Leaf Nitrogen Content and Tent Temperature as Possible Drivers of Oviposition Site Selection by the Eastern Tent Moth, *Malacosoma americanum*

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ABSTRACT

Fitness of semelparous species is highly dependent on availability of resources necessary for growth. Females of the eastern tent moth, *Malacosoma americanum* have been observed to prefer trees of smaller biomass for oviposition sites, thereby exposing their offspring to a higher risk of defoliation and thus, starvation. However, if smaller trees had higher tent temperatures and/or higher leaf nitrogen content, both of which would result in faster growth rates, than it may be advantageous for a female moth to oviposit on smaller trees. To test whether a negative correlation exists between tree biomass and nitrogen content and/or tent temperature, data was gathered for 20 trees of varying sizes, and correlation-regression tests were performed. No significant negative correlations were obtained that could suggest an advantage of oviposition preference for trees of smaller biomass. In fact, the only statistically significant results were positive correlations between tree biomass and tent temperature, most of which occurred at 9:00 pm. Therefore, it would appear that the eastern tent moths' oviposition preference for smaller trees is either maladaptive, or adaptive for other reasons yet unknown.

Introduction

Natural selection results in behavioral adaptations that increase an organism's fitness. However, due to fluctuations in the environment, certain behaviors may be adaptive under some conditions and maladaptive under others. Under such oscillating selection, behavioral adaptations may be favored because they ultimately increase fitness despite the potential for short periods of maladaptive behavior.

Grant and Gibbs (1987) observed oscillating selection in the medium ground finch, *Geospiza fortis*, of the Galapagos Islands during a long-term study of Darwin's finches. Small adult ground finches survive better than large ones during rainy seasons, but large adults are favored during drought conditions. This shift in the direction of selection is attributed to fluctuations in the types of seeds available on the island during rainy and drought conditions (Gibbs and Grant, 1987). Similar to the way that fluctuation in rainfall affects ground finch populations, fluctuating population size can change the direction of selection on certain behaviors such as those demonstrated in *Malacosoma americanum*, the eastern tent caterpillar.

The eastern tent caterpillar is a specialist that spends the entirety of its larval stage on its preferred host, *Prunus serotina*, or black cherry. *Malacosoma americanum* is native to North America, east of the Rocky Mountains. A female moth lays one egg clutch (typically containing 100-300 eggs) on the branch of a black cherry tree in late summer. The eggs later enter into diapause and remain on the tree for the duration of winter. They hatch in the early spring at approximately the same time that the tree begins to leaf out. Immediately after emerging from their eggs, the caterpillars begin to feed on the leaves, and spin a silk tent in the crotch of their host tree that they continue to build as they grow. The larvae spend most of the day and night in the tent, leaving only to feed. After approximately six weeks, the larvae reach maturity and leave the tree in order to pupate. They emerge from cocoons in July to mate, lay eggs, and then die, starting the life cycle over again (Borror, 1970).

Population outbreaks are observed every 6 to 16 years in the western tent caterpillar, a close relative of the eastern tent caterpillar (Myers, 2000). During outbreak years, host trees are often defoliated. This forces the caterpillars to leave the tree in search of another, in which case they will almost certainly perish due to predation or starvation. A female moth would be expected to oviposit on a tree that offers the highest chance of survival to her offspring, thus increasing her fitness. Since food availability is critical to the development of larvae, one might predict a female would select larger trees that offer a more abundant supply of food, thus lowering the risk of host defoliation and offspring starvation during population outbreaks. However, in a study conducted in 1989 at the University of Michigan Biological Station, Ruth Neiland concluded that, "*Malacosoma americanum* females do not oviposit randomly among cherry trees but, instead, lay their eggs preferentially on trees with specific characteristics. Overall, females appear to prefer smaller trees" (Neiland 1989, p.9). Neiland found that the number of egg masses was significantly negatively correlated with foliage volume and tree height.

