Variation in *Wyeomia smithii* with Latitude and Elevation

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A THESIS
Presented to the Honors College
of the University of Oregon in partial fulfillment of
requirements for the degree of Bachelor of Arts

January 1998

APPROVED [redacted]

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ABSTRACT

An Abstract of the Thesis of Camisha Booth for the degree of Bachelor of Arts in the Department of Biology to be taken January 1998

Title: VARIATION IN THE PITCHER-PLANT MOSQUITO *WYEOMIA SMITHII* WITH LATITUDE AND ELEVATION

Genetic variation exists within populations of the pitcher-plant dwelling mosquito *Wyeomia smithii*. Variation is also basic to the daily and seasonal cycles of the earth as well as changes in climate with latitude and elevation. I considered variations in morphological, physiological and behavioral traits in 15 populations of *W. smithii* and helped to determine the patterns of radiation into northern and alpine climates.

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CHAPTER I
INTRODUCTION

Variations exist within a given species over a geographic range. This is not surprising as particular populationss must adapt to different environmental conditions within different areas. Natural selection will favor adaptation to a combination of climactic factors impinging upon an organism. This basic ecological concept can be seen over the geographic range of the pitcher-plant mosquito *Wyeomia smithii*. Morphological, physiological and behavioral variations exist which give insight into the evolutionary history of the organism.

These variations were the focus of my research. I used fifteen populations of *W. smithii*. The populations were from sites in the eastern United States (Table 1, Figure 1). I analyzed phenotypic variations within and among populations of mosquitoes from different latitudes and altitudes. I measured differences in the morphology of anal papillae, stage of diapause, depth of diapause, and oviposition site selection behavior. These physiological and behavioral characteristics were analyzed for correlations with latitude and elevation. I will now give some background information on the life cycle and natural history of *W. smithii* to put my research in an understandable context.

Life Cycle

Wyeomia smithii lay their eggs within the water filled leaves of the pitcher-plant *S. purpurea*. The females, in fact, require this plant's leaves as stimulus to lay their eggs. The eggs hatch as larvae, which begin filter feeding in their liquid environment. As they eat, they grow. For mosquitoes, as with all insects, growth occurs through molting. Insects have an exoskeleton and are thus unable to grow in the manner vertebrates grow. They must develop an entirely new skeletal structure, discarding the old. Each stage of development is called an instar. Mosquitoes undergo this process four times in their life. Each instar results in a slightly larger larva.

It is during the larval stage that *W. smithii* enter diapause. Diapause is the insect equivalent to hibernation. It is a cessation of development during periods of unsuitable conditions. *Wyeomia smithii* overwinter in a larval diapause that is induced and maintained by short days and averted or terminated by long days. Inside the leaf, the larvae are able to wait out adverse conditions. With the cue of longer, warmer days the larvae break diapause and continue development. They then enter their brief pupal stage. This is similar to the cocoon stage in a moth. Here the animals do not eat, but unlike moths, they are mobile. Their bodies undergo drastic changes within their curled pupal shell. After a few days, the adult mosquitoes emerge through the pupal case ready to enter their new environment above the surface of the water.

The water-filled leaves of *S. purpurea* are host to a variety of organisms. Prey captured by the plant provide the nutritional material for the developing mosquito larvae. Gas and nitrogen exchange between the insects and the leaves of the plant suggest that the relationship is mutualistic (Bradshaw, 1983).

Natural History

The genus *Wyeomia* consists of about 90 species. The vast majority are distributed throughout the Neotropical and subtropical climate zones. Two species are found in peninsular Florida. A single species ranges into the temperate zone north of Florida - the pitcher plant dwelling *W. smithii* . This distribution alone suggests that the origin of the *W. smithii* is tropical. Previous research on life history traits of the mosquito support the idea that the temperate *W. smithii* has tropical ancestors (Bradshaw and Lounibos, 1977).

