Georgia Satyr Rearing Study Plan of Work

This study will investigate rearing techniques and host plant preference for Georgia Satyr (Neonympha areolata) larvae. The Georgia Satyr is a fairly common butterfly found throughout the southeastern US and is closely related to the endangered Saint Francis Satyr (Neonympha mitchellii franciscii) (Glassberg, 1999). *N. m. francisci* has a very limited distribution, restricted to locations the training grounds of Fort Bragg, NC. Previous studies have investigated population dynamics and behavior of *N. areolata* and *N. m. francisci* through studying adult members of the species, however, little is known about the larvae of these species and their development. Despite efforts by Hall and Haddad no *N. m. francisci* larvae have been observed in the wild (Hall and Haddad 2005). No *N. areolata* larvae have been observed in the wild either (Floridata, 2006). However in *N. m. francisci* oviposition has been observed. Hall et al. (2001) found that females laid eggs on several different species of plants many of which, such as cane, seem very unlikely host plants for the larvae. Therefore it is believed that the larvae move from where they hatch and select the plant hosts themselves, which makes it impossible to tell what the host plants are through oviposition (Hall and Haddad 2005).

For my study I will use *N. areolata* larvae because they are not endangered, however, hopefully the techniques and information gained from this study can be applied to learn more about the larvae of *N. m. francisci*. Such information could be valuable to the conservation of this endangered butterfly.
While no *N. areolata* larvae have been observed in the wild there is speculation about which plants might be the primary host plants for the larvae. This is based on plant distribution in the butterfly sites as well as the host plants of related butterflies. For my study I chose six of these possible host plants. Five are sedges in the genus *Carex* (*C. atlantica, C. debilis, C. lurida, C. mitchelliana, and C. stricta*) and the sixth is a grass in the genus *Calamovilfa*. Four of the sedges being used I grew last summer for a different project. The final sedge and grass species will be collected from various sites where they grow on Fort Bragg.

There will be two parts to this study. The first part investigates how well the larvae can grow and survive given one of the six possible host plants (survival study). The second part explores what the larva’s preference of plant is given the choice between two possible host plants (choice study). I expect that survival rate will differ between the six possible host plants in the survival study and that the plants showing the highest survival rate will be the plants the larvae show a preference for in the choice study.

I will obtain eggs for the study by collecting gravid females from butterfly population sites on Fort Brag. The primary collection sites for *N. areolata* will be Twig Rush Bog and the NEA Savanna. Females will be housed in oviposition chambers for 2 to 3 days while they lay eggs. The females will then be released.

The oviposition chambers will be made from plastic flowerpots. The plastic flowerpots will be covered in screen and placed into another pot with water in it. Grasses, sedges or some other type of vegetation for the butterfly to perch on will be inserted through holes in the bottom of the flowerpot with their stems extending into the water in the pot below. The oviposition chambers will be housed in ambient conditions at field house near Fort Brag. They will be place on a covered porch to protect them from rain and sun. They will be checked multiple times a day to monitor the female’s health. Once the females are released the eggs will be collected from the
flowerpots. They will be placed into plastic Tupperware containers with cheese cloth secured over them. Clippings from one of the possible host plants will be placed in the containers with eggs so that when the larvae hatch they will immediately have something to feed on. The clippings will be changed out at least once a day.

Once the eggs have hatched the larvae will be placed on the six possible host plants mentioned earlier. The plants will be growing in plastic one gallon flower pots. Using potted plants as opposed to clippings of sedge or grass leaves will hopefully eliminate any degradation in plant quality that could affect larval growth. For the survival study there will be one species of possible host plant in each pot. For the choice study there will be two species of possible host plants in each pot. There will be one pot for every combination of two of the six possible host plants. The plants will be trimmed down so that they are a reasonable size for locating larvae on them. Also they will be trimmed so that they all have approximately the same amount of vegetation.

