

**MOVEMENT AND POPULATION STRUCTURE OF  
CERAMBYCID BEETLES (COLEOPTERA: CERAMBYCIDAE)  
AND WHITE-FOOTED DEERMICE (*PEROMYSCUS LEUCOPUS*)  
ON A FRAGMENTED FOREST LANDSCAPE**

by

Emily R. Dunn

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Wildlife Ecology

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## ABSTRACT

Cerambycid beetles (Coleoptera: Cerambycidae) locate suitable hosts and mates using both pheromones and plant volatiles, many of which have been identified and are now produced synthetically for trap lures. The range over which these lures attract cerambycids within a forest, and the tendency for cerambycids to move out of a forest in response to these lures, have not been previously explored to our knowledge. We conducted two field experiments using baited and unbaited flight intercept traps in northern Delaware to investigate these questions. Within forest fragments, traps that were 2 m from the baited trap, but not 10 or 20 m, captured more beetles than control traps suggesting relatively short-range and nonspecific attraction by these lures. In contrast, male *Prionus laticollis* (Drury), which were attracted by the female-produced sex pheromone 3,5-dimethyldodecanoic acid, were rarely caught in unbaited traps at any distance from baited traps. Baited traps attracted significantly more cerambycids than unbaited traps outside of the forest at distances up to 40 m from the forest edge, with catch generally decreasing between 8 and 40 m from the forest. Some cerambycids were caught in both baited and unbaited traps at all distances from the forest edge, indicating that at least some cerambycids disperse freely outside of the forest independent of any pheromone attractants. Our results provide context to previous studies that used these pheromone lures, and offer insights into the dispersal behavior of cerambycids.

Forest fragmentation and human development undoubtedly affect wildlife movement on a landscape, but this effect likely varies by species. We analyzed single

nucleotide polymorphisms (SNPs) in two species of cerambycid beetles and in white-footed deermice (*Peromyscus leucopus*) to determine the population structure of these species on the same fragmented landscape in northern Delaware. White-footed deermice showed more population structure than either cerambycid species, indicating that cerambycids are more capable of movement among forest fragments. The cerambycid *Xylotrechus colonus* showed less population structure than *Prionus laticollis*, which may be explained by both dispersal ability and life history differences. Our study highlights the importance of using diverse study species to assess connectivity and movement on a human-dominated landscape.

## Chapter 1

### RANGE OF ATTRACTION OF PHEROMONE LURES AND DISPERSAL OF CERAMBYCID BEETLES

#### 1.1 Introduction

Longhorned beetles (Cerambycidae) are often abundant and diverse in North American forest ecosystems, with approximately 1,100 species in North America and over 35,000 described species worldwide (Yanega 1996, Švácha and Lawrence 2014). Larvae of most cerambycid species feed within dead wood and aid in decomposition and nutrient cycling (Linsley 1959); however, some species infest living trees and thus are of economic concern (Solomon 1995). The larvae typically require 1 to 3 yr to complete development, overwintering as larvae or prepupae. Adults of most species emerge during brief periods in spring and summer (Hanks et al. 2014, Handley et al. 2015). Individual adults usually live for no more than a few weeks. Location of mates is often mediated by volatile aggregation pheromones produced by males of species in the subfamilies Cerambycinae, Lamiinae, and Spondylidinae (Ray et al. 2006, Hanks et al. 2007, Mitchell et al. 2013) or female-produced sex pheromones for species in the subfamilies Prioninae and Lepturinae (Millar and Hanks 2016). Attraction to aggregation pheromones may be synergized by host plant volatiles, whereas the female-produced sex pheromones may serve as the primary attractant for males (Millar and Hanks 2016).

A number of the male-produced aggregation pheromones have been identified for cerambycid species, and have been synthesized for use as baits in traps (e.g.,

Hanks and Millar 2013, Hanks et al. 2014, Handley et al. 2015, Ray et al. 2015). Cerambycid pheromones are highly conserved among related species, with cross attraction minimized through temporal and phenological isolation (Hanks et al. 2014, Mitchell et al. 2015). Pheromones of species in different subfamilies tend to be from quite different chemical classes which in general do not interfere with one another, so that blends of multiple cerambycid pheromones can effectively attract a wide range of species, without substantial inhibition of attraction (Hanks et al. 2012, Wong et al. 2012). To further enhance attraction of many cerambycid species, plant volatiles (specifically ethanol and  $\alpha$ -pinene) can be deployed with pheromone lures, often resulting in larger and more diverse catches (Hanks et al. 2012). Ethanol is produced by a variety of woody plants when stressed (Kimmerer and Kozlowski 1982, Gara et al. 1993, Kelsey 1994, 2014, Kelsey and Joseph 2003), and  $\alpha$ -pinene is a very common plant volatile, particularly from conifers (Hanks et al. 2012). In urban forest fragments in Delaware, traps baited with both aggregation pheromone blends and ethanol attracted significantly more cerambycids than traps with only pheromone, or only ethanol (Handley et al. 2015). Racemic 3,5-dimethyldodecanoic acid (“prionic acid”), a female-produced sex pheromone or sex attractant for a number of species in the genus *Prionus* (Prioninae), can also be included in lures to attract a variety of species within that genus (Barbour et al. 2011).

Research on cerambycid dispersal has produced varying results, likely because of the diverse life histories of species within the family (Hanks 1999). For example, larger adults of the cerambycid *Phoracantha semipunctata* (F.) tend to disperse farther than smaller individuals (Hanks et al. 1998). In Delaware, the abundance of particular cerambycid species was correlated with tree species and forest fragment size,

indicating that individual species respond differently to the landscape composition and configuration (Handley 2014). Research on the dispersal of the cerambycine *Cerambyx welensii* Küster found that the majority of adults were sedentary, but averaged a dispersal distance of 200 m when they did move (Torres-Vila et al. 2013). In contrast, the lamiine *Monochamus alternatus* Hope was observed to disperse only up to 37 m in a mark-recapture experiment (Togashi 1990). Overall, the scale and frequency of dispersal can be widely variable among cerambycid subfamilies and species, and should be assessed on a species-by-species basis; studies thus far have only assessed a few species of interest (Holland et al. 2004).

