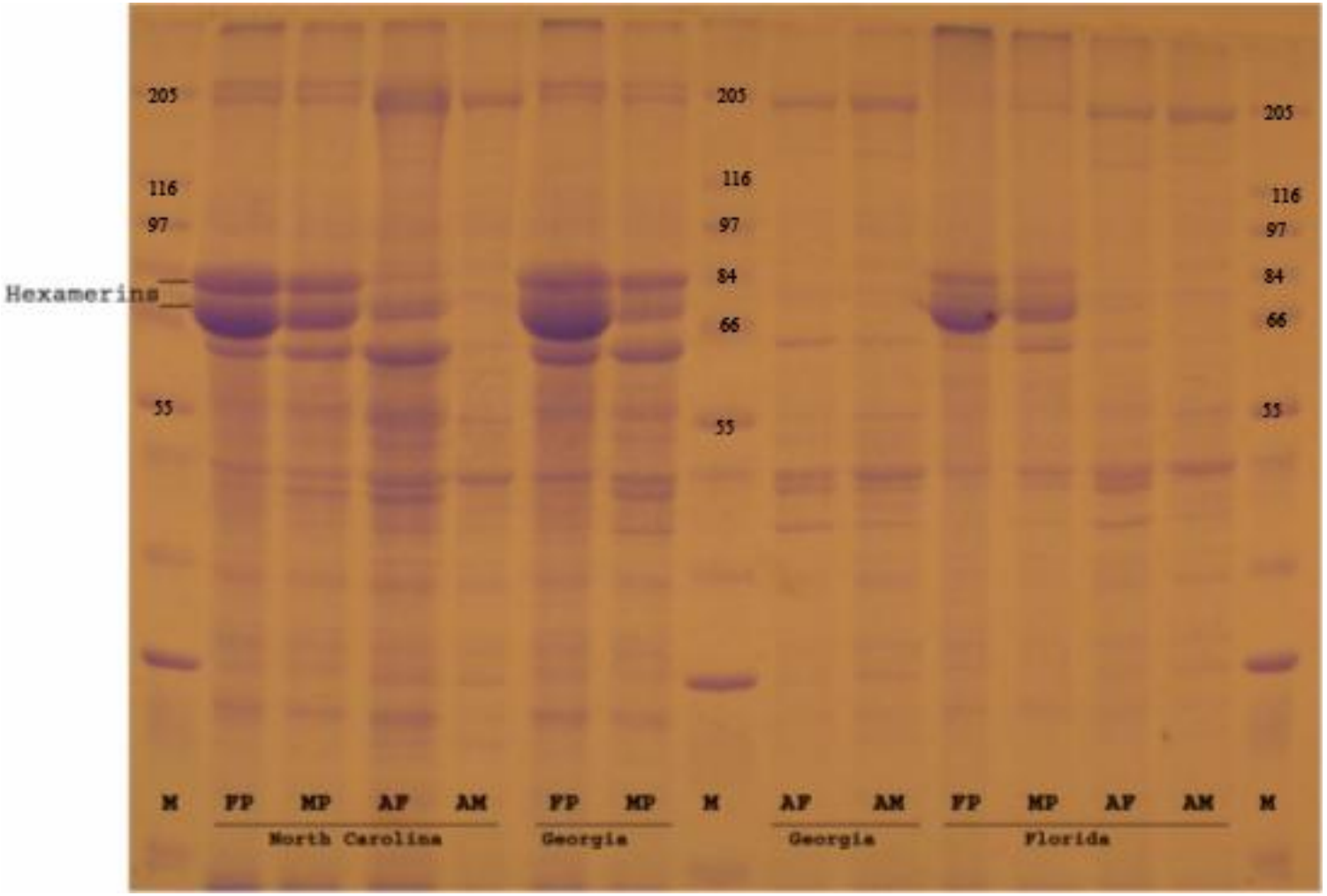
- Analysis of genetic structure begun again

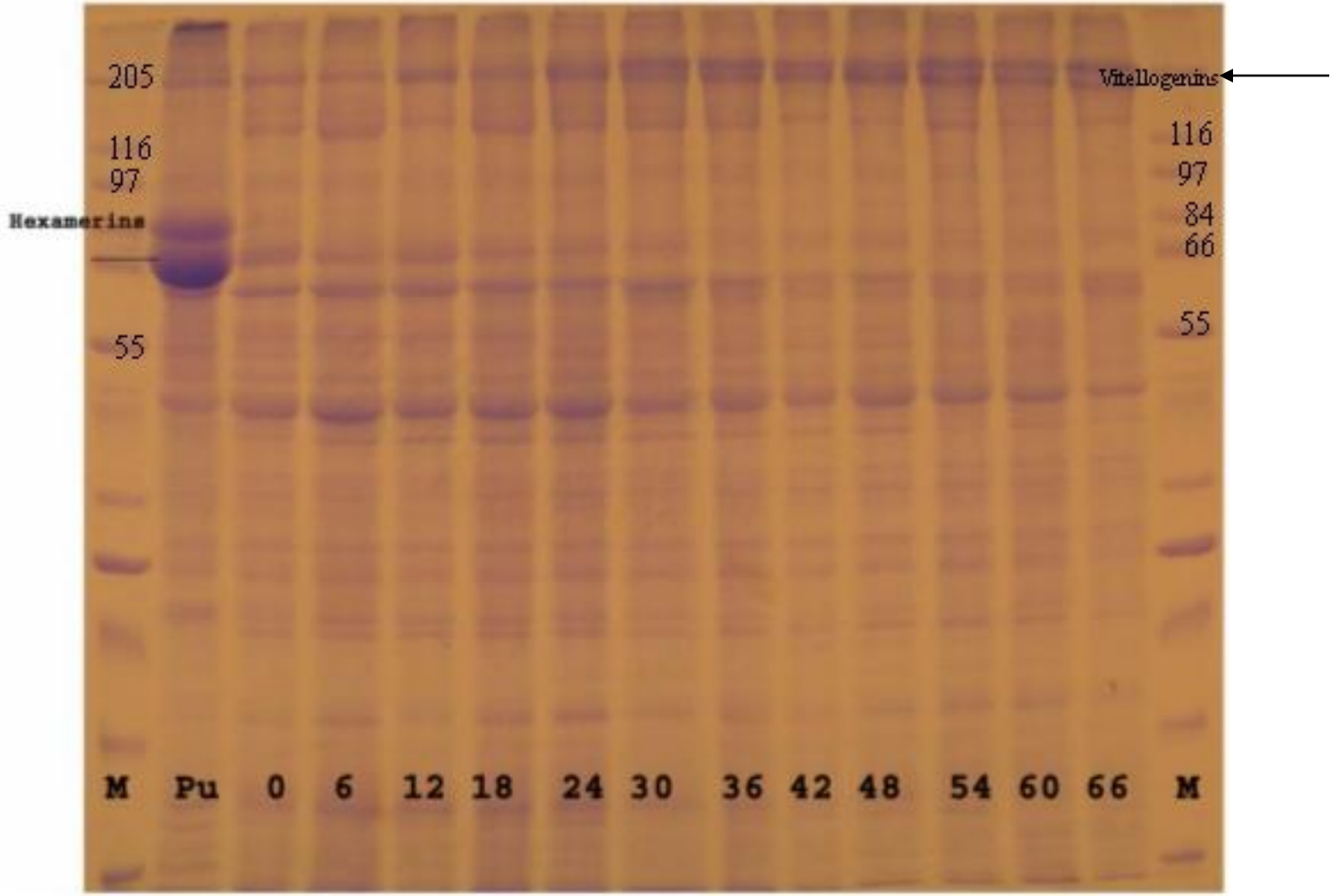
- Analysis of antennal structure initiated