Wyeomia smithii larvae live only in the water-filled leaves of the carnivorous pitcher plant *Sarracenia purpurea.* The recession of the last glacial ice sheet, about 10,000 years ago, allowed the plant to move north. The range of *W. smithii* follows that of the host plant along the eastern United States - from the Gulf of Mexico to north-central Canada. Throughout the range of the plant, the mosquito encounters varying climatic conditions. The south provides warm summers with long growing seasons, while northern populations must contend with cooler conditions and shorter growing seasons. In far northern populations, snow cover and the warmth of the bogs in which the plants grow prevents the mosquitoes within the leaves from freezing. Without this sheltered niche the mosquitoes could not survive the less than ideal climactic conditions of the temperate zone. In addition, the mosquito has adapted morphological and behavioral patterns to cope with these conditions. These characteristics were the focus of my research. I will now give background into each experiment.

Experimental Background

The first trait I considered involved variations in morphology of the anal papillae. Anal papillae are projections off the end of the larvae. They are used for salt and water balance. As seen previously by Bradshaw and Lounibos (1977), the southern populations have two long ventral and two slightly shorter dorsal anal papillae. As one moves north, the dorsal anal papillae shrink and eventually disappear in the far northern populations (Figure 2). An explanation for this change with latitude is found in the function of the anal papillae. They are used for salt and water balance. The anal papillae are also possibly used for oxygen transpiration (Gillet 1971). This suggests that the larvae do not have to surface to respire. Thus the longer anal papillae of the southern populations, with their greater surface area for oxygen/carbon dioxide exchange. As one moves north, the colder, more oxygen rich waters do not require such long or abundant anal papillae.

The next two experiments dealt with aspects of diapause. In *W. smithii*, diapause is dependent photo-periodic cues. Photoperiodism is the ability of an organism to measure day length. The mosquitoes use their biological clocks to assess the day length and react accordingly. Adaptation to varying climates is dependent on photoperiodism. It enables organisms to time their physiology and development with the changing seasons.

I considered stage of diapause in my 15 populations of *W. smithii.*. Previous studies have shown that southern animals diapause as fourth-instars while northern populations tend to diapause as third-instars. This has been hypothesized to provide the northern animals with the opportunity to enter a second, fourth-instar diapause if the harsher northern climate suddenly becomes unsuitable for development (Lounibos and Bradshaw, 1975).

I then examined the depth of diapause. I looked at the difficulty in "waking" the animals from their resting state. Previous studies have found that northern animals required longer exposure to light to break diapause and resume development. This shift is likely due to the harsher climate experienced by the northern populations. Continuation of development before winter is truly over would be fatal to emerging adult mosquitoes, thus they require greater lengths of time in "summer" conditions to break diapause.

The final trait looked at was oviposition site-selection behavior. This is basically measuring the accuracy of the female mosquitoes in placing their eggs into the *S. purpurea* leaf. Previously studies (Bradshaw and Holzapfel, unpublished) have suggested that southern populations show more accuracy in egg placement. This is an important behavior, because only those eggs deposited safely into the leaf stand a chance of survival in the natural world.

I looked at these traits in populations of *W. smithii* from a broader range of latitudes and elevations than those considered in prior studies. The intent was to determine the specific patterns of adaptive radiation.

CHAPTER II

MATERIALS AND METHODS

Collection and Maintenance

Mosquito larvae were collected from 15 sites in the Eastern United States. The collection occurred during the winter and spring when the mosquitoes were in their diapausing larval stage within the water-filled leaves of the pitcher-plant, *S. purpurea.* Larvae were maintained in plastic petri dishes (150 by 25 mm) half filled (80 mL) with distilled water. Each dish contained approximately 30 larvae. The larvae were fed an suspension of a 3:1 mixture of ground, sifted guinea pig chow and freeze-dried brine shrimp. Each dish of larvae was provided 4 mls of food a week. In this manner, the larvae were maintained in a short day room. This room provided the amount of light to simulate winter conditions (8 hours light to 16 hours of dark). In the short day room, there was a constant temperature of 21° Celsius. This room mimicked a typical winter's day and served to initiate and maintain diapause.

In contrast, the long day room had higher temperatures of 23 to 25° Celsius and a 16:8 hour light to dark ratio. This room provided summer conditions and served to break diapause. As a result, the animals continued development to adults. As the animals pupated, they were placed in a water-filled dish inside a cage. From here the pupae emerged as adults into the cage. They were provided pesticide-free raisins for nourishment. Inside the cage was a fresh *S. purpurea* leaf in a water dish. Moist paper was maintained on the bottom of the cage to prevent desiccation. Approximately two weeks after pupae were placed in the cage, they began to lay eggs. Eggs were collected into water filled dishes and the entire cycle continued.

