The effect of understory vegetation on nestbox utilization by *Peromyscus leucopus* in differently sized forest fragments.

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By

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Numerous studies of forest fragmentation have found a negative relationship between the density of *Peromyscus leucopus* (the white-footed mouse) and forest fragment size. This relationship may be caused, in part, by both more food (primary production) and more cover from predation in smaller fragments, which have more structurally complex understory vegetation than larger fragments. However, the influence of the proximity of understory vegetation on selection of nesting sites in specific locations within the fragment had not been studied. I hypothesized that nestboxes in highly vegetated areas would be utilized more often by *P. leucopus* than nestboxes in sparsely vegetated areas. I tested this hypothesis by measuring the amount of vegetation near thirty nestboxes in each of nine forest fragments. I also estimated the relative population density of *P. leucopus* in each fragment. I expected to find both a greater proportion of nestboxes occupied and a greater number of mice in nestboxes with a high amount of nearby vegetation. The structural complexity of understory vegetation was significantly greater in small forest fragments than in large and in edge habitat than interior. However, there was no relationship between any of the variables we measured and the density of mice, other than boxes being occupied more frequently in habitat where more mice were present. Additionally, none of the variables we measured were related to the probability of the nestbox being occupied. My results suggest that the complexity of vegetation immediately surrounding the nestbox may not be as important to mice as vegetation at a larger scale (e.g. throughout the individual’s territory).
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Figure 1: A comparison of the structural complexity of understory vegetation in nine forest fragments outside of Oxford, OH. Vegetation is significantly greater in small than large fragments and edge than interior habitat.
Introduction

Prior to the arrival of Europeans, forests covered much of the eastern half of the United States. However, since then, many parts of forests have been removed for farming and other purposes, leaving isolated forest fragments. Fragmentation can have a negative impact on the population dynamics of native species (Yahner, 1988). Extinctions, decreased genetic diversity, and decreased resource availability are just a few of the negative consequences of habitat fragmentation (Banks et al., 2007). Even so, some species appear to be unaffected by forest fragmentation, and some seem to benefit from it (Yahner, 1988). *Peromyscus leucopus*, the white-footed mouse, is one species for which population density is inversely associated with the size of forest fragments. This unusual relationship may be important, because white-footed mice fill several ecological roles in the forest, including those of prey, predator, and competitor (Marcello et al., 2008).

Several studies have shown that there is a negative relationship between the density of *P. leucopus* and forest fragment size; in other words, smaller fragments have more mice per hectare than larger fragments (Anderson et al., 2003; Nupp and Swihart 1996, 1998, 2000; Yahner 1992). This is thought to be due, in part, to the fact that small forest fragments have a greater complexity of understory vegetation than do larger fragments (Anderson and Meikle, 2006). Vegetation plays a fundamental role in the survival of *P. leucopus*, as it provides both food, in the form of nuts, fruits, and insects, and shelter from predators (Anderson et al., 2003; Gehlhausen et al., 2000; Graves et al., 1988; Kaufman et al., 1983; Ranney et al., 1981). In fact, individuals tend to avoid areas
of their home ranges that do not provide sufficient cover from predators (Barnum et al., 1992; Kaufman et al., 1983). Trees and shrubs provide covered pathways for travel, vertical escape routes from predators, and locations for nest construction (Barnum et al., 1992; Graves et al., 1988; Kaufman et al., 1983; Kaufman et al., 1985; Yahner, 1985), which results in a higher rate of reproduction and population density for *P. leucopus* in areas of greater vegetation complexity (Anderson et al. 2003; Wilder and Meikle 2005, 2006).

In addition to living vegetation, coarse woody debris (e.g., fallen logs and stumps) is beneficial to *P. leucopus*. Mice tend to choose large (> 5 cm) logs for travel, because they can move without auditory detection by predators by avoiding leaf litter on the ground, and they can easily keep their balance on the wider logs (Barnum et al., 1992). Additionally, mice use coarse woody debris as orientation guides as they navigate through the habitat (Barry and Francq, 1980). Coarse woody debris also can provide food in the form of insects and fungi (Greenberg, 2002). In general, the microdistribution of *P. leucopus* is positively influenced by the presence of coarse woody debris (Greenberg, 2002).

Because Anderson and Meikle (2006) argue that a greater complexity of understory vegetation leads to a greater relative density of mice, and because previous research has indicated that microhabitat use by *P. leucopus* is related to the surrounding vegetation (Barnum et al., 1992; Kitchings and Levy, 1981), I hypothesized that the immediate surrounding vegetation would affect the occupancy rate of each nestbox in our study sites. I predicted that more mice would be found in the nestboxes near the greatest
amount of vegetation. I also predicted that more mice would be captured in nestboxes near the greatest amount of coarse woody debris.