In order to use pheromone- and ethanol-baited traps to study cerambycid dispersal, we must first understand the response of cerambycids to these traps. While the traps are useful to assess diversity and permit collection of species that would otherwise be very difficult to locate, their overall efficacy has yet to be assessed. In particular, the range over which a baited trap attracts cerambycids generally is not known for male-produced aggregation pheromones, and probably varies by species. A rough estimate of 10 m has been suggested based on field experience (L.M.H. and J.G.M., pers. obs.), but this has not been tested or supported by published data. Thus, in our first experiment, we aimed to more precisely determine the range at which cerambycid activity is affected by a blend of male-produced aggregation pheromones. Our second experiment sought to gauge the dispersal tendency of cerambycids from forest fragments, both in response to pheromones and by random movement. This dispersal tendency is especially important in the context of the highly fragmented forests prevalent in the coastal eastern United States, where our research took place.

## **1.2 Materials and Methods**

### **1.2.1 Pheromone Range Experiment**

Our aim was to determine the spatial range at which cerambycid activity is affected by a pheromone lure; thus, we sought to define the distance at which a pheromone-baited trap led to significantly greater cerambycid catch at surrounding unbaited traps, compared to equivalent unbaited traps not in the vicinity of a pheromone lure. Study sites were part of the FRAME (Forest Fragments in Managed Ecosystems) system, a collaboration between the University of Delaware and the U.S. Forest Service that aims to better understand urban and suburban forest fragment dynamics to improve ecosystem management (<http://sites.udel.edu/frame/>). The experiment was conducted within two FRAME forest fragments (Folk and Glasgow 1; Fig. 1.1) from 22 May to 26 August 2014. Within each site, two arrays of traps were established (Fig. 1.2), each with a central trap surrounded by concentric rings of unbaited traps at distances of 2 m (4 traps), 10 m (8 traps), and 20 m (12 traps), for a total of 24 unbaited traps. Traps 2 m away from the center were 2.8 m apart, those 10 m away were 7.7 m apart, and those 20 m away were 10.4 m apart.

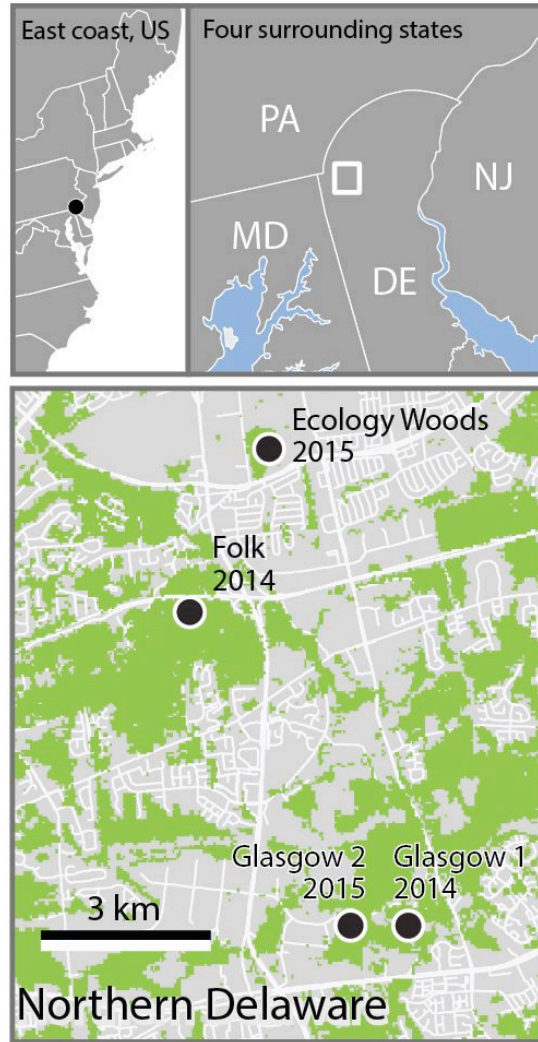


Figure 1.1: Location of study sites in northern Delaware and the year of the experiment for which they were used.