Given this observed preference for oviposition on smaller trees, our study aims to address whether this apparently maladaptive behavior is actually an adaptive behavior, and if so, what the selective force(s) favoring it may be. Greater nitrogen availability and higher temperature both strongly accelerate insect development (USGS, 2000). Nitrogen is known to enhance the survival and fitness of many insects, especially herbivores (Mattson, 1980). For instance, higher levels of nitrogen resulted in increased body weight and better cocoon production for larvae of the silkworm, *Bombyx mori* (Mahmood, 1989 in Gondal and Javed, 2002). This can also be seen in *Manduca sexta* caterpillars, in which consumption of high-nitrogen diets during early instars led to a 20% increase in growth rates when compared to those consuming low-nitrogen diets (Woods, 1999).

Eastern tent caterpillars maintain higher body temperatures than are common for ectotherms of a similar size, and increased body temperature promotes more rapid rates of both digestion and overall growth. A shortened larval development period is often assumed to mean less exposure to predators, parasites, and parasitoids (Buttemer et al. 1988). One study found eastern tent caterpillar growth to depend heavily on temperature (Knapp and Casey, 1986).

It may be that eastern tent moth females select oviposition sites based in part upon leaf nitrogen content and tent temperatures. For instance, smaller trees may possess leaves with higher nitrogen content and may allow for increased temperatures within the tents since there are usually fewer leaves to obstruct the direct sunlight received by the tent. If so, these benefits of increased nitrogen and temperature can provide an explanation for the female moth's apparently maladaptive preference for ovipositing on smaller trees. Accordingly, in this study we ask:

1) Is leaf nitrogen content greater in smaller trees?

2) Is tent temperature higher in smaller trees?

Materials and Methods

Study Site

All experiments in this study were carried out in a field on the south side of Riggsville road in Pellston, Michigan, just west of the terminal moraine. There are approximately 50 trees in the area, the great majority of which are black cherry trees. About half of the black cherry trees contained tents, ranging from one to three tents per tree.

Effect of Tree Biomass on Nitrogen Content

To determine whether tree size is significantly related to the nitrogen content of leaves, we sampled 20 trees that reflected a representative variation in size. We used an inclinometer to measure the height and DBH tape to measure the diameter of each tree. Since some of the trees were too small to measure at actual breast height, the DBH of each tree was taken at one and half feet from the ground. DBH measures in inches were then used with the following allometric equation (Brennerman et al, 1978), specific to black cherry trees, to determine the amount of dry, above-ground biomass in kilograms:

$$(1.8082*(DBH^{2.6174}))/2.205$$

We collected 12 larger, fully developed leaves and 12 smaller, newly-budded leaves from each tree in order to provide a representative sample of the food source that the caterpillars are likely to encounter. Each set of 12 leaves was placed in a separate glassine envelope and stored in a -80 degree freezer for 36 hours. Leaves were then lyophilized overnight, ground to a fine powder, and placed in a mass spectrometer to determine the nitrogen content. A regression was used to determine if nitrogen content of small or large leaves is correlated with tree biomass.

Effect of Tree Biomass on Tent Temperature

To determine whether a difference in temperature exists between interiors of the caterpillars' tents on smaller trees versus larger trees, we measured the temperature of all 12 accessible tents within our site. We placed a Thermochron i-button in the middle of each tent to monitor temperature at ten minute intervals throughout the day for three and a half days. We also measured the percentage of total tent surface area facing south in order to determine if southern exposure might further affect tent temperature. We approximated the total surface area by measuring the length and width of each face of the tent (north, south, east, and west) to obtain a surface area for each aspect. We then divided the south-facing area by the total to get a south-facing percentage.

The data were analyzed using a regression-correlation to determine how tent temperature is correlated with tree biomass. The regressions were performed with temperature data from 12:00 am, 6:00am, 9:00am, 12:00 pm, 3:00 pm, 6:00 pm, and 9:00 pm in order to ensure that we had results with representative variations in ambient temperatures throughout each day. A stepwise regression was then carried out with percent south-facing forced at step 1 in order to correct for any temperature variation that might be attributed to tent orientation. The remaining variation could then be more accurately accredited to difference in tree biomass.

Results

Effect of Tree Biomass on Nitrogen Content

Correlation analysis revealed no significant relationship between tree biomass and the nitrogen content of either small or large leaves (Figures 1a and 1b). When the relative influence of each tree on the correlation coefficient was taken into account, we could see that tree 12 had a disproportionate effect on the correlation coefficient for small leaves (Figure 1c). When this tree was removed from the sample, we observed a significant positive correlation between tree biomass and nitrogen content ($R = 0.6$, $p = 0.014$).