Experiment 1

The first variable considered was anal papillae. Twenty-five fourth instars from each cohort were preserved in Kahle's solution. There were 5 cohorts from each population. A cohort refers to a group of individuals within a population. Dividing into cohorts is useful in increasing statistical power during data analysis. The larvae were fixed on a glass slide dorsal side down while the ventral anal papillae were measured. An ocular micrometer lens of a calibrated dissecting scope was used for measurements. The larva was then rotated to ventral side down and the dorsal anal papillae, if present, were measured.

Experiment 2

The next variable measured was stage of diapause. I obtained 125 eggs from each population. These were divided into 5 cohorts of 25 eggs each. The eggs were placed in 3 mL wells with 1 mL distilled water and evenly mixed food. The food was drawn with a pipette while blending to ensure precise food to water ratio in each of the wells. The protocol for food is shown on Table 2. The animals were placed on short days and observed for stage of diapause. Stage of diapause was determined through head capsule size and coloration.

Experiment 3

The next experiment considered the depth of diapause. Each population was broken into cohorts of 25 animals each. They were kept in the short day room until all animals reached diapause. They were then exposed to a predetermined number of long days, ranging from 1 to 10 days. Three cohorts for each number of long days exposed were used to ensure accuracy. After set exposure time, the animals were returned to short days. I then waited 30 days from initial exposure to ensure ample pupation time. The dishes were scored twice a week and the number of pupae was recorded. These data were analyzed to determine the number of days necessary to induce 50% pupation in each of the populations.

Experiment 4

Oviposition site selection behavior was the final trait considered. This refers to the accuracy of egg placement into leaves by the female mosquitoes. Five cohorts of 30 animals from each population were placed in petri dishes in a long day room This resulted in pupation and development to adults. The adults flew about, mated and laid eggs. Eggs were collected three times a week and their location recorded as in the leaf, in the pupal dish, or on the paper. Later the data from three days of maximum oviposition was used for statistical analysis.

The data from all four experiments were analyzed for correlations with latitude and elevation using SAS. In all analysis involving percentages, the arcsin transformation was used.

CHAPTER III

RESULTS

The length and number of anal papillae found in *W. smithii* decrease overall as one moves north. The results of statistical analysis show a highly significant correlation of length of dorsal anal papillae with both latitude and altitude (Figure 3a). The ventral anal papillae length show a highly significant correlation with latitude and a significant correlation with altitude (Figure 3b).

The stage of diapause is also clearly correlated with latitude (Figure 4a). The alpine populations show an abrupt shift to the more northern phenotypes. Depth of diapause, too, shows a correlation with latitude. Here the effect of altitude is not as clear, as the alpine populations require similar number of days to break diapause as lowland populations from the same latitude (Figure 4b).

Oviposition site selection behavior was the final experiment. Here the females from southern population show more accuracy in placing their eggs into leafs. The correlation is significant (Figure 5a). There is, however, no significant correlation with altitude. The percent eggs in the pupal dish is correlated significantly with elevation and there is a highly significant correlation with latitude.

CHAPTER IV
DISCUSSION

Overall, each of my experiments clearly supports the idea of an adaptive radiation from south to north. The specific details of the movements of individual populations and their comparative relatedness are more difficult to ascertain.

The morphology of the anal papillae matches that previously seen - the southern mosquitoes show two long ventral and two slightly shorter dorsal anal papillae. As one moves north, the dorsal anal papillae shrink and eventually disappear in the far northern populations. Populations in the south have longer, more abundant anal papillae and this is correlated with their warmer, less oxygenated, water. The change involves a gradual shift in size and number with geography. The alpine populations show the phenotype of the northern animals, which makes sense as the water higher up would most likely be cooler, and thus more oxygen rich. This is clearly seen when comparing the lowland and alpine North Carolina populations.

The population from Maryland at 38.3° N is the northern most population to show dorsal anal papillae. They were extremely small and often difficult to see at all. This perhaps could be some sort crucial transition point. Unfortunately, this location is not significant when considering the other traits.