Comparison of hexamerins in male and female pupae and adults from three geographically separate populations



Time course of disappearance of hexamerins in female mosquitoes after adult emergence

1) Highlands, North Carolina



Wyeomyia smithii larvae

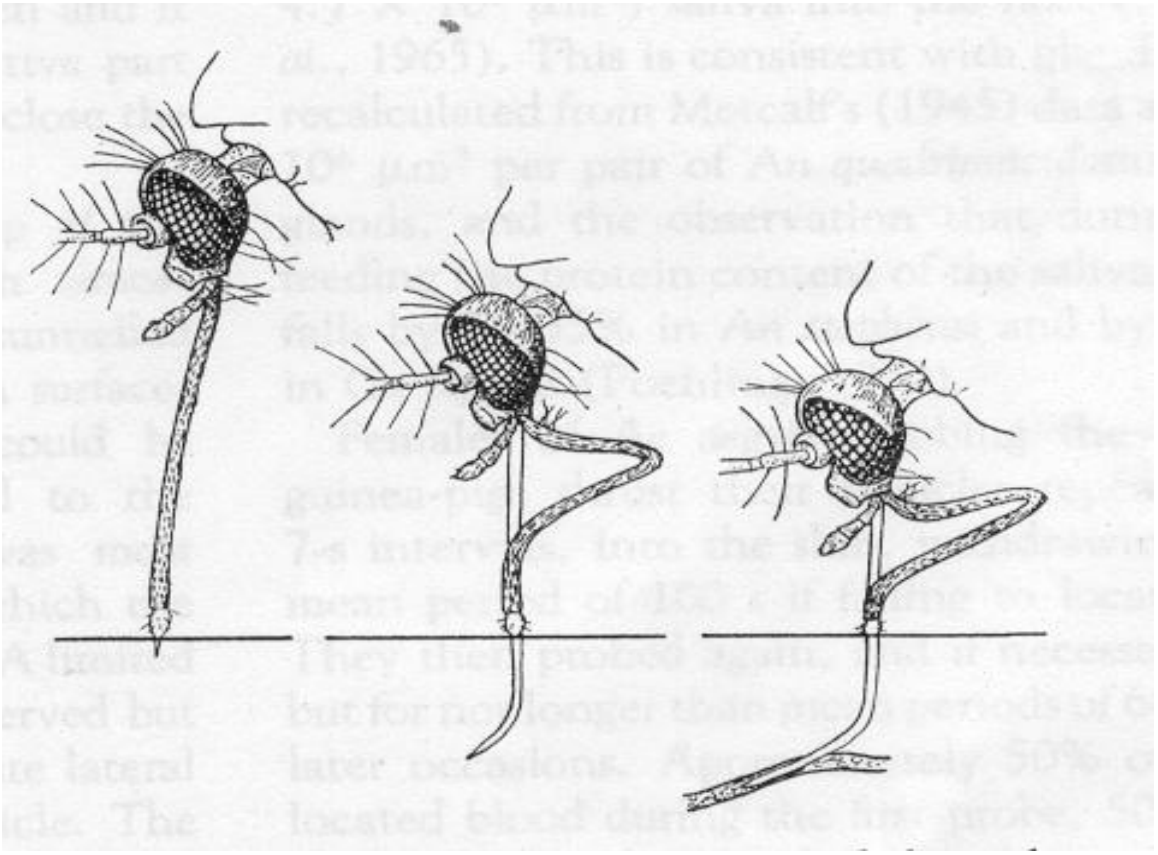
Comparison of feeding rates (palpal brushing) of larval *Wyeomyia smithii* from 3 populations

Population	Average number of brushings/min (all instars combined)
Highlands	75.3
Tattnall	103.3
Apalachicola	48.4

