Comparison of Ant (*Lasius neoniger* and *Prenolepis imparis*) Nesting Sites at Pierce Cedar Creek Institute and the Influence of Temperature on *L. neoniger* Activity

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Abstract

Our study focuses on three objectives: surveying ant biodiversity on Pierce Cedar Creek Institute trails, comparing nesting site characteristics in *P. imparis* and *L. neoniger*, and testing the effect of soil surface temperature on activity levels of *L. neoniger.* We identified twenty-one species of ants on the trail system at PCCI, of which only four were previously documented in Barry County, MI, the site of our study. With regard to nest characteristics, we found that ant hole density in the nests of *L. neoniger* was significantly greater than in *P. imparis*. In addition, percent canopy cover in *L. neoniger* was significantly lower than in *P. imparis.* Finally, soil surface temperature was shown to have a significant influence on the number of ants excavating throughout the course of a day in one of two *L. neoniger* nesting sites studied.

Introduction

Ants are abundant. Ants and termites comprise 1/3 of the rainforest's entire animal biomass in the Amazonian *terra firme* rainforest; there are 8 million ants and 1 million termites in each hectare of soil (Hölldobler and Wilson 1990). Closer to home, Mary Talbot found 87 ant species in 23 genera in 5.6 square kilometers of the Edwin S. George reserve in Livingston, Michigan (Talbot 1975, cited in Hölldobler, 1990). Because ants are abundant, they profoundly influence ecosystems; in one study, over half of the sampled seeds in a New York state beech-maple forest were dispersed by ants (Handel et al. 1981). *Lasius neoniger*, the cornfield ant, overturns the top 0.3 m of soil on the edge of a cornfield in about 1,000 years (Wang et al. 1995). The profound environmental impact of ants makes them a worthy topic of study.

Michigan ant surveys have identified 113 ant species in the state (Wheeler et al. 1994). Of these, twelve ant species have been documented in Barry County (Wheeler et al. 1994). Pierce Cedar Creek Institute (PCCI) is located in Barry County but no ant survey has previously been conducted specifically on the institute's property. Thus, we chose to conduct a foundational study of the institute's ant population. Our study had three components. We first developed a working list of the Institute's ant species. Next, we analyzed nesting sites of the two most recurrent species with respect to soil composition, canopy cover, and hole-density. We hypothesized that preferred nesting characteristics would differ between species. Canopy cover affects the soil surface temperature, and surface temperature affects foraging behavior (Cole et al. 2010); we included canopy cover as a variable to see if it was significantly different between species. Finally, we analyzed the effect of temperature on the foraging behavior of *Lasius neoniger,* a prominent species on the trails at PCCI. It has been shown that temperature influences ant behavior (Yamamoto et al. 2008; Ruano et al. 2000). According to Bert Hölldobler and Edward O. Wilson (1994), most worker ants do not function well at temperatures below 20$^{\circ C}$ and without a certain amount of heat, ants cannot perform foraging, egg laying, and brood-rearing tasks. We hypothesized that surface soil temperature determines the number of ants (*Lasius neoniger*) excavating and foraging at a given time of day.

Materials and Methods

Biodiversity

We surveyed the biodiversity of ants using two collection methods along the 11.6 km trail system of Pierce Cedar Creek Institute in Barry County, Michigan. Our decision to survey only the trail system was made in an effort to make the scope of the study manageable and to provide information about ants that would most frequently be encountered by visitors to the Institute. Habitat types in which we collected ants included: constructed prairie, oak/hickory and beech/maple forests, mixed hardwood and tamarack swamps, and fens. From mid-May through mid-July 2011, we collected specimens by-hand, using forceps and test tubes containing isopropyl alcohol. During this collection period, we covered the entire trail system twice and visited certain areas three to four times. All samples were collected between the hours of 9:00 AM and 5:00 PM.

After finding that for large portions of the trail system, our manual collection method had not provided any specimens, we decided to supplement our collection with the pitfall trapping method. Being constrained by budget, we were able to select only twelve sites to collect additional specimens using pitfall traps. These sites were selected by viewing the distribution of sites already sampled on a GIS map of the property and determining the twelve areas where sample points were most scarce. The traps were constructed of plastic cups filled with approximately an inch of a salt and dish-soap solution and covered with paper plates (to keep mammals out) according to the method described by Laub et al. 2009. After forty-eight hours, the traps were collected and ants were removed.