The larvae will be transferred directly on the leaves of the possible host plants. Four to six larvae will be placed in each pot. In the choice study pots two or three larvae will be placed on each plant. After the larvae have been placed, half of a tomato cage will be inserting into each pot. Cheese Cloth will be used as screening to cover the plants. The cheese cloth will go over the tomato cage and be secured around the edge of the pots with wire. In addition duct tape will be used to seal off the edge of the cheese cloth against the side of the pots. This will insure that no insects or other predators enter the pots.

The pots will be housed in ambient conditions at a field house near Fort Bragg. The screening will be removed and the larvae checked every other day for the first week to week and a half. The larvae will be checked less frequently as they mature and their chances of survival
increase. For the survival study checking the larvae will entail locating all the larvae that were originally placed on the plant or spending 10 minutes searching for them. The number or larvae observed will be recorded as well as their position on the plant. When measuring the larvae’s position on the sedge we will look at what section of the leaf it is on, bottom, middle, or top third. We will also record whether the larvae are on the dorsal or ventral surface of the blade. We will also measure the height they are from the soil in the pot. We will measure the length of the larvae to the nearest half-millimeter, using a ruler. Along with these measurements we will record general observations about the larvae, their behavior and the conditions of the sedges. For the choice study we will simply record the number of larvae observed on each of the two possible host plants. As with the survival study observers will locate all larvae placed on the plant or spend 10 min searching.

We will continue to regularly observe the larvae through pupation. Any adult *N. areolata* that emerge will be released back to the site where the eggs were collected from. *N. areolata* are bivoltine thus there will be two flight periods to collect eggs and perform this experiment.

The objectives of this study are to develop effective methods for rearing *N. areolata* larvae and to gain insight into how they grow and develop. To see if larvae feeding on a range of several possible host plants exhibit significantly different growth and/or survival rates. To see which plant the larvae prefer to feed on and also to look at where the larvae spend the majority of their time. Ultimately the goal is to try to develop a greater understanding of how larvae would act in their natural environment.

Learning more about the behaviors and preferences of *N. areolata* larvae will hopefully aid in finding and observing larvae in the wild. Evaluating how larvae grow in their natural habitat can give insights into habitat preferences and limitations. Also the methods used in this study can be used with
the endangered Saint Francis Satyr. The results of a study with the Saint Francis Satyr could be important in creating and implementing conservation techniques.

Sources


Examples of Posters

Posters – Both are good although they both have more text than is recommended.
Larval Development and Host Plant Preference in Neonympha areolata
Abstract

The Georgia Satyr (*Neonympha areolata*) is a butterfly found throughout the southeastern US that is closely related to the endangered Saint Francis Satyr (*Neonympha mitchellii francisci*). *N. m. francisci* has a very limited distribution, restricted to locations on Fort Bragg, NC. While there have been previous studies of adult *N. areolata* and *N. m. francisci*, little is know about the larvae of either species. We conducted a study with *N. areolata* larvae to establish rearing techniques and to investigate host plant preference and behavior of the larvae. We collected eggs from *N. areolata* females found at Fort Bragg during each of the two *N. areolata* flight periods in the summer of 2006. We then reared the larvae on six different potential host plant species of sedge or grass. For the larvae collected during the first flight period we found that all the potential host plant species we tested were able to support larvae to pupation. In addition we examined larval period and found that there was some variation between plant species. Also all the larvae appeared to emerge as adults in a time period consistent with the time adults were emerging in the wild. For the larvae collected during the second flight period we found that all species of possible host plants tested had larvae survive over winter. The rearing techniques developed in this study will be used this summer in a similar project studying *N. m. francisci* larvae. The information obtained from that study could be directly applied to conservation of the *N. m. francisci* through habitat restoration and augmentation.