Optimal foraging theory prediction: A > T > H

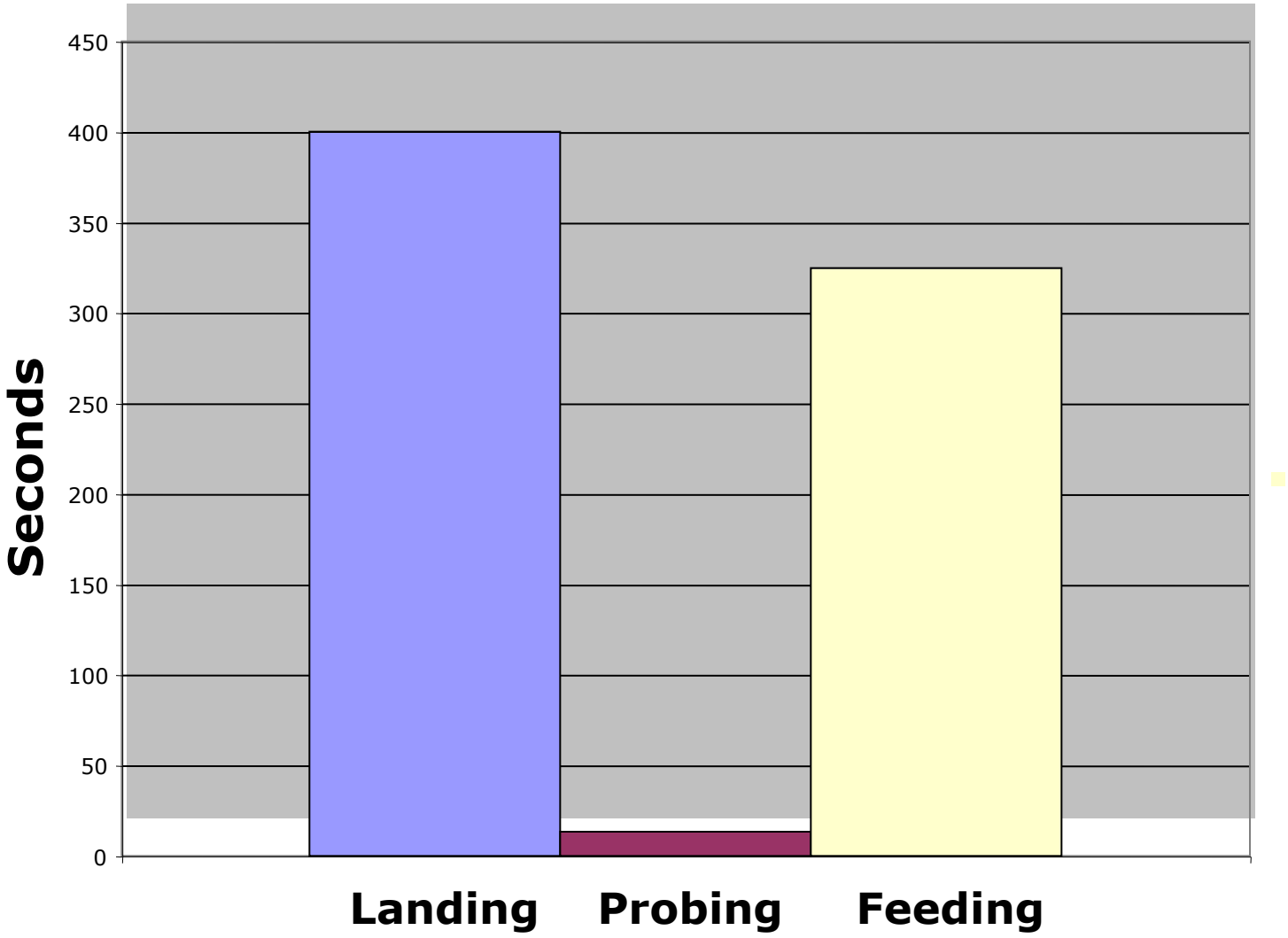
Results: ? Tattnall mosquitoes more stressed by drought ?

Timed Measures of Blood-feeding Stages



Landing → Exploration Probing Feeding

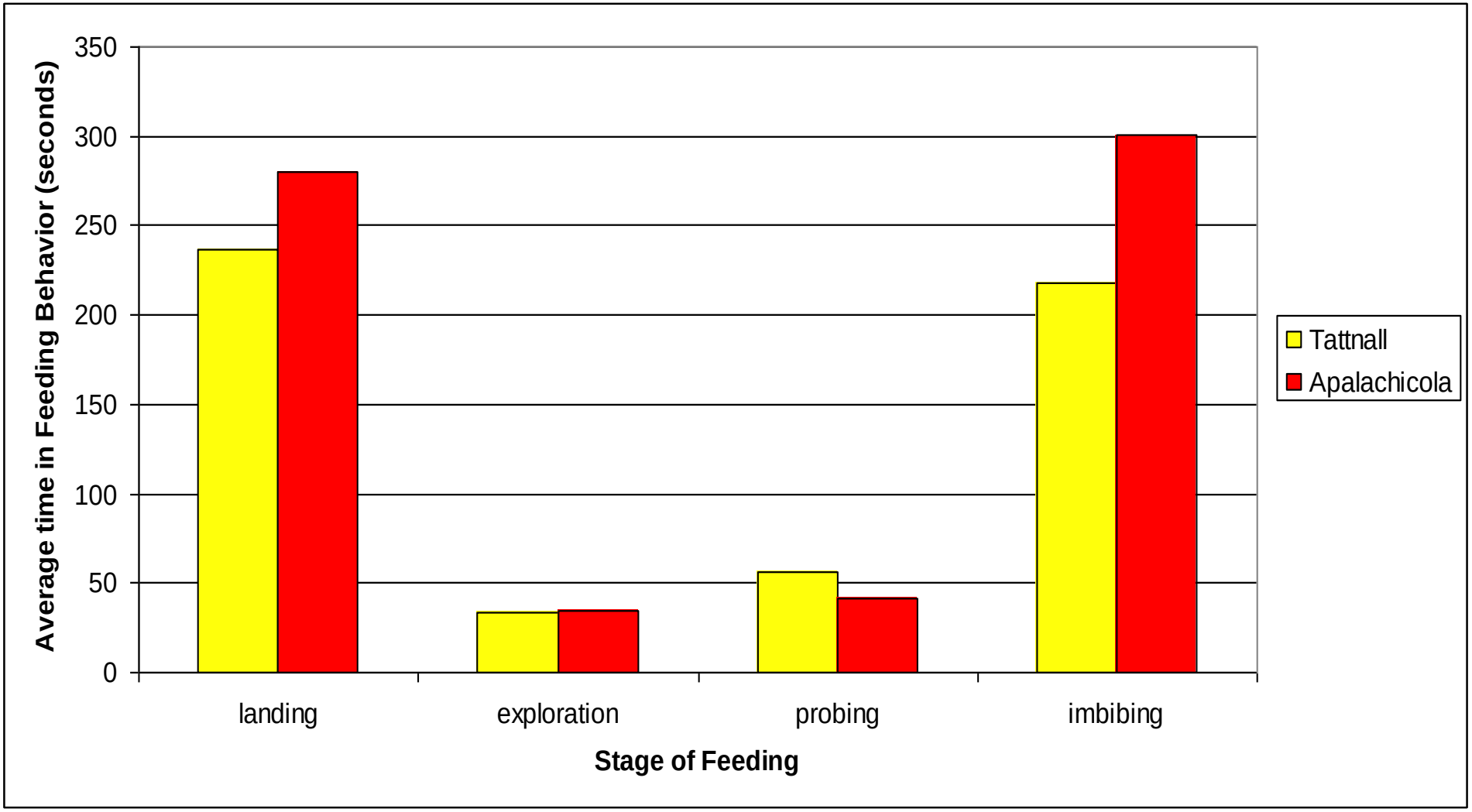
Mean Duration of Feeding Behaviors, 2004