In an effort to learn ant anatomy and taxonomy, we attempted to identify some ant specimens to genus using dissection microscopes and an online dichotomous key to the family, *Formicidae* (Linksvayer 2011). Brian Fisher's *Ants of North America: A Guide to the Genera* was also consulted for additional help identifying specimens. After our initial attempt, all specimens were sent to Dr. Grzegorz Buczkowski of the Purdue University Ant Behavioral Ecology and Genetics Laboratory for identification to the species level. After completion of the study, collected ant specimens will be housed at Pierce Cedar Creek Institute.

Nesting Site Characterization

For the second portion of our study, we measured nesting site characteristics for each of fifty-nine sites at which specimens were collected manually and a single species was present. This allowed data that were collected for the nest site to be matched with the species that was collected from the site. Parameters we measured at each nesting site included: canopy cover, soil composition, and hole-density. Canopy cover was measured using a spherical densiometer and calculated according to the manufacturer's protocol. Soil samples (collected at a depth of 13 cm) were air dried for three to fifteen days, ground with mortar and pestle, and sieved into fractions of organic matter, gravel (>1,680 microns), and three sand fractions (420 to 1,680, 250 to 420, and 125 to 250 microns). Percent by mass was then calculated for each fraction of each soil sample. The hole-density of each nesting site was calculated using a quadrat of 10 m length by trail width (approximately 2 m in most places). We included in the hole-density calculation any hole contained within the section which was surrounded by cones of excavated soil, had an ant emerging from it, and/or was highly similar to holes previously seen with ants emerging from them. Characterization of nest sites was done during two weeks: one in late June, and one in mid-July, due to weather and time constraints. We collected data at all fifty-nine nests, which included nests of eleven species. The number of nests sampled for each species ranged from one to twenty-two (Figure 1). Statistical analysis was only performed on species for which at least ten nests were sampled. For this reason, T-tests were performed on only *Prenolepis imparis* and *Lasius neoniger*. For *Prenolepis imparis*, data from eighteen nests were included in the calculations, and for *Lasius neoniger*, data from twenty-two nests were included.

Foraging Behavior

The final portion of our study involved observing foraging behavior at two nesting sites, White Trail (W) and Yellow Trail (Y) (approximately 1.29 km apart), of *Lasius neoniger*. Both sites were located on sandy trails at Pierce Cedar Creek Institute: one in an open field and the other in a semi-open prairie. Because ant colonies operate as super-organisms, the number of ants leaving or excavating a nest can be used as an indicator of colony activity level (Yamamoto et al. 2008). At each site, we counted the number of ants leaving the nest for an eight-minute period every hour as well as the number of ants excavating the hole during a separate two-minute period of every hour, from approximately 6:00 AM until 9:00 PM on each of four days. For hours in which rain prevented ant activity, we completed counts one or two days later at roughly the same times. At the beginning of each ten-minute period in which leaving or excavating ants were counted, we also measured the temperature next to the ant hole just under the surface of the soil using a digital thermometer. This ensured that a measurement of soil temperature (and not ambient temperature) was obtained (Cole et al. 2010). Because it was impossible to follow ants which left the nest and confirm that they were foraging, any ant seen leaving the excavated radius around the nest opening was assumed to be foraging. Our results were then analyzed by polynomial regression to determine whether soil surface temperature played a major role in determining how many ants left the nest or excavated at a given time.

Results

Biodiversity

Our survey of the ant biodiversity of the PCCI's trail system is detailed in Table 1. Figure 2 shows the GPS locations of manually sampled points on the trail system. We collected a total of twenty-one species in thirteen different genera. The two most prevalent species from our collection were *Lasius neoniger* and *Prenolepis imparis* (Figure 1). Manual collection provided the greatest biodiversity of ant species, and pitfall collection provided more locations of some species which had previously been gathered elsewhere by hand. Two species, *Formica lasioides* (Emery) and *Lasius claviger* (Ward) were separated from their respective pitfall traps without proper labeling during analysis of pitfall debris, so the trail locations of their collection cannot be determined. GPS points were taken at the sites of manual collection and the sites of the pitfall traps. These data are on record at PCCI.