Introduction

The Saint Francis Satyr (*Neonympha mitchellii francisci*) is an endangered butterfly with very limited distribution, restricted to locations the training grounds of Fort Bragg, NC. The Georgia Satyr (*Neonympha areolata*) is a closely related non endangered species. Previous studies have investigated population dynamics and behavior of *N. areolata* and *N. m. francisci* through studying adult members of the species, however, little is known about the larvae of these species and their development. Despite efforts by Hall and Haddad, no *N. m. francisci* larvae have been observed in the wild (Haddad, et al.)
2003). However oviposition in *N. m. francisci* has been observed. Hall et al. (2001) found that females laid eggs on several different species of plants, many of which seem very unlikely host plants for the larvae. Therefore it is believed that the larvae move from where they hatch and select the plant hosts themselves. This makes it impossible to tell what the host plants are through oviposition (Hall and Haddad 2005).

*N. areolata* and *N. m. francisci* have two flight periods each year, one in early summer and one in late summer. Larvae produced during the first flight period develop over the next 2 months and emerge as adults during the second flight period. Second flight period larvae over winter as larvae and then pupate and emerge as adults during the first flight period of the subsequent summer.

While little is know about the feeding behavior of *N. areolata* and *N. m. francisci* larvae, there is speculation about which plants might be their primary host plants. This is based on plant distribution in the butterfly sites as well as the host plants of related butterflies. This study tested six of these possible host plants. Five were sedges in the genus *Carex* (*C. atlantica, C. debilis, C. lurida, C. mitchelliana, and C. stricta*) and the sixth was a grass in the genus *Calamovilfa* (*C. Brevipilis*). Four of the sedges used were grown last summer for a different project. The other sedge and grass species were collected from various sites where they grow on Fort Bragg.

There were two parts to this study. The first part investigated how well the larvae can grow and survive given one of the six possible host plants (survival study). The second part explored larval plant preference given the choice between two possible host plants (choice study). The objectives of this study were to develop effective methods for rearing *N. areolata* larvae and to gain insight into how they grow and develop. To see if larvae feeding on a range of several possible host plants exhibit significantly different growth and/or survival rates. To see which plant the larvae prefer to feed on and also to look
at where the larvae spend the majority of their time. Ultimately the goal was to try to develop a greater understanding of how larvae would act in their natural environment.

A previous study conducted by S.P. Hall and N. M. Haddad used clippings from possible host plants to rear *N. areolata* and *N. m. francisci* larvae. They successfully reared *N. areolata* larvae on three (*C. mitchelliana*, *C. brevipilis*, and *C. glaucescens*) out of seven plant species and *N. m. francisci* larvae on one (*C. mitchelliana*) out of seven plant species (Hall and Haddad 2005). However, they found that it was hard to maintain the quality of the plant clippings and that some plant species’ clippings deteriorated more quickly than others.

For this study potted plants were used in order to sustain higher quality of the plants. Only *N. areolata* larvae were used, because they are not endangered, however, the techniques and information gained from this study will be applied to learn more about the larvae of *N. m. francisci*.

**Methods**

During the first flight period eggs were obtained from gravid females collected from the NEA Savanna on Fort Bragg on 6/19/06 and 6/21/06. Three females were collected each time. The females were housed in oviposition chambers for up to three days and then released. The oviposition chambers were made from two plastic flowerpots, one inserted into the other with water in the lower pot. Grasses, sedges or some other type of vegetation for the butterfly to perch on were inserted through holes in the bottom of the flowerpot with their stems extending into the water in the pot below (Figure 1). The pots were covered with screening.
The oviposition chambers were housed in ambient conditions at a field house in Carthage, NC. They were placed on a covered porch to protect them from rain and sun. They were checked multiple times a day to monitor the female’s health. Once the females were released, the eggs were collected from the flowerpots. About 160 eggs were collected.