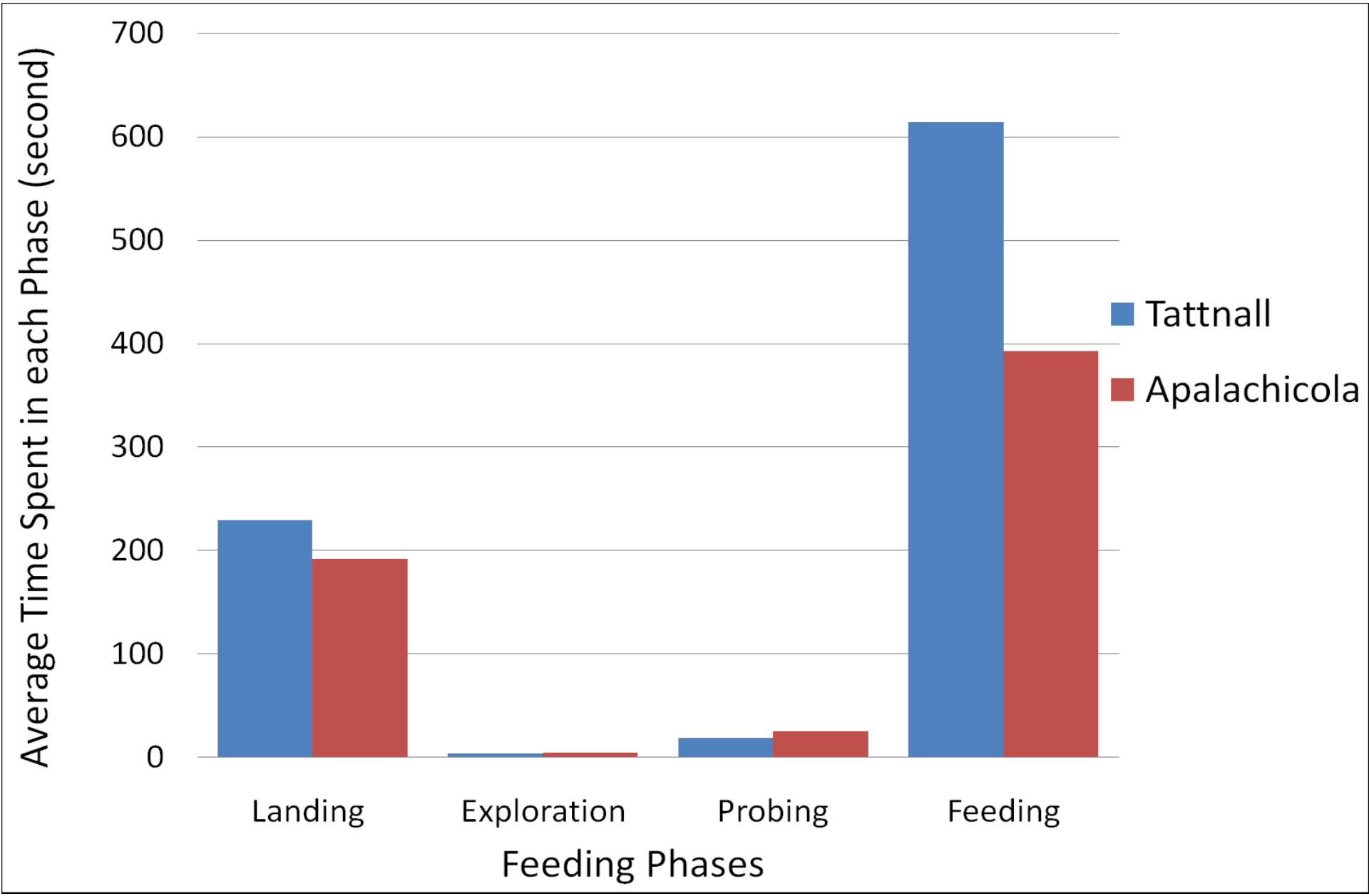
Mean Duration of Phases of Blood Feeding: 2004

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	

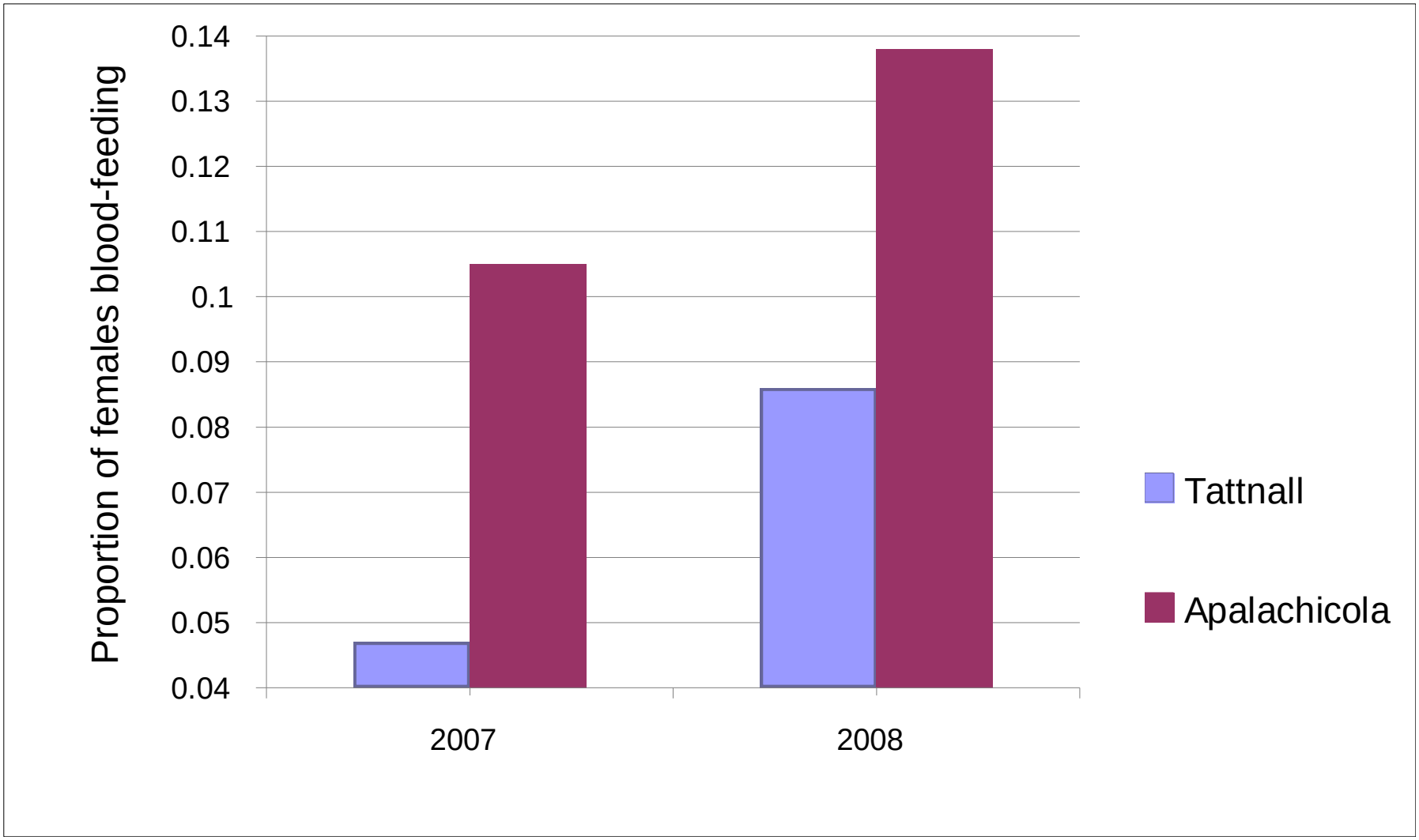
Data other than for *Wy. smithii* from Clements, 1999.