Nest Characterization

Of all the species sampled in the biodiversity portion of the study, we selected nests of *Prenolepis imparis* and *Lasius neoniger* to characterize. T-Tests were performed on the data collected from twenty-two nesting sites of *L. neoniger* and eighteen nesting sites of *P. imparis* (Table 2). The t-Test showed that the mean hole density of *L. neoniger*'s nesting sites is significantly greater than the mean hole density of *P. imparis'* nesting sites ($t = 3.86$, $df = 35$, $P = 0.0005$). A second t-Test showed that the mean canopy cover of *L. neoniger*'s nesting sites is significantly less than the mean canopy cover of *P. imparis'* nesting sites ($t = -6.39$, $df = 29$, $P = 5.5 \times 10^{-7}$). The t-Tests comparing soil composition showed no significant differences.

Foraging Behavior

A significant relationship was observed between soil surface temperature and the number of ants excavating at site W ($P = 0.01$) and at both sites combined ($P = 0.01$) when data were analyzed by polynomial regression. Results of all polynomial regressions performed are listed in Tables 1 and 2. Scatter plots of the number of ants excavating versus soil-surface temperature show the largest number of ants excavating in the median temperature range of 20-36°C (Figures 3 & 4). Ants were seen excavating at all of the lowest temperatures at which data were collected; however, no ants were seen excavating at temperatures above 39.2°C.

Discussion

Biodiversity

Based on a list of Michigan ant species, we expected to find at least twelve species at Pierce Cedar Creek Institute (Wheeler et al. 1994). By collecting ants manually and through the use of pitfall traps, we were able to obtain samples of twenty-one species on the trail system alone. The species we identified are listed by subfamily in Table. 1. Future research is needed before the ant biodiversity of PCCI's entire property can be known. Other projects could focus on sampling the forest and prairie ecosystems, utilizing a variety of sampling methods such as malaise traps, fogging, and sifting through forest ground cover.

Nest Characterization

Lasius neoniger's habit of selecting finer particles for their nest walls significantly modifies soil composition; this may impact nutrient leaching processes (Wang et al. 2000). Because of the abundance of *Lasius neoniger* and its importance to ecosystems in soil modification, we focused on characterizing the nests of this species at Pierce Cedar Creek Institute to see if they significantly differed from other species' nests in terms of soil composition, hole density in the quadrat around the hole from which the species was sampled, and canopy cover. We found no significant difference between the soil composition of *Lasius neoniger* sites and *Prenolepis imparis* sites. Future research could explore soil composition using more sophisticated instruments. We observed more holes around the nesting sites of *Lasius neoniger* than around the nesting sites of *Prenolepis imparis.* Our study did not examine whether the holes counted were all ant holes or even the same species; further research is required to determine the implications of this relationship.

Foraging Behavior

The significance of the relationship between soil surface temperature and the number of ants excavating at site W and at both sites combined reinforces the established idea that soil surface temperature influences ant activity patterns (Cole et al. 2010). What our study points out, however, is that the influence of soil surface temperature on excavating activity may differ between ant nesting sites, even within the same species. Though the scope of our study is not wide enough to draw definitive conclusions about this possibility, it opens up topics for further study. Knowing that the location of a nest is in itself a factor in determining when (at what soil surface temperatures) ants excavate, one could develop a study to determine which other variables, unique to a specific site, are related to excavating schedules. These variables might include the foraging schedules of other insects living nearby, time of year, and surrounding terrain. Furthermore, because our study did not reflect a significant relationship between soil surface temperature and foraging activity in *L. neoniger*, additional studies are necessary to determine whether this can be attributed to experimental error, a unique characteristic of the species, or a unique set of conditions at the study site.

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Tables and Figures

Table 1. Ant Biodiversity of Pierce Cedar Creek Institute's Trail Network
Collection method denoted. M = Manual, PT = Pitfall Trap
Species listed in green were not previously documented in Barry County.