The eggs were placed in plastic Tupperware containers with screening over them. Clippings from *C. mitchelliana* and *C. brevipilis* were kept in the containers and changed daily to provide food for the larvae once they hatched. The first larva hatched the evening of 6/25/06. The larvae were initially placed on *C. atlantica*, *C. brevipilis*, *C. lurida*, and *C. mitchelliana* planted in pots (survival study). There were two pots of each species of plant. The pots were one gallon plastic pots. The plants were sections of larger plants transplanted into the one gallon pots prior to placing larvae. The plants were all washed and trimmed so that they all had approximately the same amount of vegetation. Small clumps of plants were used as opposed to whole plants to facilitate observation of larvae. Sections of wire tomato cages were inserted in the pots so that they extended one to one and half feet above the tops of the pots.
Four larvae were placed on plants in each of the eight pots on 6/26/06. They were transferred from the Tupperware containers to the leaves of the plants using the clippings in the containers. The pots were covered with screening, which was secured by wire around the base of the pot. The pots were housed at the field house in Carthage. On 06/27/06 larvae were placed on plants in pots set up for the choice study. These pots were constructed similarly to the above pots the only difference being that these pots had two species planted in them. Six larvae were place in each of three pots containing combinations of *C. atlantica* and *C. brevipilis*, *C. atlantica* and *C. lurida*, and *C. brevipilis* and *C. lurida*. Three larvae were placed on each plant in the pots. On 06/28/06 it was discovered that some type of insect, perhaps in the assassin bug family, had entered the pots and was preying on the larvae. All pots were checked and larvae were replaced so that all the survival pots (those with one plant species) had four larvae and choice pots (those with two plant species) had two larvae on each plant in the pots. A total of 17 larvae were replaced in the survival pots and 7 larvae in the choice pots (choice pots were replaced with a total of four larvae per pot instead of the original six). The pots were temporarily covered with two layers of screening to prevent predation until a finer cheese cloth covering was available. From that point on the screening/cheese cloth was also duct taped around the base of the pot in addition to the wire (Figure 2).
On 6/28/06 larvae were placed in the same manner in three more choice pots with combinations *C. atlantica* and *C. mitchelliana*, *C. debilis* and *C. lurida*, and *C. lurida* and *C. mitchelliana*. On 06/30/06 another choice pot containing *C. brevipilis* and *C. debilis* had larvae placed on it. In addition a single larva was placed on *C. debilis* in a survival pot.

The pots were uncovered and checked two to three times per week. For the survival study checking the pots entailed observers locating all the larvae that were originally placed on the plant or spending 10 minutes searching for them (this was shortened to 8 minutes starting 07/13/06 when the larvae had grown larger and easier to find). The number of larvae observed was recorded as well as their position on the plant. The larva’s position was measured in three ways: the section of the leaf they were on (bottom, middle, or top third), whether the larvae were on the dorsal or ventral surface of the leaf, and the height they were from the soil in the pot. The length of the larvae was also measured to the nearest half-millimeter, using a ruler. Along with these measurements general observations about the larvae, their behavior and the conditions of the sedges were recorded. For the choice study observers recorded the number of larvae found
on each of the two possible host plants. As with the survival study observers located all larvae originally placed on the plant or spent 10 min (also shorten to 8 minutes) searching.

If plants became unhealthy or died sections of new plants of the same species were transplanted into the pot or the larvae were transferred to another pot. In two cases with *C. debilis* and one pot of *C. brevipilis* the plants died and the larvae were transferred to a pot of *C. mitchelliana*. All larvae were regularly checked until pupation and then were released back at the NEA Savanna upon emergence.

During the second flight period three female *N. areolata* were collected from the NEA Savanna in a similar fashion as the first flight period. About 110 eggs were obtained. Six survival pots were set up with *C. atlantica, C. brevipilis, C. debilis, C. lurida, C. mitchelliana,* and *C. stricta* (*C. stricta* was not used during the first flight period because of the health of plant). Larvae were placed on these plants between 08/22/06 to 08/25/06. Six larvae were placed on each plant. There were six choice pots with all possible combinations of *C. atlantica, C. brevipilis, C. lurida, and C. mitchelliana*. Three to four larvae were placed in these pots on 08/25/06 and 08/26/06. The larvae were placed in between the two plants on the soil. The pots were checked about once a week initially and then less frequently as the larvae matured. During checking all larvae originally placed were located or the observer spent at least seven minutes searching. Lengths were recorded for the larvae in survival pots and the number of larvae on each plant species was recorded in choice pots.