Effect of Tree Biomass on Tent Temperature

Correlation analysis revealed that tree biomass was significantly positively correlated with tent temperature at 3:00 pm on Thursday and at 9:00 pm on all four days while at all other times there were no significant correlations. Once again, tree 12 disproportionately influenced the correlation coefficient. Due to its location near the road where there is greater retention of heat by asphalt, it is likely that ambient as well as tent temperatures for this tree were artificially elevated. Therefore, it was removed from the analysis. Tree 39 was also located near the road, so, despite its unremarkable influence on the correlation coefficient, it too was removed. Reanalysis without these two trees revealed that the majority of the significant correlations occurred at 9:00 PM (Table 1).

When percent south-facing surface area was taken into account, all p-values increased slightly, causing the nearly significant results to become non-significant (Table 2). However, all of the remaining correlations were still positive, and thus still could not provide any explanation for why females may have adapted a preference for ovipositing on smaller trees.

In order to ensure that variation in tent size between large and small trees was not a confounding variable, a regression test was performed to determine if there was any correlation between tree biomass and total surface area of the tent. The results of this regression test were not significant ($p=0.86$, $R=0.06$) showing that the variation in tent size between large and small trees is equal.

A regression test was also run between total tent surface area and tent temperature at the seven time periods during each day to determine if the size of a tent affects its temperature. The results showed positive correlations between tent size and tent temperature in the time period ranging from 12:00 am to 9:00 am (Table 3). However, in our sample, tent size is not correlated with tree biomass so the correlation between tent size and tent temperature will not bias our results.

Discussion

The results of this study indicate that no correlation exists between tree biomass and nitrogen content within the leaves. Therefore there is no advantageous nitrogen-based explanation for the female moth's oviposition preference.

The positive correlations observed between tree biomass and tent temperature also do not suggest that the observed oviposition preference is an adaptive behavior. It is unclear why tent temperatures were higher in larger trees almost exclusively at 9:00 pm. Possibly, the wood content of larger trees retains more heat that is given off in the evening around 9:00 pm. The specific heat of air is lower than the specific heat of wood, allowing the wood to cool more slowly than the air (Dunlap, 1912 in Campion et al., 2004). During the early night the wood content of larger trees may stay warm enough to maintain an elevated tent temperature. It might also be that heat rising at night takes longer to filter through the canopies of larger trees. The heat may become temporarily trapped in the pockets of the foliage, allowing the air temperature within the canopy to stay elevated longer than the air outside of the canopy. This could permit the tents under larger canopies to maintain higher temperatures, specifically in the early night.

One possible explanation for the observed oviposition preference is that the behavior is simply maladaptive. The current existence of such maladaptive behavior may be due to fluctuations in the environment; ovipositing on smaller trees may have been advantageous under different conditions in the past, however, under current conditions they may now appear maladaptive. For example, interspecific competition for larger trees may have resulted in resource partitioning, driving the eastern tent caterpillar toward a preference for smaller trees. Alternatively, in the past a disease may have infected only larger black cherry trees, decreasing their nutritional quality enough that selection would favor oviposition on smaller, healthier trees to increase the moth's fitness.

This seemingly maladaptive behavior may also be due to the fluctuation cycles in the eastern tent caterpillar population, known to occur approximately every 12 years. These fluctuations can cause the population to surge one year and cause a severe bottleneck to ensue the following year. Specifically in 1989, the local eastern tent caterpillar population experienced an outbreak that resulted in about 99.9% of the caterpillars being parasitoidized (Karowe, 2005). This caused the population to rapidly plummet, which would potentially allow the allele for small tree oviposition preference to be fixed. A mutation may have arisen favoring the oviposition preference for larger trees, but genetic drift may have eliminated the allele.

Conversely, the preference for ovipositing on smaller trees could be adaptive for reasons yet unknown to us. The eastern tent caterpillar is unusually social compared to other species of caterpillar. The caterpillars habitually bask in groups, allowing them to reduce their surface area to volume ratio and thereby achieve higher body temperatures than they would on their own (Knapp and Casey, 1986). Therefore, occupying a smaller tree might facilitate their social behavior because there is less surface area for the caterpillars to disperse themselves.