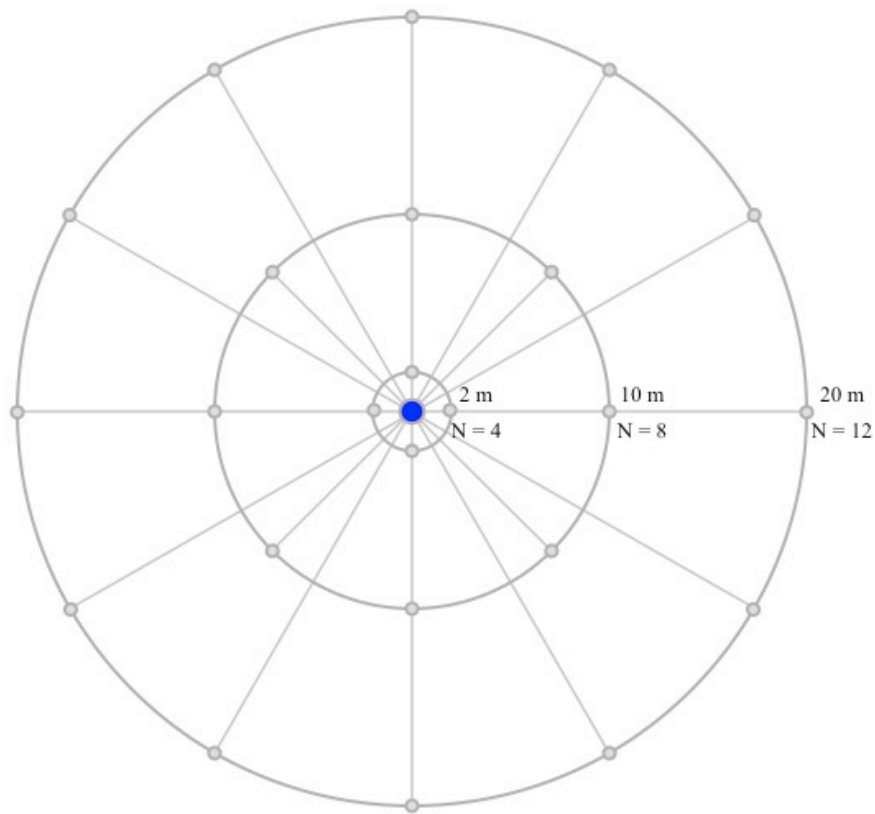


Figure 1.2: Arrangement of traps for cerambycid pheromone range experiment in 2014. Traps were positioned in concentric circles around a central trap at distances of 2 m ( $N = 4$ ), 10 m ( $N = 8$ ), and 20 m ( $N = 12$ ). Pheromone was placed at the central trap at one of the arrays at each site, and all other traps were left without a pheromone lure.

At each site, the central trap of one array was baited with pheromone (referred to as the pheromone array), while the central trap of the other array was unbaited (the control array). From center to center, pheromone and control arrays were separated by 158 m at Folk and 112 m at Glasgow 1. Treatments were switched between pairs of trap arrays (within study sites) at intervals of 9 wk, resulting in three sampling periods, which were treated as temporal replicates.

We used black cross-vane panel traps (corrugated plastic, 1.2 m high x 0.3 m wide, Alpha Scents Inc., West Linn, Oregon) coated with Fluon<sup>®</sup> (Insect-a-Slip, Bioquip Products, Rancho Dominguez, CA) and suspended from 2-m tall frames constructed of PVC pipe. Beetles were captured alive by replacing the supplied collection jars with 1.89-L plastic jars (General Bottle Supply, Los Angeles, CA) with the bottom replaced with fiberglass screen (New York Wire, Grand Island, NY) to allow precipitation to drain and air to circulate. Jars were joined to 20.3-cm-diameter funnels (US Plastic Corp., Lima, OH) that were attached to trap bottoms. Nonbiodegradable plastic packing peanuts (~2.5 cm; CPI Packaging, Inc., Somerset, NJ) were placed in each jar as a neutral substrate on which trapped beetles could climb.

Pheromone lures consisted of clear low-density polyethylene press-seal sachets (5.1 x 7.6 cm, 0.05 mm wall thickness, Cousin Corp., Largo, FL) that were suspended at trap centers. The synthetic pheromone blend was similar to that of previous studies (Handley et al. 2015), and formulated to contain 25 mg of each isomer per 1 ml of solvent carrier (91% isopropanol) per lure: racemic 3-hydroxyhexan-2-one (50 mg/lure), monochamol (25 mg), racemic (*E*)-fusicumol and (*E*)-fusicumol acetate (50 mg each), all from Bedoukian Research (Danbury, CN), *syn*-2,3-hexanediol (50 mg; synthesized as described by Lacey et al. 2004), and racemic 2-methylbutan-1-ol (50 mg; Sigma-Aldrich, St. Louis, MO). Also included in the blend were citral (50 mg; Aldrich Chemical Co.), an isomeric blend of neral and gerial, which are pheromone components of the cerambycine *Megacyllene caryae* (Gahan), and racemic prionic acid (1 mg; synthesized as described by Rodstein et al. 2009), a sex pheromone or attractant for many species in the genus *Prionus* (Barbour et al. 2011). Pheromone

emitters were replaced every 2 wk. Ethanol lures consisted of 10.2 x 15.3 cm polyethylene sachets (Cousin Corp.) containing 100 ml of ethanol (100%), also clipped to trap centers. Ethanol lures can last the entire season, but were monitored each week and replaced periodically throughout the summer to ensure relatively constant release rates.

Traps were set up and baited (when appropriate) on 22 – 23 May 2014. Traps were checked and specimens collected twice per week, replacing the collection jar with an empty one. All traps were frozen at approximately -1°C to kill specimens. Captured cerambycids were identified to species (Yanega 1996, Lingafelter 2007); taxonomy follows Lingafelter (2007).

### **1.2.2 Dispersal Experiment**

Our aim for this second experiment was to establish at what distance cerambycids would travel out of a forest fragment in response to a pheromone attractant; thus, we sought to determine how cerambycid catch changed with distance from the fragment, and at what distances pheromone-baited traps caught significantly more cerambycids than their corresponding control traps. We again used two sites that were part of the FRAME system (Fig. 1.1). We placed traps in open fields adjacent to two sites (Ecology Woods and Glasgow 2) from 2 June to 11 August 2015. At Ecology Woods, alfalfa dominated the majority of the field; at Glasgow 2, the field was turf in a business complex. At each site, trap pairs were spaced 10 m apart along the edge of the forest fragment at distances of 2, 4, 8, and 40 m from the dominant canopy drip line (Fig. 1.3). Each pair of traps included one trap baited with pheromone and ethanol, and an unbaited trap as a control. Traps within a pair were spaced 5 m apart. Within each replicate, the order of these distances was randomized but

pheromone and control traps were always alternated. We placed three replicates at each site, also separated by 10 m. At each site, we placed a sonic anemometer (DS-2 model, Decagon Devices, Pullman, WA) at a central location within the experimental design to measure wind speed and direction. Wind direction as measured every 5 minutes during each trial was compiled for every 10° range to determine the dominant wind direction relative to the forest fragment at each site. We used the same traps, pheromone blend lures, and ethanol lures as in the previous experiment.