The pots were housed in ambient conditions in Apex, NC. The pots were covered with cheese cloth in the same manner as pots from the first flight period, however this time they were not under a covered porch and were thus exposed to rain and full sun. Over winter the pots were buried in the ground from 01/03/07 to 03/11/07 to provide insulation.
**Results: Survival**

First Flight Period

All species of possible host plants tested with larvae from the first flight period were able to support larvae to pupation (Figure 3). All larvae that survived to pupation successfully emerged as adults.

**Figure 3:** Survival rates or larvae from the first flight period to pupation.

The survival rates in the graph above are the average of two pots for all species except for *C. brevipilis* which was a single pot. The other *C. brevipilis* plant died and its larvae were transferred to *C. mitchelliana* (not included in the survival rates). The error bars seen in the graph are exact binomial confidence intervals based on the averaged survival rates for each species. They do not take into account any variation between pots. However there was substantial variation between pots (Figure 4).
Survival of larvae from first flight period to pupation by pot

![Survival of larvae from first flight period to pupation by pot](image)

**Figure 4:** Survival rates of larvae from individual pots.

Except for *C. brevipilis*, which only had one pot, all the other plant species had very different survival rates between the two pots. This is seen most drastically with *C. mitchelliana* which had a 75% survival rate in one pot and 0% in the other. The pot of *C. mitchelliana* that had all its original larvae die had the larvae from the dead *C. brevipilis* and *C. debilis* plants transferred to it. In the end it had two larvae pupate on it (not included in graph).

There was significant variation in larval period across plant species (Figure 5). Analysis of variance was used to determine if the days to pupation for the larvae varied significantly between plant species and between individual pots. There was significant variation for both species and pots with p-values of 0.0319 and 0.0392 respectively. However, because the larvae were not checked every day it was impossible to tell exactly when the larvae pupated. To get an estimate for the days to pupation an average between the two dates (last date observed as larva and first date observed as pupa) was used. If a larva was starting to pupate when observed, it was counted as pupating the following day.
Average Larval Period Across Plant Species

![Bar chart showing average days to pupation for different plant species.]

Figure 5: Variation in larval period.

In the above graph the error bars are 95% confidence intervals based on standard deviation.

All larvae including choice and transferred larvae pupated within 30 to 52 days. The distribution was more linear and did not have the shape of a normal s-curve distribution (Figure 6). This would support the finding that there was variation between species and individual pots.
Figure 6: Distribution of larval periods for all larvae that pupated.

Larvae feeding on *C. lurida* were some of the first to pupate. This is consistent with figure 4 where *C. lurida* had the shortest average larval period. Some of the choice pot larvae took the longest to pupate, however, the combination of *C. lurida* and *C. mitchelliana* supported the larva with the shortest larval period. All larvae that made it to pupation emerged and were released during the same time period that wild *N. areolata* adults were emerging.

The behavioral data collected showed that on average larvae feeding on all plant species were observed more often on the ventral surface of the leaf. It was also found that all larvae had a mean foraging height of about 11.5 cm off the ground (Figure 7).
<table>
<thead>
<tr>
<th>Plant species</th>
<th>Mean foraging height (cm)</th>
<th>% observations on ventral side of leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. atlantica</em> (pot 1)</td>
<td>10.03448</td>
<td>67.86</td>
</tr>
<tr>
<td><em>C. atlantica</em> (pot 2)</td>
<td>11.26724</td>
<td>79.59</td>
</tr>
<tr>
<td><em>C. brevipilis</em> (pot 1)</td>
<td>9.690476</td>
<td>52.38</td>
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<tr>
<td><em>C. brevipilis</em> (pot 2)</td>
<td>14.27419</td>
<td>53.85</td>
</tr>
<tr>
<td><em>C. debilis</em> (pot 1)</td>
<td>5.7</td>
<td>80</td>
</tr>
<tr>
<td><em>C. lurida</em> (pot 1)</td>
<td>11.55556</td>
<td>81.25</td>
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<td><em>C. lurida</em> (pot 2)</td>
<td>16.26471</td>
<td>84.375</td>
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<tr>
<td><em>C. mitchelliana</em> (pot 1)</td>
<td>12.44643</td>
<td>60</td>
</tr>
<tr>
<td><em>C. mitchelliana</em> (pot 2)</td>
<td>13.51163</td>
<td>84.615</td>
</tr>
</tbody>
</table>