Subfamily	Species	Where Collected
Myrmicinae	*Aphaenogaster rudis* (Emery)	Green Trail (M&PT)
Myrmicinae	*Crematogaster cerasi* (Fitch)	Yellow Trail (M)
Myrmicinae	*Monomorium minimum* (Buckley)	Red Trail (M)
Myrmicinae	*Myrmica americana* (Weber)	Red Trail (Ml), Yellow Trail (M)
Myrmicinae	*Myrmica pinetorum* (Wheeler)	Blue (PT), Green (PT), Orange (PT), Red (M&PT), White (M&PT), and Yellow (PT) Trails
Myrmicinae	*Myrmica lobifrons* (Pergande)	Fern along Red Trail (M)
Myrmicinae	*Temnothorax curvispinosus*	White Trail (PT)
Myrmicinae	*Tetramorium caespitum* (Linnaeus)	Orange (M), Red (M), and White (M) Trails
Formicinae	*Camponotus chromaiodes*	Red Trail (M)
Formicinae	*Camponotus pennsylvanicus* (DeGeer)	Blue (M&PT), Green (PT), Orange (PT), and Red (PT) Trails
Formicinae	*Formica aserva* (Forel)	Red Trail (M)
Formicinae	*Formica glacialis* (Wheeler)	Red Trail (M)
Formicinae	*Formica lasioides* (Emery)	Unknown (PT)
Formicinae	*Formica neogagates* (Emery)	Red Trail (M)
Formicinae	*Lasius alienus* (Foerster)	Red Trail (M)
Formicinae	*Lasius claviger* (Ward)	Unknown (PT)
Formicinae	*Lasius neoniger* (Emery)	Blue (PT), Green (M), Loop (M), Orange (PT), Red (M&PT), White (M&PT), and Yellow (M&PT) Trails
Formicinae	*Prenolepis imparis* (Say)	Blue(M), Orange(M), Red(M), White(M), and Yellow(M) Trails
Dolichoderinae	*Dolichoderus taschenbergi* (Mayr)	White Trail (M)
Dolichoderinae	*Dorymyrmex grandulus* (Forel)	White Trail (M)
Dolichoderinae	*Tapinoma sessile* (Say)	Red (M), Green (PT), and Yellow (PT) Trails