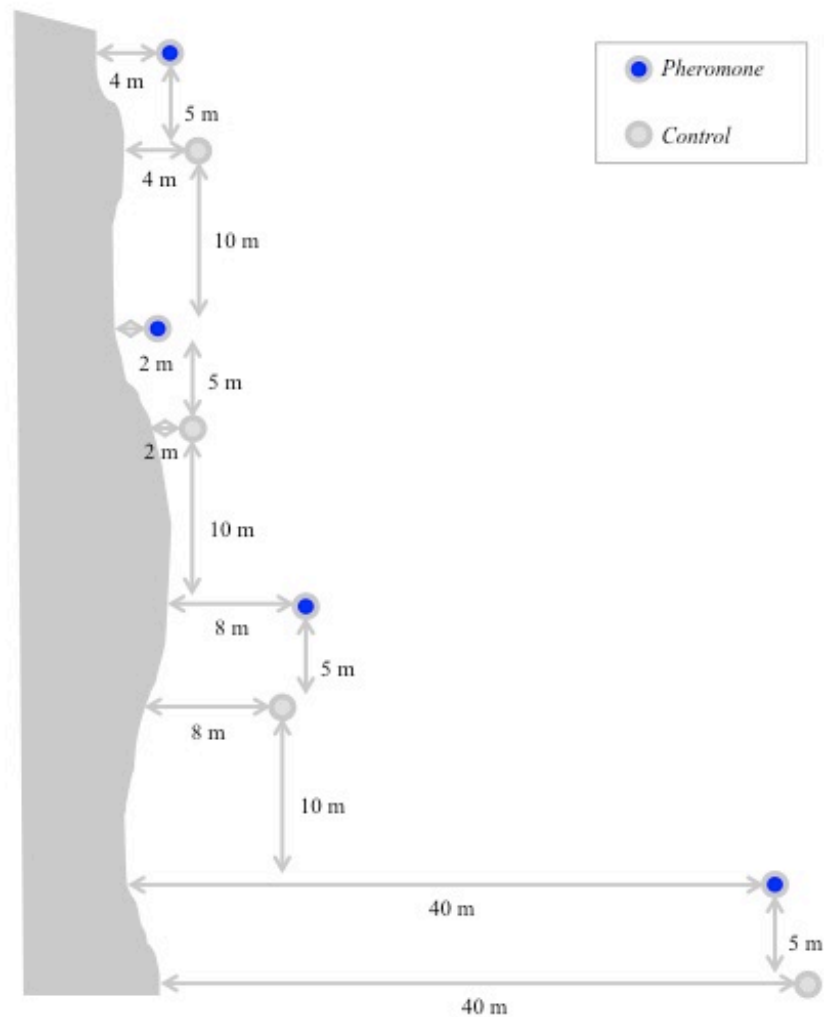


Figure 1.3: Experimental design for the cerambycid field dispersal experiment in 2015. Pairs of pheromone baited and unbaited traps, separated by 5 m, were positioned every 10 m at distances of 2, 4, 8, and 40 m from the canopy edge, in random order. Three replicates were placed at each study site.

Beginning 2 June 2015, traps were checked twice per week for 9 collections, at which point traps containing pheromone and those with no lures were swapped.

Trapping continued twice per week for an additional 11 collections. Traps at Ecology Woods had to be removed for one week (21 – 28 July) to allow mowing of the alfalfa field. For this reason, we omitted this week from analysis, so that each trial in effect lasted 9 collections. Specimens were collected and identified as described above.

### **1.2.3 Statistical Analyses and Anticipated Results**

We conducted all analyses in R (R Core Team 2015). For each trial in both experiments, we calculated the total number of cerambycids captured at each trap to eliminate daily variation due to flight phenology or weather. Means of totals per treatment were compared by analysis of variance (ANOVA) after first confirming that data did not violate the assumptions of that test with the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance. Data that violated these assumptions were log transformed prior to analysis, which brought them into accord with ANOVA (*X. colonus*, pheromone and control). Differences between pairs of means were tested with Tukey's HSD test. Analyses were applied to total cerambycids and to the most abundant individual species. Treatment effects for individual species used data from the trial in which that species was most abundant, while for total cerambycids all trials were included as replicates.

For the pheromone range experiment, pheromone treatment and distance effects were tested with two-way ANOVAs. We expected significantly more beetles in the pheromone array than in the control array, with a distance effect expected only in the pheromone array. Numbers of beetles caught in the control array were expected to provide an indication of the extent to which cerambycids are actively moving within forests, with capture due either to random interception by the flight traps, or possibly visual attraction by the traps' dark silhouette (e.g. Morewood et al. 2002). Within the

pheromone array, we expected the most beetles to be captured in the central baited trap. If higher numbers (compared to the control array) were also caught at 2, 10, or 20 m from the center, this would suggest that the pheromone was causing greater than normal beetle activity at those distances.

For the dispersal experiment, treatment effects were tested by a two-way ANOVA by block (replicate) and distance, with pheromone and control traps analyzed separately. We also conducted paired t-tests to test the pheromone effect for each combination of site and distance treatment.

Because most, if not all, of the cerambycid species attracted by the pheromone mixture depend on woody material in the larval stage, and there was no such material in the open fields next to the forest fragments, most of the beetles caught in the baited traps in the dispersal experiment would likely have been attracted out of the forest by the pheromone. However, others may have been traveling between forest fragments seeking a new host. If more beetles were found in the pheromone traps that were closest to the woods, this would suggest that the majority were coming from the woods. Beetles caught in the unbaited traps would either represent by-catch from beetles attracted to the paired pheromone traps (located 5 m away at each distance from the edge), or beetles traveling through the field to another forest fragment. As with the pheromone range experiment, beetles could be caught in unbaited traps through random interception or visual response to trap silhouettes. If more beetles were caught in the pheromone than in the control traps at 2, 4, 8, or 40 m from the edge of the forest, this would suggest that the pheromone was attracting beetles from at least that far away from the forest.