My results show a clear correlation between stage of diapause and geography, with southern populations diapausing as fourth instar larvae and northern populations as thirds. As stated earlier, this is likely due to the needed possibility of a second fourth-instar diapause in the northern populations if the winter conditions persist. The alpine populations exhibit the phenotype of their more northern counterparts. The switch from third to fourth diapausing populations is abrupt. Unlike anal papillae characteristics, there is no gradual transition, but two clearly defined phenotypes. The change is discontinuous with the percent fourths dropping dramatically at around 35° N.

Depth of diapause shows a more gradual transition. Northern populations do take longer to break diapause than southern populations, but there is no definite latitude that any major change occurs. Rather the change is continuous. This is one trait that shows no clear correlation with altitude. Two of the alpine populations show a tendency towards northern populations in that they require slightly more light exposure to break diapause, however, the third alpine population requires less. Thus the correlation is loose and there are clearly other factors influencing depth of diapause.

The photoperiodic controls which control both depth and stage of diapause are key here. The southern most populations with their fourth instar diapause and shallow depth of diapause seem less dependent on environmental conditions in controlling their development.

Oviposition site selection behavior considered the accuracy of the adult females in placing their eggs into the leaves. The southern populations had a higher percentage of leaf egg laying overall. Alpine populations did not show any clear trends. Possibly the harsher climate of the north was off set by greater density of mosquitoes in the south (Bradshaw and Holzapfel 1989). More mosquitoes result in greater competition for the available resources within the leaf. Figure 5b shows that the percent eggs laid in the pupal dish increases significantly with latitude and altitude. This also supports the influence of density in this behavior. The pupal dish would contain pupae and the molt left in the water. Adult females likely use visual cues to decide to avoid the overcrowded leaves.

The inconsistencies in correlations with geography from trait to trait are likely due to trade offs in the mosquito's genetic make up. An organism's life style and life history must be a compromise allocation of the resources that are available to it (Begon 1996). A "trade-off" is a relationship between two life history characteristics in which increased benefits from one are associated with decreased benefits from the other because of limitations in the total resource available to be allocated to them. So in the case of *W. smithii* a denser southern population with a tendency to live in overdrowded conditions will put more effort into accurate egg placement while some other life history trait may suffer.

This study was designed to confirm the adaptive radiation of populations of *W. smithii* and to give insight into details of this radiation, specifically the alpine populations. The overall tendency toward northern phenotypes in alpine populations suggests that perhaps the initial movement north was followed by a later move back south. However it is not as though the animals simply packed up and moved one day and their bodies just made the necessary adjustments. The location of *W. smithii* is ultimately dependent on the location of its host plant. Over time, major climactic changes presented fresh suitable habitats for *S. purpurea*. As the host plant branched out into new environment the mosquito exploited these new environments. In the case of *W. smithii* the movement of the plant and hence the mosquito was likely controlled by the movement of the ice sheets. When looking at a map, some of the obvious cutoff lines, particularly in stage of diapause, lie along latitudes that the ice sheet lingered the longest.

From my results it seems that the alpine North Carolina populations are most closely related to their northern counterparts. These questions will be answered definitively as current genetic studies reach completion.

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POPULATION	LOCATION	Latitude (°N)	Altitude (M)
CB	North Carolina	35.2	925
CR	Florida	30.7	40
DB	North Carolina	35.0	925
GS	North Carolina	34.2	20
HK	North Carolina	35.1	910
HV	New Jersey	39.7	10
KC	Maine	46.2	366
LI	Alabama	30.5	15
ML	Wisconsin	46.0	500
MM	New Jersey	39.8	10
NP	Maryland	38.3	18
PB	New Jersey	39.9	10
PM	North Carolina	34.9	10
TH	Pennsylvania	41.2	610
WI	Florida	30.1	10

Table 1. Geographic locations of *Wyeomia smithii* populations.

Instar	Food Concentration
I	.5
II	.
III	1.5
IV	2.5

Table 2. Food provided each larval instar (g/L) in 10µL/L PhotoFlo - for experiment #2.