**Methods**

**STUDY SITE**

Nine fragments of secondary-growth deciduous forest within 25 km of Oxford, Ohio, U.S.A. were used for the study. The trees in the forest patches were of similar age, as estimated by mean basal area of trees among patches (Anderson et al., 2006). As a part of another study, the fragments were designated as either large (>100 ha) or small (~1-2 ha), and were separated from the nearest study fragment by at least 1 km and from other forested areas by at least 50 m (Marcello et al., 2008).

**RELATIVE POPULATION DENSITY OF MICE**

Each fragment had fifteen nestboxes in the edge and fifteen nestboxes in the interior, for a total of 270 boxes for the nine fragments. Edge habitat was defined as being within 15 m of the field/forest transition and was based on differences in the complexity of the understory vegetation (Anderson et al., 2003; Marcello et al., 2008; Wilder and Meikle, 2005). The nestboxes were approximately 15 m apart, and each was hung on a tree at a height of ~1.5 m. Nestboxes (15 x 15 x 15 cm) were made of wood with two 2.5 cm openings. In addition, nestboxes were filled with a polyester fiberfill for mouse bedding (Marcello et al., 2008). The use of nestboxes allowed the capture of mice with a minimum of stress to the animals (Wilder and Meikle, 2005).
Censusing and collection of data on mice were performed as outlined in Marcello et al. (2008). Mice were captured individually in a plastic bag and scanned for the presence of a passive integrated transponder (PIT tag; AVID®). Data for each mouse included weight, body and tail length, sex, and reproductive status. New captures (if ≥ 8 g, no PIT tag present) were lightly anesthetized with isoflurane and injected with a PIT tag subcutaneously in the interscapular area (see Anderson et al., 2003; Marcello et al., 2008; Wilder and Meikle, 2005). Animals less than 8 g were not tagged or included in the census. Instead, their collective weight was recorded with the data of the reproductive female found in the same nestbox (Marcello et al., 2008). The nestboxes were checked from April 2008 until November 2008, for a total of six censuses. The Institutional Animal Care and Use Committee of Miami University had approved these methods (protocol number 726).

COMPLEXITY OF UNDERSTORY VEGETATION

The vegetation measurement used in our study was performed in August 2008. For each nestbox, four measurements were obtained 2 m from the nestbox (to the north, south, east, and west) in a method similar to Anderson and Meikle (2006). A 2 m vertical rod marked at half-meter intervals was placed at each of the four compass points around the nestbox tree. The number of the four sections on the pole that were contacted by vegetation was recorded for a possible total of four. For example, a vegetation score of two meant that the pole was contacted by vegetation somewhere in two of the four half-meter sections. If one of the standard measured points was in the matrix (e.g. corn,
soybeans, pasture), the measurement was not included in the average score for that nestbox. Vegetation scores for each nestbox were averaged to obtain a total vegetation score for each nestbox for comparison with scores of other nestboxes. Coarse woody debris was also recorded at each compass point by counting all woody debris greater than 1 cm in a six-centimeter radius around the bottom of the vegetation pole. It was then summed for the four points the rod was placed. In addition, diameter at breast height was obtained for each tree that contained a nestbox, and a soil moisture measurement was taken using a Lincoln soil moisture meter at the base of each nestbox tree.

STATISTICAL ANALYSIS

The relationship between microhabitat characteristics and relative density of mice was tested in two ways. In the first analysis, only data from the two population censuses immediately before and after the vegetation check were used (a census in July and a census in August). In this analysis, we assumed that the vegetation only represented a narrow window of time encompassed by censuses before and after the vegetation measurement. In the second analysis, data from all six *P. leucopus* censuses were used. For this second analysis, it was assumed that the values for the single measurement of vegetation complexity were representative of the relative complexity of vegetation throughout the year.

The relative density of mice (for two censuses and for six censuses) was analyzed using an analysis of variance (ANOVA) with fragment size, habitat type (edge or interior), coarse woody debris, diameter at breast height, mean vegetation score, mean
soil moisture, and number of mice in the habitat as factors all in the same model (ANOVA; PROC MIXED, SAS Institute). A logistic regression was performed (PROC GENMOD, SAS Institute) to determine whether any of the above factors influenced the probability of each box being occupied. Mean vegetation score, coarse woody debris, diameter at breast height, and mean soil moisture were individually analyzed using an ANOVA with fragment size, habitat type (edge or interior), and number of mice in the habitat as factors in the model. Only statistically significant interactions are reported.

**Results**

When analyzing the data obtained from the relative population density censuses immediately before and after the vegetation measurement, I found that nestboxes in habitats (edge or interior) with a greater population density of *P. leucopus* were more likely to be occupied ($X^2 = 4.75$, df = 1, $p < 0.0001$) and were occupied by more mice simultaneously ($F_{(6, 244)} = 3.14$, $p = 0.006$). However, there was no relationship between any of the other variables we measured (vegetation, coarse woody debris, diameter at breast height, or soil moisture) and the relative density of mice in relation to the nestbox (all $p > 0.1$). Likewise, none of the variables we measured were related to the probability of the nestbox being occupied (all $p > 0.1$). The structural complexity of understory vegetation was significantly greater in small forest fragments than in large ($F_{(1, 253)} = 4.64$, $p = 0.03$) and in edge habitat than interior ($F_{(1, 253)} = 35.11$, $p < 0.0001$; Fig 1).