### 1.3 Results

#### 1.3.1 Pheromone Range Experiment

We collected 1856 cerambycids of 29 species from 4 subfamilies during the 13.5-week experiment (Table 1.1). The majority of cerambycids were of the subfamily Cerambycinae (60.2%), followed by the Lamiinae (24.7%), Prioninae (11.3%), and Lepturinae (3.8%). The total number of cerambycids that were captured declined over the course of the season (Fig. 1.4), with 911, 683, and 262 beetles captured during the first trial (27 May – 24 June), second trial (27 June 27 – 25 July), and third trial (29 July – 26 August), respectively. The three most abundant species were the cerambycine *Xylotrechus colonus* (F.), the lamiine *Urographis fasciatus* (Degeer), and the prionine *Prionus laticollis* (Drury). *Xylotrechus colonus* was the most abundant species, and was caught in greatest numbers during the first trial but persisted throughout the season (Fig. 1.4). *Urographis fasciatus* showed a similar broad activity period. The flight period of *P. laticollis* was much more restricted during mid summer (Fig. 1.4).

Table 1.1: Cerambycids captured during the pheromone range experiment in 2014 by species and location, in order of abundance within subfamilies.

Subfamily and species	Total	Folk – Site 1	Folk – Site 2	Glasgow 1 – Site 1	Glasgow 1 – Site 2
Cerambycinae					
<i>Xylotrechus colonus</i> (F.)	828	251	173	122	282
<i>Cyrtophorus verrucosus</i> (Olivier)	136	46	24	30	36
<i>Anelaphus villosus</i> (F.)	89	23	7	15	44
<i>Neoclytus acuminatus</i> (F.)	29	9	5	1	14
<i>Phymatodes testaceus</i> (L.)	14	8	1	0	5
<i>Neoclytus mucronatus</i> (F.)	12	1	0	2	9
<i>Phymatodes amoenus</i> (Say)	4	0	1	1	2

Table 1.1 continued.

<i>Megacyllene caryae</i> (Gahan)	3	2	1	0	0
<i>Eburia quadrigeminata</i> (Say)	1	0	0	0	1
<i>Neoclytus scutellaris</i> (Olivier)	1	1	0	0	0
Lamiinae					
<i>Urographis fasciatus</i> (Degeer)	266	47	47	67	105
<i>Psenocerus supernotatus</i> (Say)	147	45	20	77	5
<i>Styloleptus biustus</i> (LeConte)	29	9	5	4	11
<i>Astylopsis macula</i> (Say)	15	2	2	6	5
<i>Liopinus misellus</i> (LeConte)	1	0	1	0	0
<i>Hyperplatys aspersa</i> (Say)	1	0	0	1	0
Lepturinae					
<i>Gaurotes cyanipennis</i> (Say)	23	8	2	5	8
<i>Bellamira scalaris</i> (Say)	14	0	2	4	8
<i>Typocerus velutinus</i> (Olivier)	11	4	2	0	5
<i>Necydalis mellita</i> (Say)	10	7	1	0	2
<i>Brachyleptura rubrica</i> (Say)	4	1	1	1	1
<i>Leptorhabdium pictum</i> (Haldeman)	3	2	1	0	0
<i>Trigonarthris proxima</i> (Say)	2	0	1	0	1
<i>Judolia cordifera</i> (Olivier)	1	0	0	0	1
<i>Strophiona nitens</i> (Forster)	1	0	0	0	1
<i>Trigonarthris minnesotana</i> (Casey)	1	1	0	0	0
<i>Typocerus lugubris</i> (Say)	1	1	0	0	0
Prioninae					
<i>Prionus laticollis</i> (Drury)	158	6	45	97	10
<i>Orthosoma brunneum</i> (Forster)	51	21	4	7	19
Total	1856	495	346	440	575

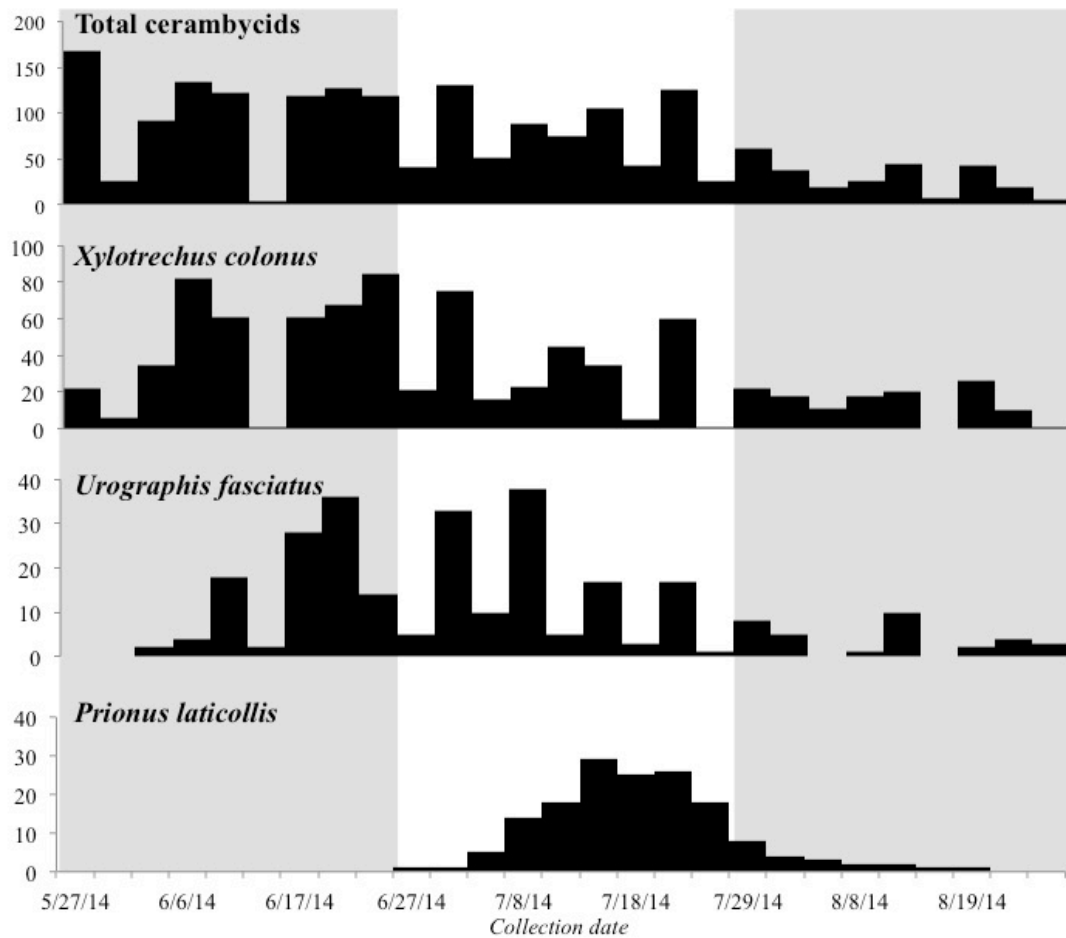


Figure 1.4: Phenology of total cerambycids and the three most abundant species captured during the pheromone range experiment in 2014. Gray shading indicates separate trials.