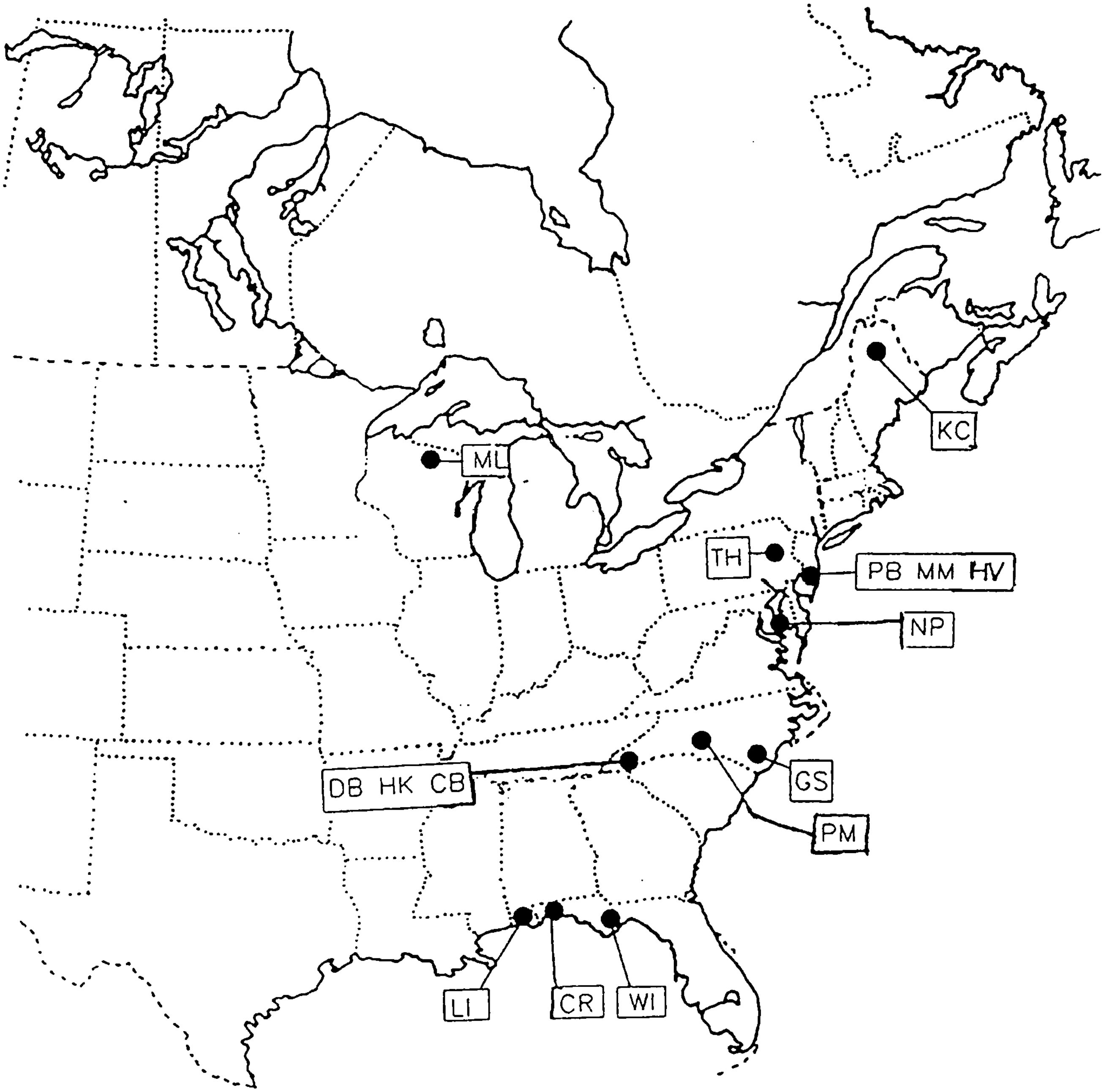
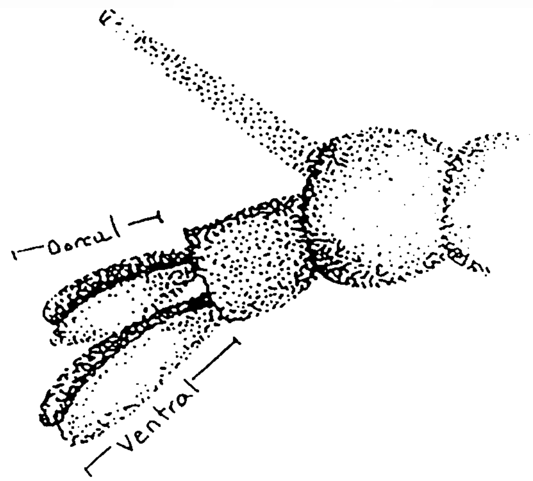
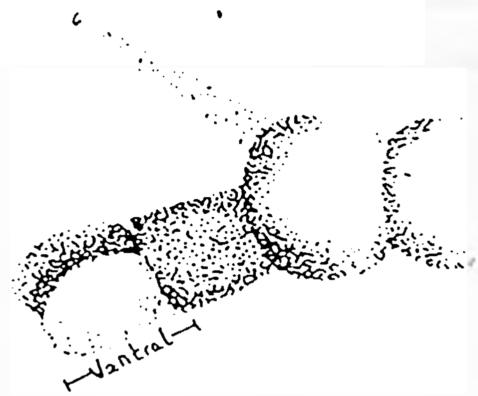