When analyzing all six censuses in relation to the vegetation measurement, we obtained similar results. Nestboxes in habitats (edge or interior) with a greater population
density of *P. leucopus* were more likely to be occupied ($X^2 = 8.24$, df = 1, p = 0.004). Additionally, there was no relationship between any of the other variables we measured (vegetation, coarse woody debris, diameter at breast height, or soil moisture) and the relative density of mice in relation to the nestbox (all p > 0.1). Likewise, none of the variables we measured were related to the probability of the nestbox being occupied (all p > 0.1).

![Figure 1](image.png)

**Figure 1.** A comparison of the structural complexity of understory vegetation in nine forest fragments outside of Oxford, OH. Vegetation is significantly greater in small than large fragments and edge than interior habitat.

### Discussion

The greater complexity of understory vegetation in the edge versus the interior in this study is consistent with previous findings (Anderson *et al.*, 2003; Anderson and Meikle, 2006; Burke and Nol, 1998). Although understory vegetation complexity was related to both fragment size and habitat type, the population density of *Peromyscus leucopus* was not related to the structural complexity of understory vegetation, either in
the forest fragment overall or specifically near the nestbox. No relationship was found between the population density, the nestbox occupancy, and the other variables tested, such as coarse woody debris. There could be several explanations for these findings.

A factor contributing to the lack of relationship between nestbox occupancy and the relative density of *P. leucopus* may be that the 2 m area around each nestbox tree was too small an area to determine if the vegetation in the immediate area surrounding the tree had any effect on occupancy rates. The home ranges of *P. leucopus* are much bigger than the area sampled (averaged around 590m²; Wolff, 1985), and *P. leucopus* is known to travel throughout its territory to find food. Perhaps the vegetation immediately surrounding where *P. leucopus* nests is not indicative of its nesting choices; instead, there may be other factors influencing the choice of nest location.

Research by Barnum *et al.* (1992) indicates that *P. leucopus* prefer logs that are greater than 5 cm in diameter for path selection. This could have implications for the current project in two ways. When measuring coarse woody debris in this study, all logs and sticks that were at least 1 cm in diameter were counted. In future studies, only logs greater than 5 cm should be enumerated, as those are of the most importance to *P. leucopus* (Barnum *et al.*, 1992). In addition, I found that there was no influence of the diameter of the nestbox tree on the distribution of mice. Perhaps *P. leucopus* prefers all woody vegetation greater than 5 cm in diameter, and as all of the nestbox trees were much greater in diameter than this, we did not see an effect.

Another factor influencing these results could be that our estimate of vegetation complexity is based on one measurement of vegetation. This measurement was done in
August, when the growing season is coming to an end in the study area. Vegetation is likely to vary over the course of the growing season and may influence the choice of microhabitat over a longer time period (e.g., spring to fall). Various herbaceous species are present at different times over the growing season, and cover utilized as protection may change. In future studies, vegetation should be measured at each population census for *P. leucopus*, in order to test for a correlation between the nestbox occupancies and the amount of vegetation at each check.

Future studies should focus on the effects of vegetation in close proximity to nesting sites over a longer time period. This would provide a better estimate of the effects of each aspect of the environment on densities of *P. leucopus* and of preferential nestbox locations. Observing nestbox occupancies over time in relation to variable vegetation growth, soil moisture, and coarse woody debris would eliminate possible biases created by a single check. Overall, in the 270 nestboxes, I found that mice appear to be influenced by vegetation, but possibly at a larger scale than the small area immediately adjacent to their nesting sites. The vegetation located in their entire home range may be more influential than that in the immediate area.

**Implications for Personal Development**

Creating my own research project and experiencing the honors thesis process has done much to aid both my development as a researcher and how I think about hypotheses and data. Up until this point, I have read scientific literature and performed experiments in classroom laboratories that may have taken two to three hours, or a day or two to
complete. Completing my own project has helped me to realize how much effort it takes to see a scientific project from conception to the end product. I gained an appreciation for the difficulties faced by individuals who make research their life’s work. It is time consuming, often frustrating, and as in my case, the result may not be what is expected. However, research is highly beneficial; almost everything we know about the world, from mouse population patterns to breakthrough medical procedures are a product of research. Oftentimes we can be inspired by something that did not go the way we expected, and we can formulate new hypotheses based on the new information. This is another thing I have learned from completing an honors thesis.

While my career goal is to practice medicine, I believe that my research in ecology has taught me a great deal. I have learned the research process in a way that can never be taught in a course- by doing it myself, in a full-scale project. I have discovered the creativity needed to think outside what has already been done in a scientific field; how to take what has been studied already one step further. I have learned how to write a scientific article in a way that could be submitted for publication in a journal. Most importantly, I have learned that no matter the outcome, something can always be learned from research.
References