Total cerambycids were caught in significantly higher numbers in the pheromone arrays than in the control arrays (Fig. 1.5). Distance from the center affected trap catch; the interaction between treatment and distance was also significant in all trials, reflecting the lack of distance effect in the control arrays and the strong distance effect in pheromone arrays (Fig. 1.5). Significantly more cerambycids were

caught in the unbaited traps that were 2 m from the central pheromone-baited traps compared to traps in the control arrays, but not in the unbaited traps that were 10 m or 20 m from the pheromone traps. An average of 2.3 – 4.6 beetles were caught in the unbaited control traps and 4.1 and 4.2 in the traps that were 10 and 20 m from the pheromone-baited traps, respectively. *Xylotrechus colonus* and *Urographis fasciatus* showed a similar result (Fig. 1.6). However, with *P. laticollis*, only the central baited trap contained significantly more beetles, with very few caught 2, 10, or 20 m from the baited trap, and none caught in any of the unbaited traps in the control array (Fig. 1.6).

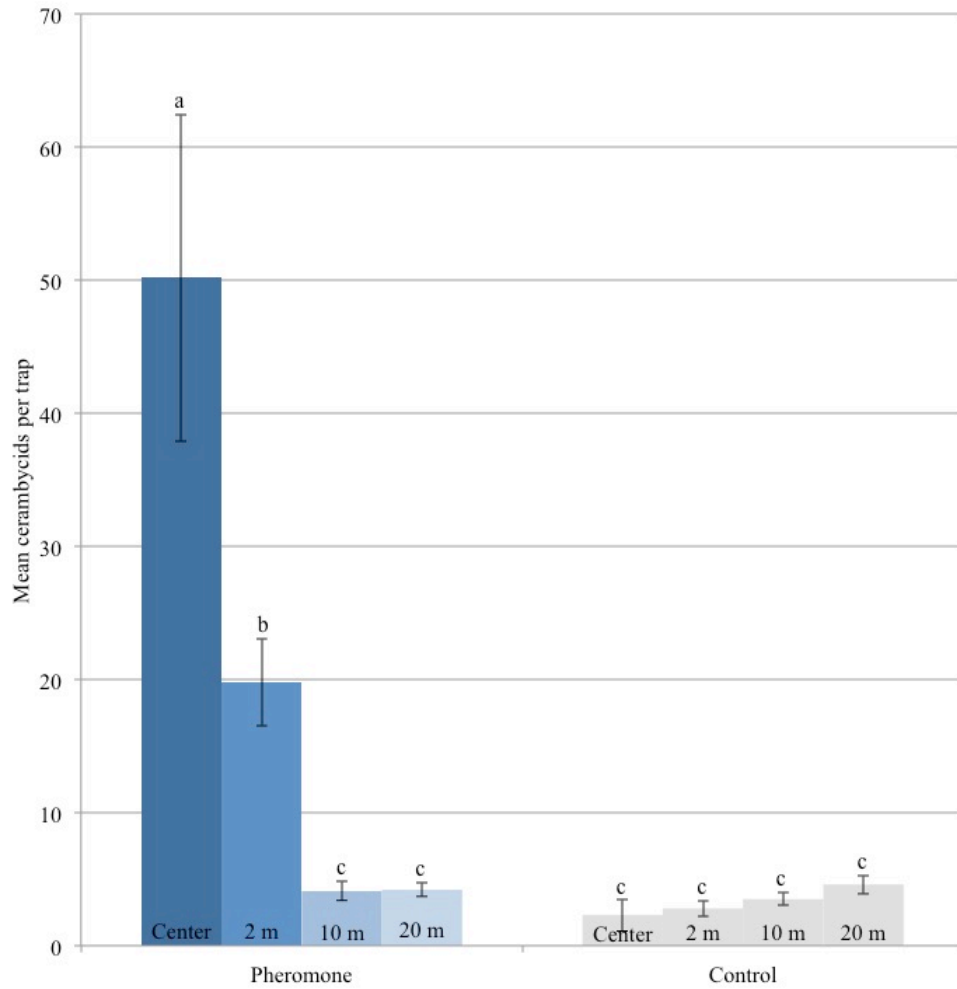


Figure 1.5: Mean  $\pm$  SE cerambycid catch per trap in the pheromone range experiment in 2014 at each combination of treatment and distance ( $N = 6$ , with three trials at each of two sites as replicates). Letters indicate significant differences at  $P < 0.05$  as determined by a two-way ANOVA (treatment:  $F = 29.5$ ,  $df = 1$ ,  $P < 0.001$ ; distance:  $F = 43.4$ ,  $df = 3$ ,  $P < 0.001$ ; treatment $\times$ distance:  $F = 53.3$ ,  $df = 3$ ,  $P < 0.001$ ) and Tukey test.