Figure 1. Map of Locations of 15 Populations



A. Southern phenotype for anal papillae



B. Northern phenotype for anal papillae

Fig. 2 The southern and northern phenotypes for anal papillae. Southern populations have two ventral and two shorter dorsal, while northern populations have only two short dorsal.

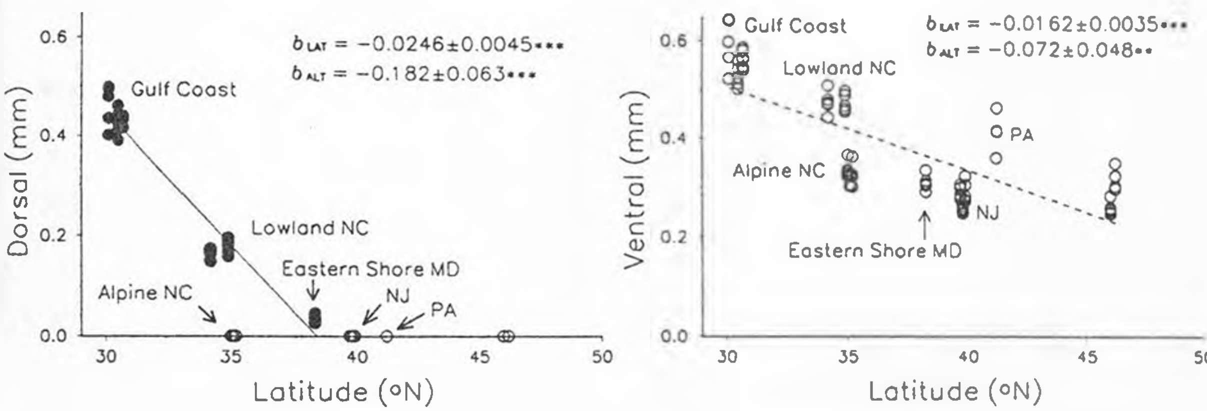


Figure 3. Anal Papillae Results: a) Length of Dorsal vs. Latitude
b) Length of Ventral vs. Latitude

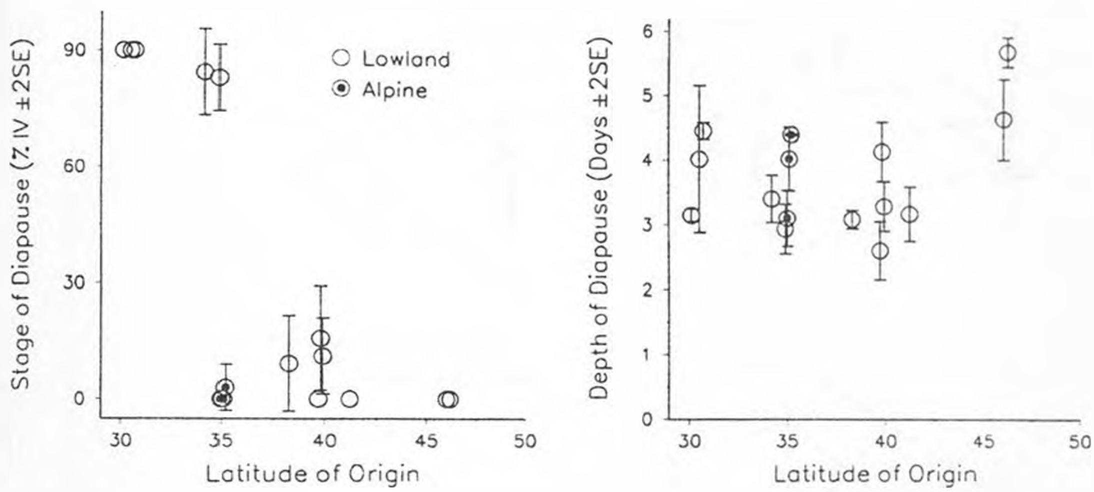


Figure 4a. Stage of Diapause Results: % IVth's vs. Latitude
4b. Depth of Diapause Results: Days in Long Day vs. Latitude