Wyeomyia smithii feeding behaviors in cage trials, 2007



Wyeomyia smithii feeding behavior in cage trials, 2008



Results of cage blood-feeding trials of *Wyeomyia smithii* in 2007 and 2008

Results of cage feeding trials* for *Wyeomyia smithii* in 2004-2008

Population	Ave. % Feeding: 2004	2007	2008
Apalachicola	ND	10.5	13.8
Tattnall	1.1	4.7	8.6
Highlands	ND	0	0

* 6+ 15 minute feeding trials/population, 50-125 female mosquitoes/trial, all blood-fed specimens removed after each trial

Overall results, 2004-2008:

- Georgia mosquitoes are increasing:

    - rate of depletion of hexamerins

    - frequency of blood-feeding (1.1 → 8.6%)

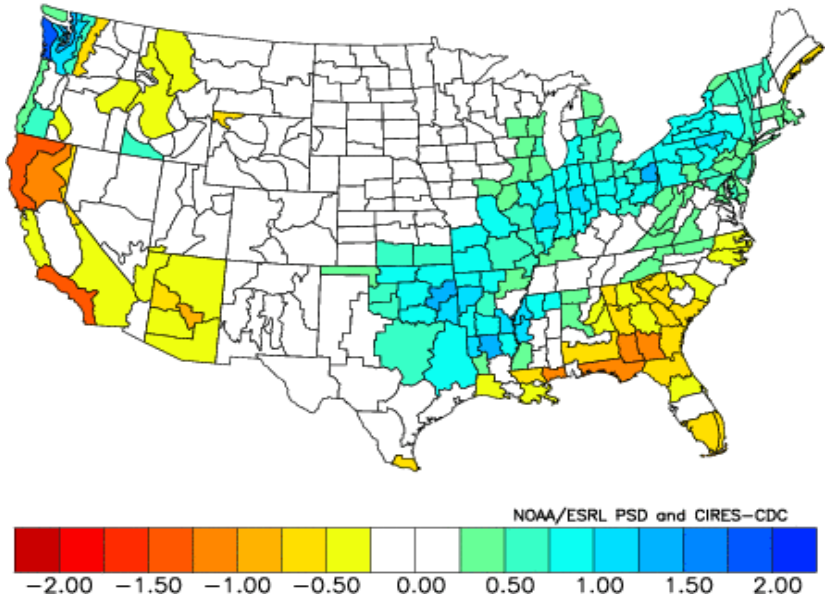
    - success at blood-feeding (albeit slowly):

        - 2004: partially engorged, ¼ contain clear fluid
        - 2007: fully engorged, 1/10 contain clear fluid
        - 2008: fully engorged, all contain blood

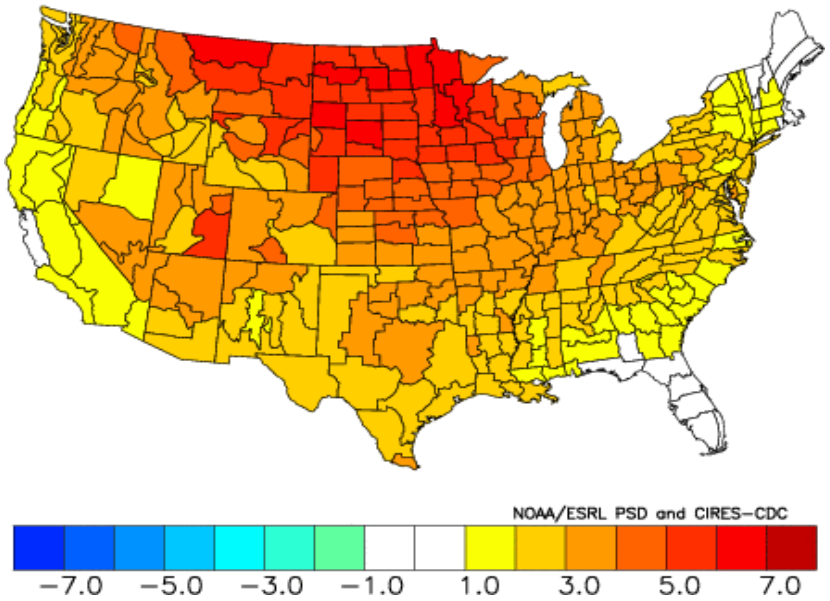
- Blood-feeding rate increasing in Florida as well!