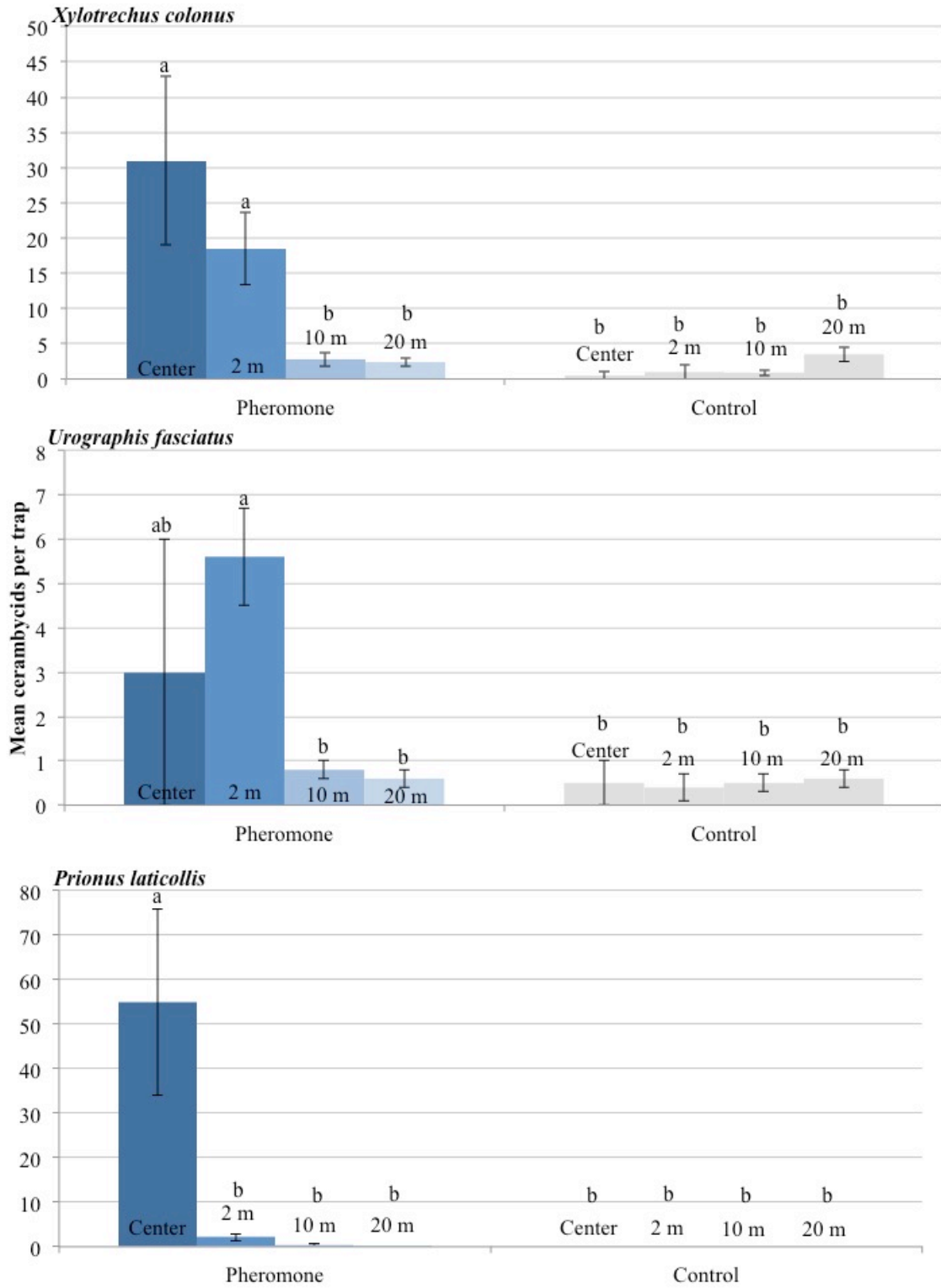


Figure 1.6: Mean  $\pm$  SE *Xylotrechus colonus*, *Urographis fasciatus*, and *Prionus laticollis* collected in the pheromone range experiment at each combination of treatment and distance (N = 2, with one trial at two sites). Letters indicate significant differences at the  $P = 0.05$  level as determined by a two-way ANOVA and Tukey test for *X. colonus* (treatment:  $F = 13.2$ ,  $df = 1$ ,  $P < 0.001$ ; distance:  $F = 13.7$ ,  $df = 3$ ,  $P < 0.001$ ; treatment $\times$ distance:  $F = 19.2$ ,  $df = 3$ ,  $P < 0.001$ ), *U. fasciatus* (treatment:  $F = 17.0$ ,  $df = 1$ ,  $P < 0.001$ ; distance:  $F = 16.4$ ,  $df = 3$ ,  $P < 0.001$ ; treatment $\times$ distance:  $F = 18.9$ ,  $df = 3$ ,  $P < 0.001$ ), and *P. laticollis* (Treatment:  $F = 18.6$ ,  $df = 1$ ,  $P < 0.001$ ; distance:  $F = 94.5$ ,  $df = 3$ ,  $P < 0.001$ ; treatment $\times$ distance:  $F = 94.5$ ,  $df = 3$ ,  $P < 0.001$ ).

### 1.3.2 Dispersal Experiment

We collected 1686 cerambycids of 37 species from 4 subfamilies during the 9-wk experiment (Table 1.2). Again, most cerambycids were of the subfamily Cerambycinae (76.3%), followed by Lamiinae (19.8%), Prioninae (3.6%) and Lepturinae (0.4%). Total cerambycid catch once again declined during the experiment (Fig. 1.7), with 1039 and 644 cerambycids caught during the first trial (5 June – 3 July) and second trial (7 – 21 July, 31 July – 11 August). *Xylotrechus colonus* was again the most abundant species captured in the traps (Table 1.2; Fig. 1.7).

Table 1.2: Cerambycids captured during the field experiment in 2015 by species and location, in order of abundance within subfamilies.

Subfamily and species	Total	Ecology Woods	Glasgow
Cerambycinae			
<i>Xylotrechus colonus</i> (F.)	657	102	555
<i>Elaphidion mucronatum</i> (Say)	239	13	226
<i>Neoclytus mucronatus</i> (F.)	229	25	204
<i>Sarosesthes fulminans</i> (F.)	65	2	63
<i>Anelaphus villosus</i> (F.)	38	0	38
<i>Neoclytus scutellaris</i> (Olivier)	20	3	17
<i>Anelaphus pumilus</i> (Newman)	14	0	14

Table 1.2 continued.