Average of all larvae:  

Average of all larvae:  

11.63830178  

71.54666667

**Figure 7:** Behavioral data

Some plant species such as *C. debilis* were small and grew lower to the ground which would account for the much lower foraging height. Also different species had varying broadness of leaves which could possible make a difference in how much time larvae spent on the ventral surface of the leaf.

Second Flight Period

All species of possible host plants tested with larvae from the second flight period had larvae survive over winter (Figure 8).
Survival of Larvae from 2nd Flight Period Through Winter

Figure 8: Survival rate of larvae over winter. Number of larvae last recorded 04/15/07.

The pot with *C. brevipilis* had some *C. lurida* added on 10/30/07 after *C. brevipilis* appeared to be dying. As in figure 3 the error bars in this graph are 95% exact binomial confident intervals. There was only one pot per species. This may account for the larger variation in survival rate between species. Throughout the fall and winter the larvae continued to feed on warm days. Larval growth (measured by length of larva) slowed and even reversed in November and December, however, larvae began growing again in the spring.

**Results: Choice**

There were a number of problems with the choice study with both larvae from the first and second flight periods. In a number of pots there was disparity in the health of the two plant species. In a few cases one species died leaving the larvae with no choice of host plant. The limited number of larvae was also a problem. At most there were four larvae originally placed in a pot (some pots from the second flight period only had three). No pot had more than two larvae survive to pupation or over winter. Multiple pots from both flight periods had no larvae survive. Pots from the first flight period had larvae directly placed on plants which could have affected their choice. Also larvae from both flight periods were given
clippings from *C. mitchelliana* to feed on immediately upon hatching. This initial feeding could have affected their choice. Because of these issues no significant pattern that would indicate larval host plant preference was found. However, in a couple cases larvae were found to move back and forth between the two plant species.

**Discussion**

Using potential host plants grown in pots, *N. areolata* larvae were successfully reared on a variety of plants in time periods similar to their development rates in the wild. Hall and Haddad had larvae survive to pupation on *C. mitchelliana* and *C. brevipilis* using plant clippings. However, their survival rates were much lower (~10% for *C. mitchelliana* and ~17% for *C. brevipilis*) than those observed in this study (37.5% for *C. mitchelliana* and 50% for *C. brevipilis*). The average larval period observed by Hall and Haddad in *C. mitchelliana* (41.2 days) was similar to what was observed in this study (43.17 days). In *C. brevipilis* the average was much longer (51.8 days vs. 38 days) in Hall and Haddad’s study (Hall and Haddad 2005).

Being able to successfully rear larvae on potted plants in time periods consistent with that of wild larvae is significant for both studying larvae and rearing larvae for introduction into new habitat. This is especially important for the endangered *N. m. francisci*. The techniques developed in this study will be used in the summer of 2007 to study *N. m. francisci* larvae. Initially only survival given a single plant species will be tested. More repetitions of pots of a single species will be used to endeavor to gauge whether variance in survival rate is due to different species or different pots.

The choice study may be repeated with *N. areolata* larvae during the first flight period of the summer of 2007 with changes to its design. Some changes would include using more larvae per pot, having back up plants to ensure plant quality remains high, and placing eggs directly in the pots between plants to give newly hatched larvae the choice of host plant. If this new design is successful a choice
study using *N. m. francisci* larvae may take place during the second flight period. Learning more about how *N. m. francisci* larvae grow in their natural habitat can give insights into habitat preferences and limitations. These insights could be critical for habitat restoration/augmentation and for guiding the management of this endangered species.

Sources