- Why? → Changes in precipitation and temperature? (1950-1995 vs. 1998-2007)

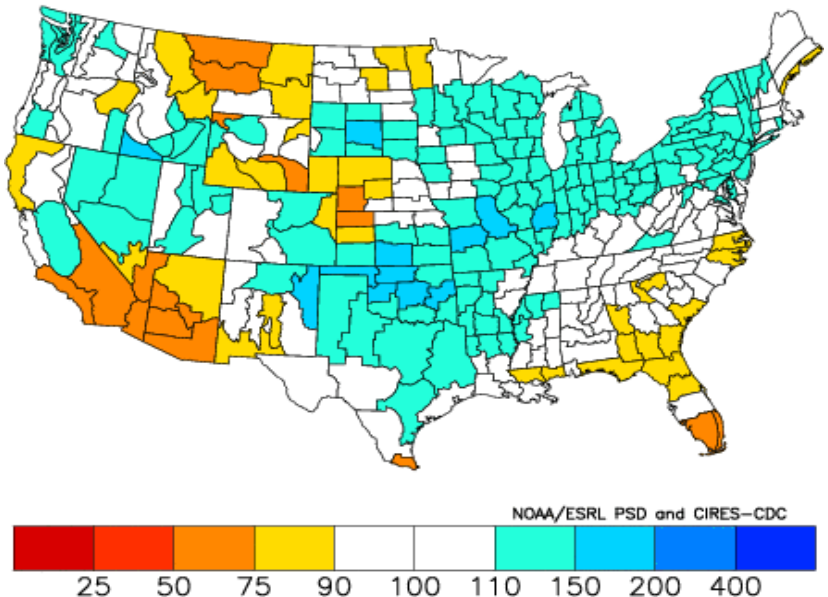
Composite Precipitation Anomalies (inches)
Jan 1998 to 2007
Versus 1950–1995 Longterm Average



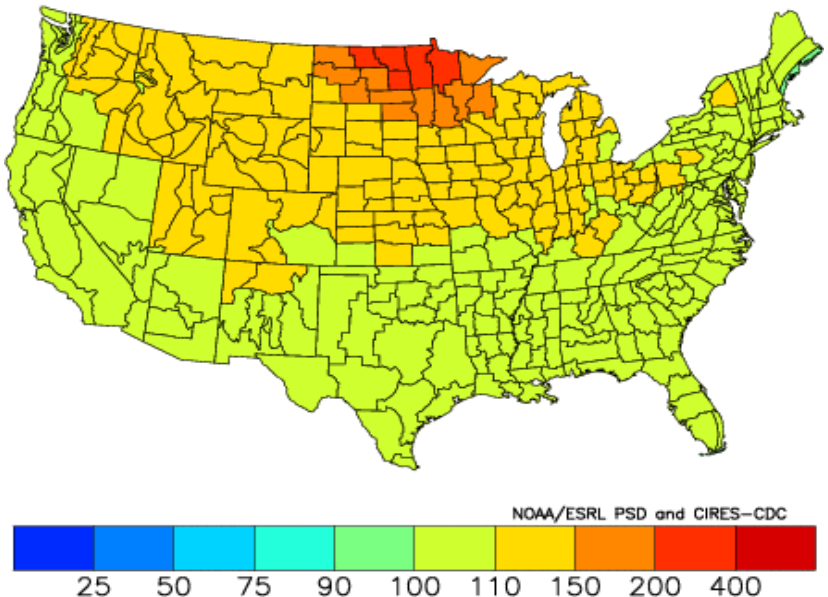
Composite Temperature Anomalies (F)
Jan 1998 to 2007
Versus 1950–1995 Longterm Average



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



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



Climate Trends: 1998 -2007 vs. 1950-1995

- **Rainfall anomalies**: persistent drought conditions in 1998-2007

→ decreased pitcher fluid volume, especially in FL

→ **greater competition**

- **Temperature anomalies**: higher average monthly and annual temperatures in 1998-2005

→ increased metabolic rates, especially in GA, NC

→ **greater competition**

→ **Pitcher plant mosquitoes are re-evolving blood-feeding in response to climate change!**

Bradshaw, WE, Holzapfel, CM. 2001. **Genetic shift** in photoperiodic response with **global warming**. *PNAS.* 98: 14509-14511.

* * *

- **larval diapause** (hibernation) of pitcher plant mosquitoes is induced and terminated by a **critical photoperiod**
- critical photoperiod is **latitude dependent** (> in N; < in S)
- photoperiod response is **genetically determined** (non-plastic) (heritability of 15-70%)
- 30 years of studying mosquito populations show:
    - shift towards **shorter** photoperiods throughout range (FL to Canada)
    → more southerly phenotype & genotype
    - correlation with > daily and annual temperature minima

→ 1st demonstration of natural populations exhibiting adaptive **evolutionary response** to **global warming**