Additionally, the eastern tent caterpillar has the ability to ingest the cyanide in the leaves of the black cherry tree, which stays in the gut of the caterpillar until it is expelled as waste (Fitzgerald et al., 2002). The cyanide within the caterpillar serves as a defense against small predators that are unable to neutralize the toxin. Younger, smaller leaves found at the tips of growing branches contain significantly higher levels of cyanide (Fitzgerald et al., 2002). Perhaps ovipositing on smaller trees that may have more of these younger leaves creates a food source for the caterpillars that enhances their anti-predator defense.

Any of these potential benefits of ovipositing on smaller trees could outweigh the negative consequences, such as defoliation, that may result from this preference. In this case, a preference for oviposition on trees of smaller biomass might still be an adaptive behavior.

Table 1. Summary table of R-values and p-values for significant (*) and nearly significant (†) correlations between tent temperature and tree biomass.

Day		12 AM	6 AM	9 AM	NOON	3 PM	6 PM	9 PM
Wednesday	R						0.60	0.89
	p						0.07†	0.001*
Thursday	R					0.766		0.710
	p					0.010*		0.021*
Friday	R							0.837
	p							0.003*
Saturday	R			-0.630				
	p			0.051†				

Table 2. Summary table of R-values and p-values for significant correlations of stepwise regression (percent south-facing forced at step 1, tree biomass forced at step 2).

Day		12 AM	6 AM	9 AM	NOON	3 PM	6 PM	9 PM
Wednesday	R							0.929
	p							0.001
Thursday	R					0.769		0.799
	p					0.044		0.028
Friday	R							0.854
	p							0.010
Saturday	R							
	p							

Table 3. Summary table of R-values and p-values for significant (*) and nearly significant (†) correlations between tent temperature and total tent surface area.

Day		12 AM	6 AM	9 AM	NOON	3 PM	6 PM	9 PM
Wednesday	R		0.642					
	p		0.045*					
Thursday	R		0.669	0.669				
	p		0.034*	0.034*				
Friday	R	0.646	0.602	0.587				
	p	0.044*	0.065†	0.074†				
Saturday	R		0.714					
	p		0.020*					

Figure 1a. Relationship between leaf nitrogen content of large leaves and total tree biomass where R = 0.07 and p = 0.77. Relative influence of each individual within the sample is denoted by point size.

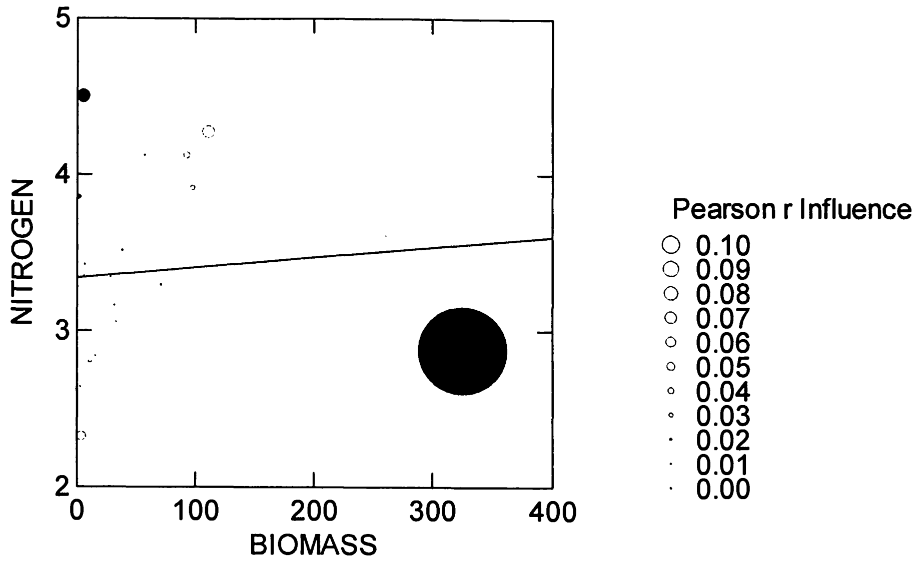


Figure 1b. Relationship between leaf nitrogen content of small leaves and total tree biomass where R = 0.08 and p = 0.73. Relative influence of each individual within the sample is denoted by point size.