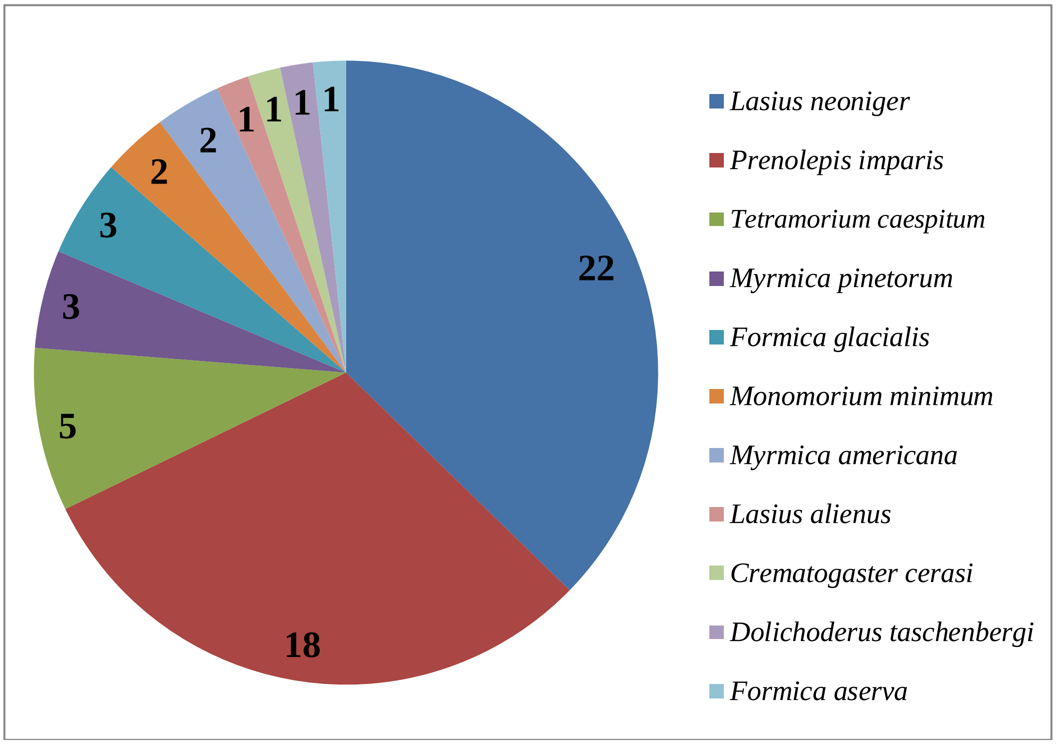

Figure 1. Number of nest sites characterized for eligible ant species

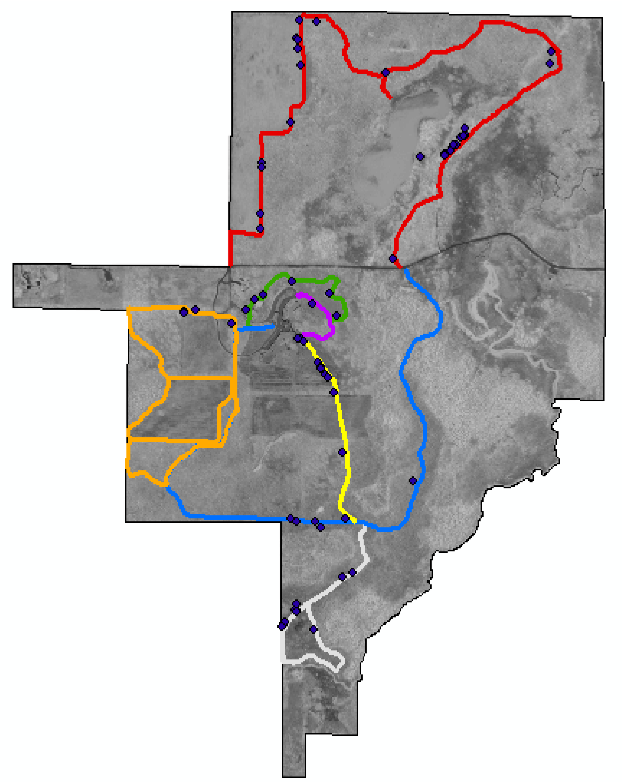

Figure 2. GPS locations of manually sampled ant nest sites at Pierce Cedar Creek Institute.

Table 2. Significant P values from t-Tests for *L. neoniger* and *P. imparis* nest characteristics

Parameter	*L. neoniger* Mean	*P. imparis* Mean	df	t Stat	P(T<=t) two-tail	Significant
Hole Density (Holes/m^2)	1.44	0.54	35	3.86	0.0005	Yes
Canopy Cover (%)	34.8	84.2	29	-6.39	5.5E-07	Yes
Soil Organic Material (%)	0.51	0.54	27	-0.175	0.86	No
Soil Gravel (%)	2.84	3.90	35	-0.854	0.40	No
Soil Sand Fraction 1 (%)	25.97	31.26	24	-1.09	0.29	No
Soil Sand Fraction 2 (%)	33.78	28.57	37	1.738	0.09	No
Soil Sand Fraction 3 (%)	36.20	35.38	30	0.165	0.87	No

Foraging Behavior

Table 3: Polynomial regression results for number of ants excavating vs. soil surface temperature in sites Y, W, and both sites combined.

Site	P-Value	Significant
Y	0.19	no
W	0.01	yes
W&Y	0.01	yes

Table 4: Polynomial regression results for number of ants foraging vs. soil surface temperature in sites Y, W, and both sites combined.