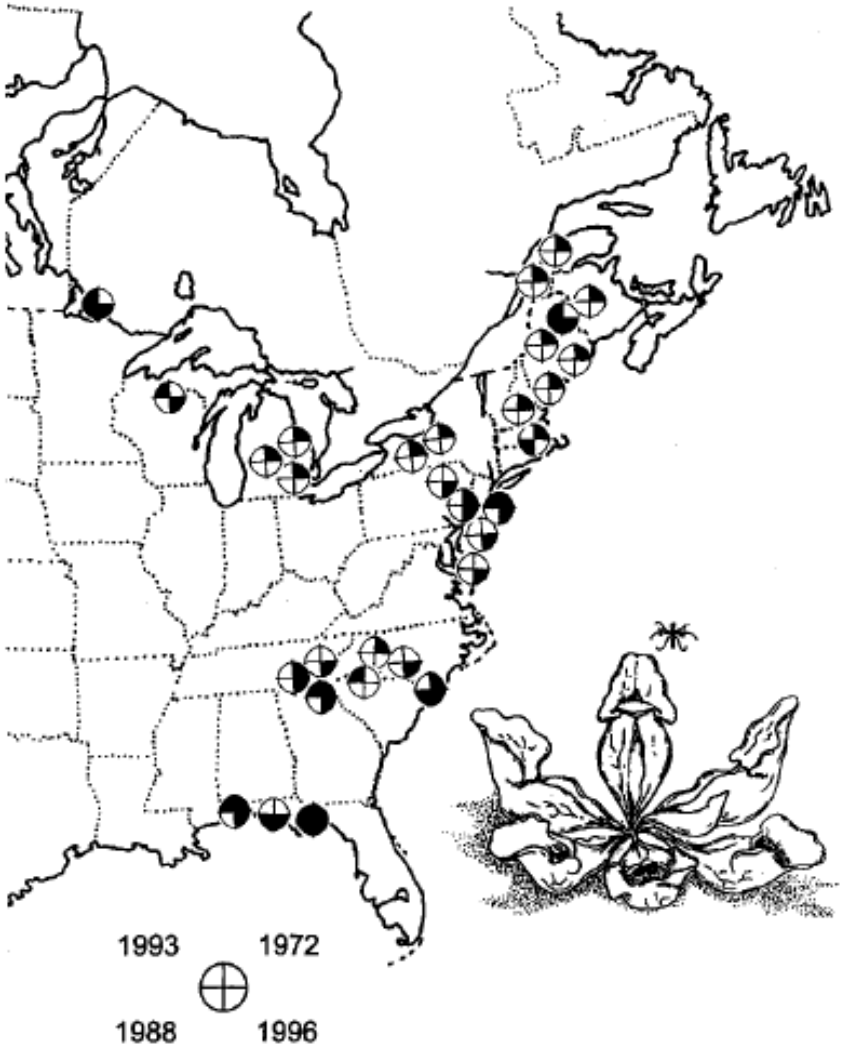


Fig. 1. Localities at 30–50°N latitude from which *W. smithii* were collected from *S. purpurea* (*Inset*) from the overwintering generation in 1972, 1988, 1993, and 1996. For each pie diagram, a blackened quadrant indicates a year that larvae were collected at that locality.

Table 1. Analysis of covariance for critical photoperiod with altitude-corrected latitude (ACL) from pooled 2-year samples as a covariate and year sampled (Yr) as the treatment, using type III sum of squares in the GLM procedure of SAS (26)

	1972 vs. 1996		1988 vs. 1993	
Treatment	$F_{1,34}$	P	$F_{1,10}$	P
ACL	975.24	<0.001	546.45	<0.001
Yr	4.17	0.049	3.41	0.095
ACL × Yr	7.85	0.008	7.35	0.022

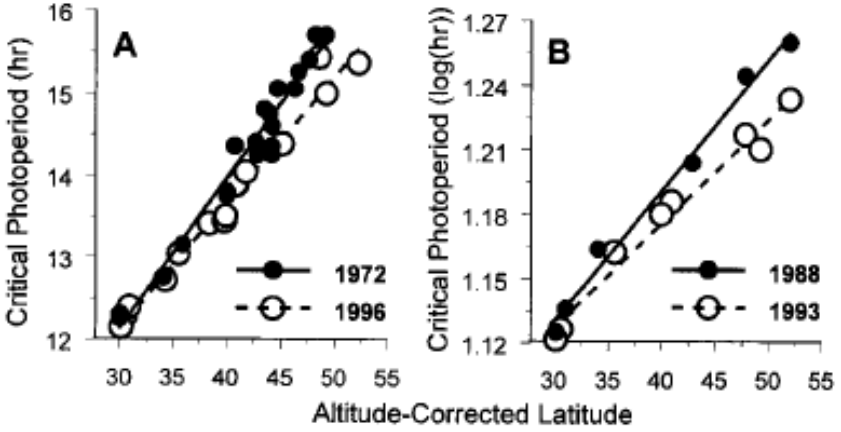
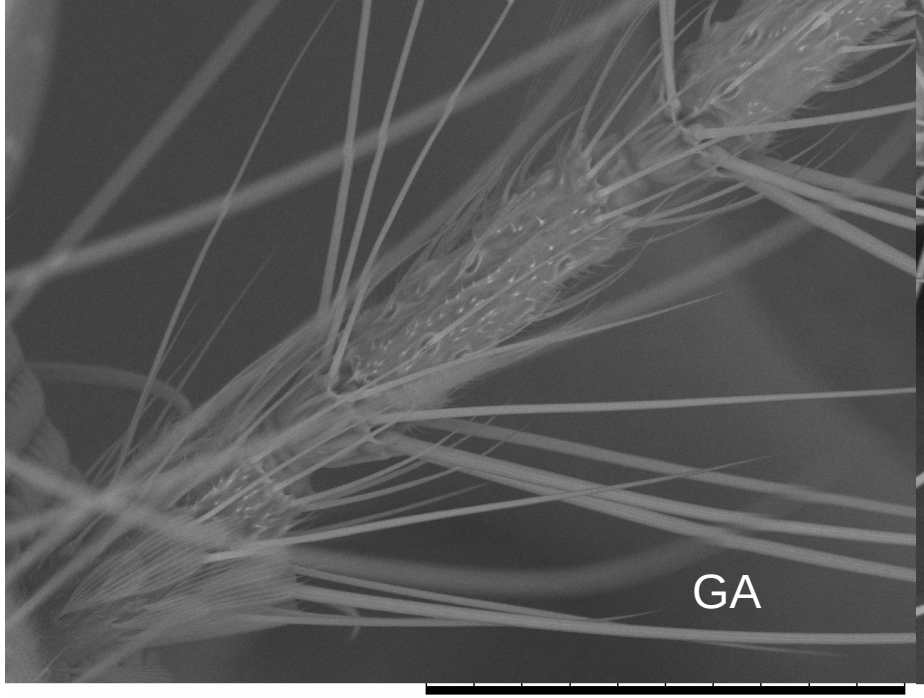
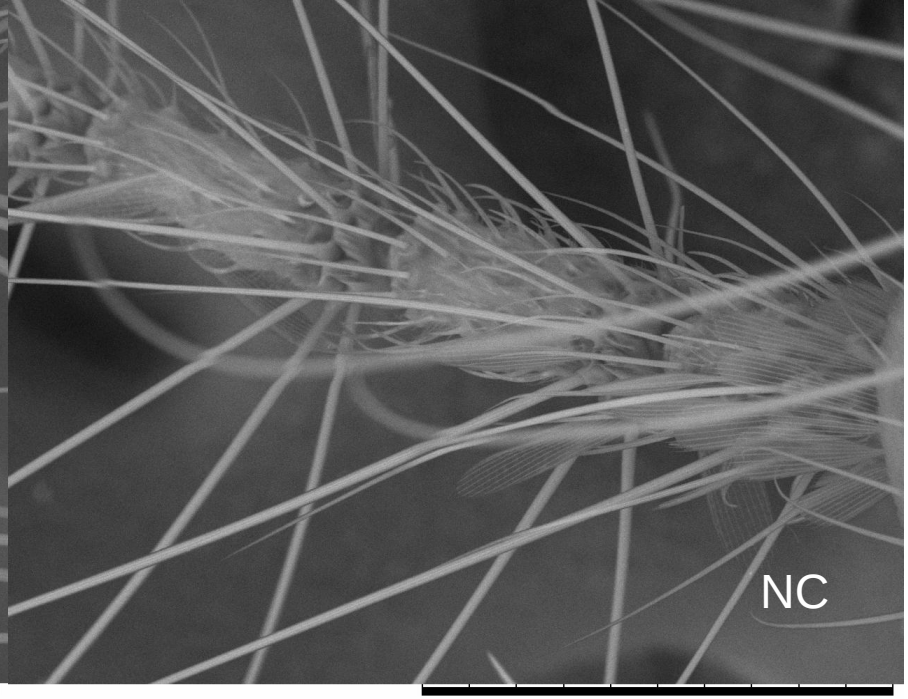
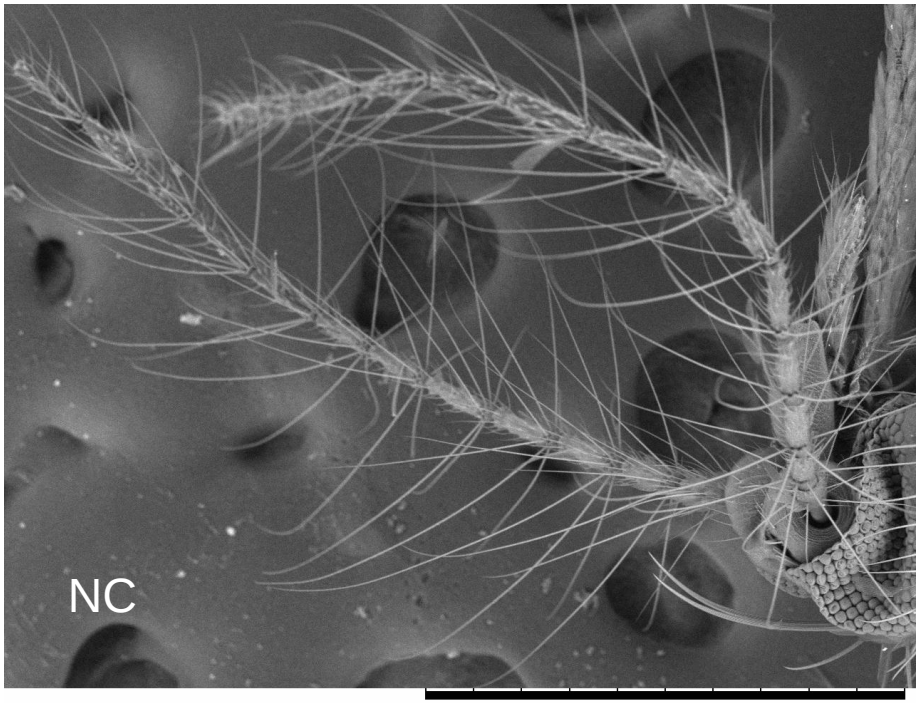