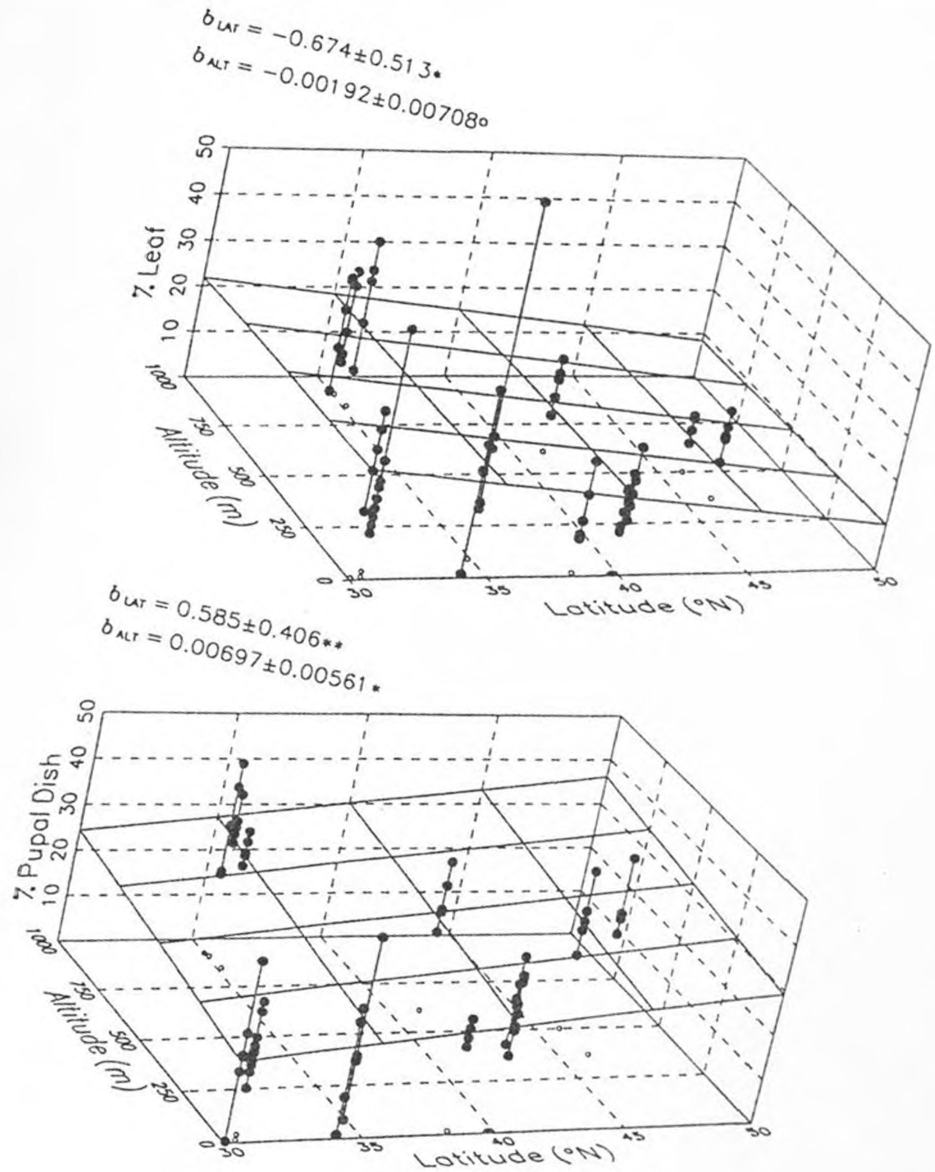


Figure 5. Oviposition Site Seletion Behavior Results
a) Percent Eggs in Leaf
b) Percent Eggs in Pupal Dish