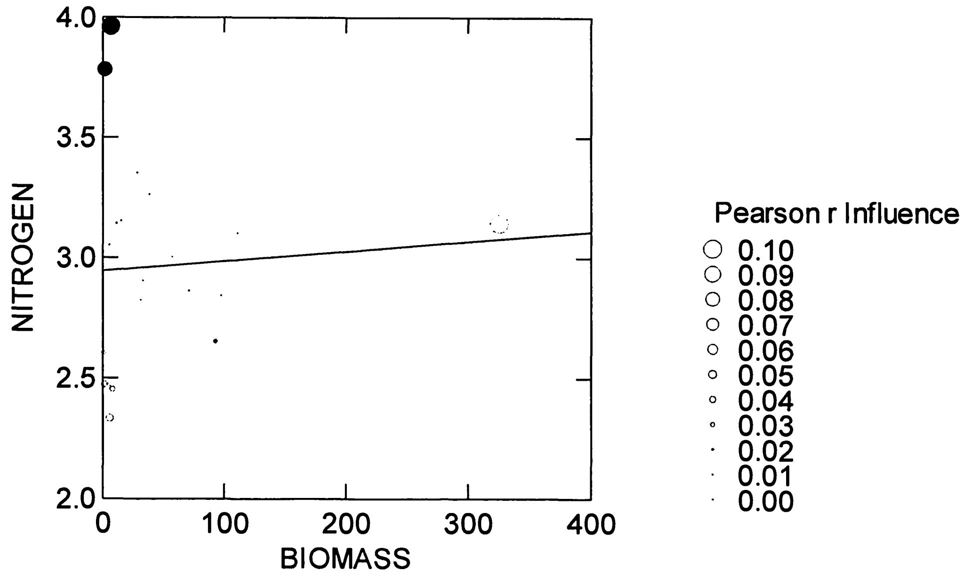
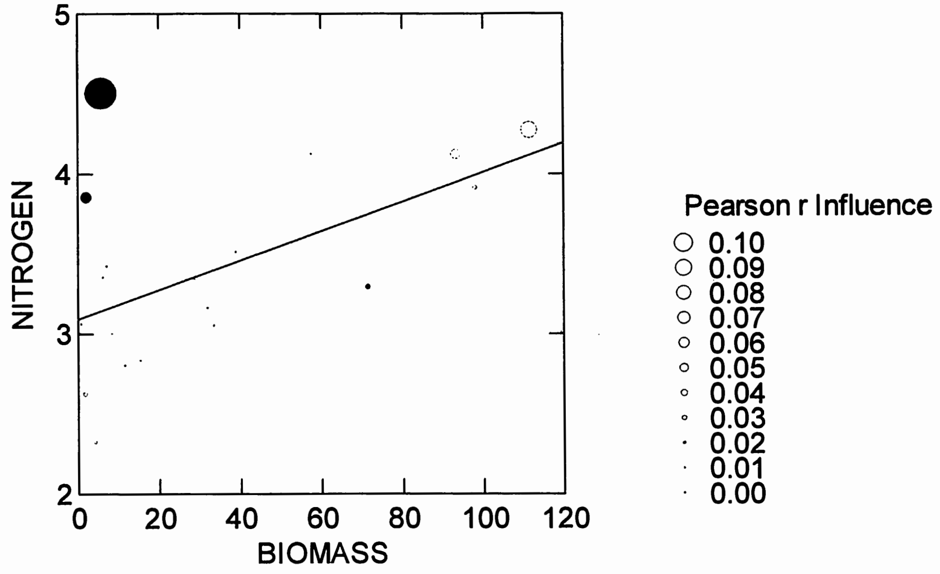


Figure 1c. Relationship between leaf nitrogen content of small leaves and total tree biomass when tree is removed from sample. R = 0.55 and p = 0.014. Relative influence of each individual within the sample is denoted by point size.



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