Fig. 2. Critical photoperiods of *W. smithii* collected during the overwintering generation from 1972 to 1996 determined from static (1972, 1996) or changing (1988, 1993) photoperiods. Analysis of covariance (Table 1) indicated significantly steeper slopes for the earlier year in each comparison, meaning that shifts toward shorter critical photoperiods (more southern phenotypes) increased with latitude.

Bradshaw & Holzapfel, 2001.



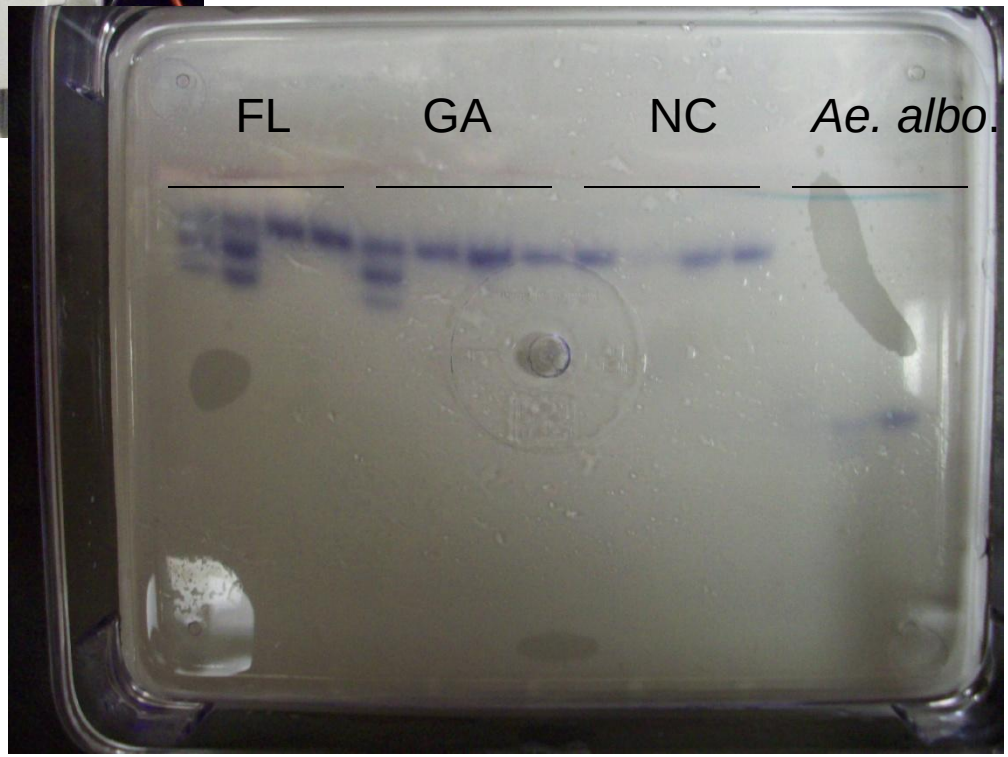




Future work: comparison of antennal morphology in female pitcher plant mosquitoes from NC, GA, and FL from 1997-present

Are density and types of chemoreceptive setae changing in conjunction with behavioral changes?

Sample isozyme gel from 2009:
→ isocitrate dehydrogenase (IDH)



Ongoing work: reanalysis of genetic structure based on isozymes

Are Georgia mosquitoes becoming more “southerly”?

Acknowledgments:

Graduate students:
- Helena Cantrell
- Sagar Reddy

Undergraduate students:
- Damilola Olowoyo
- Michael Newman (Emory Univ.)
- Ryan Carter
- Stacia Bennett