Site	P-Value	Significant
Y	0.22	no
W	0.70	no
W&Y	0.24	no

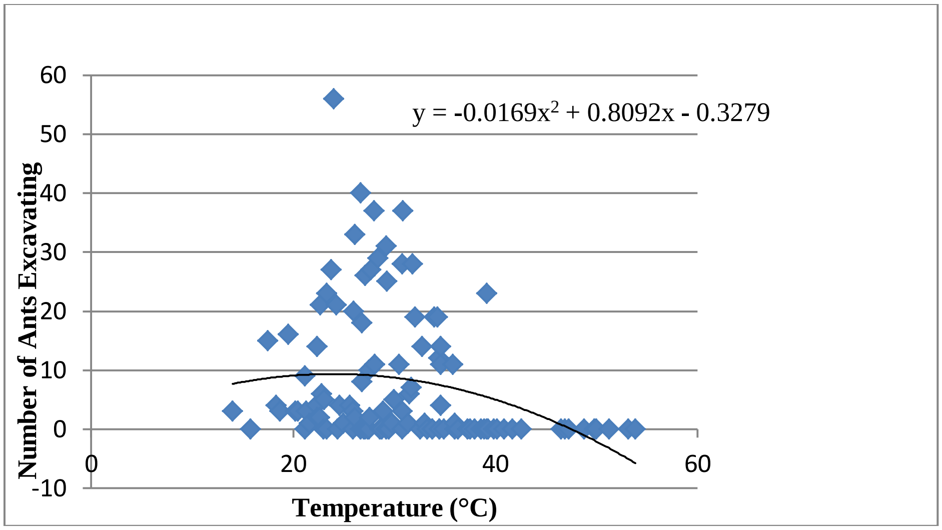

Figure 3: Polynomial regression for number of ants excavating at sites W & Y for various soil surface temperatures over a 15-hour period. The equation for the regression line is shown.

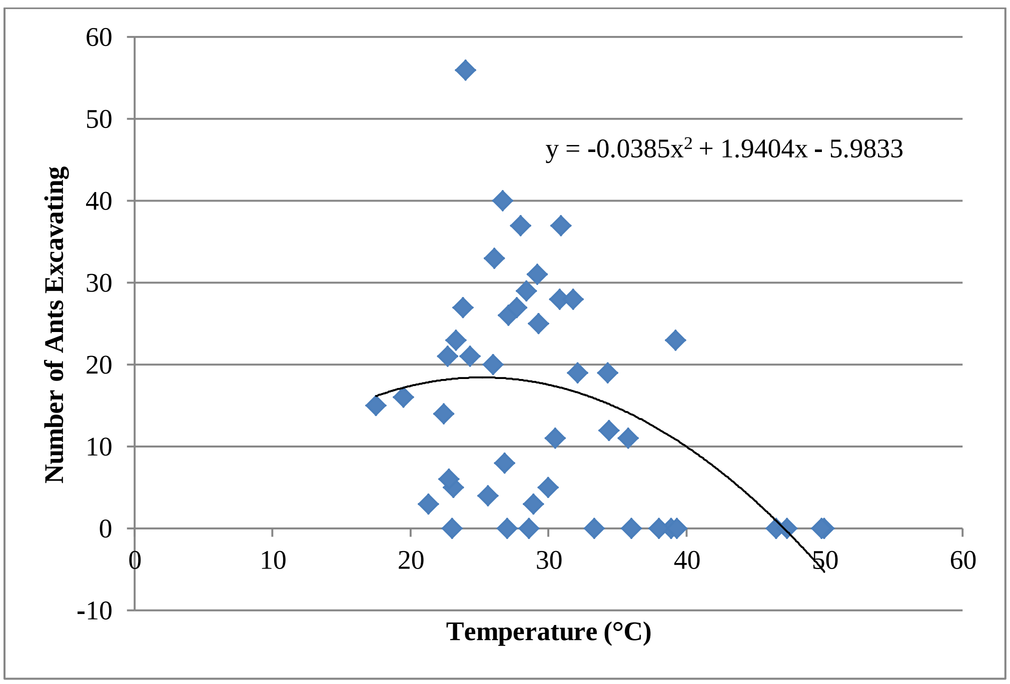

Figure 4: Polynomial regression for number of ants excavating at site W for various soil surface temperatures over a 15-hour period. The equation for the regression line is shown.