<i>Neoclytus acuminatus</i> (F.)	6	2	4
<i>Curius dentatus</i> Newman	6	0	6
<i>Phymatodes amoenus</i> (Say)	4	4	0
<i>Cyrtophorus verrucosus</i> (Olivier)	2	1	1
<i>Anelaphus parallelus</i> (Newman)	2	0	2
<i>Eburia quadrigeminata</i> (Say)	2	0	2
<i>Euderces picipes</i> (F.)	1	0	1
<i>Smodicum cucujiforme</i> (Say)	1	0	1
Lamiinae			
<i>Liopinus alpha</i> (Say)	103	12	91
<i>Aegomorphus modestus</i> (Gyllenhal)	77	5	72
<i>Urographis fasciatus</i> (Degeer)	62	5	57
<i>Ecyrus dasycerus</i> (Say)	25	0	25
<i>Liopinus mimeticus</i> (Casey)	10	0	10
<i>Dectes sayi</i> Dillon & Dillon	10	0	10
<i>Liopinus misellus</i> (LeConte)	9	5	4
<i>Liopinus punctatus</i> (Haldeman)	9	1	8
<i>Lepturges confluens</i> (Haldeman)	8	2	6
<i>Astylopsis macula</i> (Say)	6	2	4
<i>Leptostylus transversus</i> (Gyllenhal)	4	0	4
<i>Sternidius variegatus</i> (Haldeman)	3	1	2
<i>Psenocerus supernotatus</i> (Say)	2	1	1
<i>Astylopsis collaris</i> (Haldeman)	1	0	1
<i>Styloleptus biustus</i> (LeConte)	1	0	1
<i>Lepturges angulatus</i> (LeConte)	1	0	1
<i>Eupogonius pauper</i> LeConte	1	0	1
<i>Hippopsis lemniscata</i> (F.)	1	1	0
Lepturinae			
<i>Bellamira scalaris</i> (Say)	3	0	3
<i>Typocerus velutinus</i> (Olivier)	2	0	2
<i>Analeptura lineola</i> (Say)	1	0	1
Prioninae			
<i>Prionus laticollis</i> (Drury)	61	7	54
Total	1686	194	1492

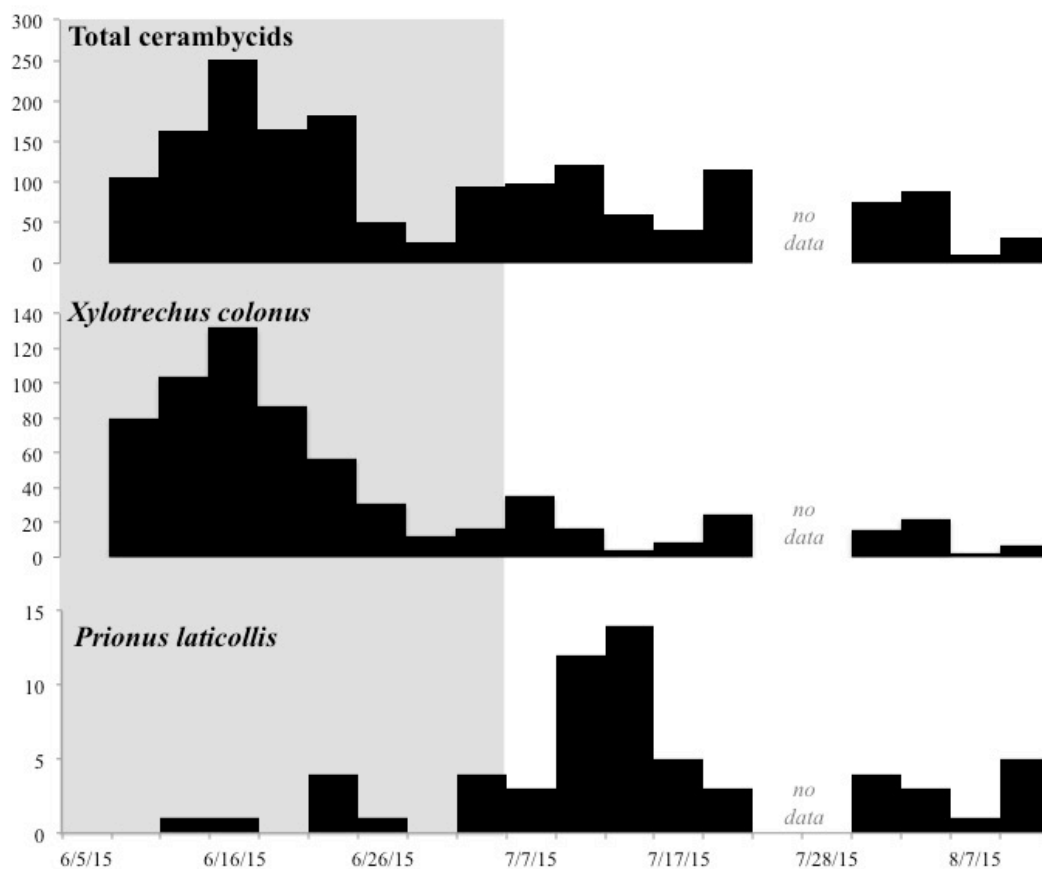


Figure 1.7: Phenology of total cerambycids, *Xylotrechus colonus*, and *Prionus laticollis* captured during the field dispersal experiment in 2015. Gray shading indicates trial 1.

Total cerambycid catch by pheromone traps decreased significantly between 8 and 40 m from the forest edge (Fig. 1.8). Significantly more total cerambycids were caught in the pheromone-baited traps than in their paired control traps at all distances (Fig. 1.8). Cerambycid catch at control traps gradually decreased with distance from the forest edge (Fig. 1.8).

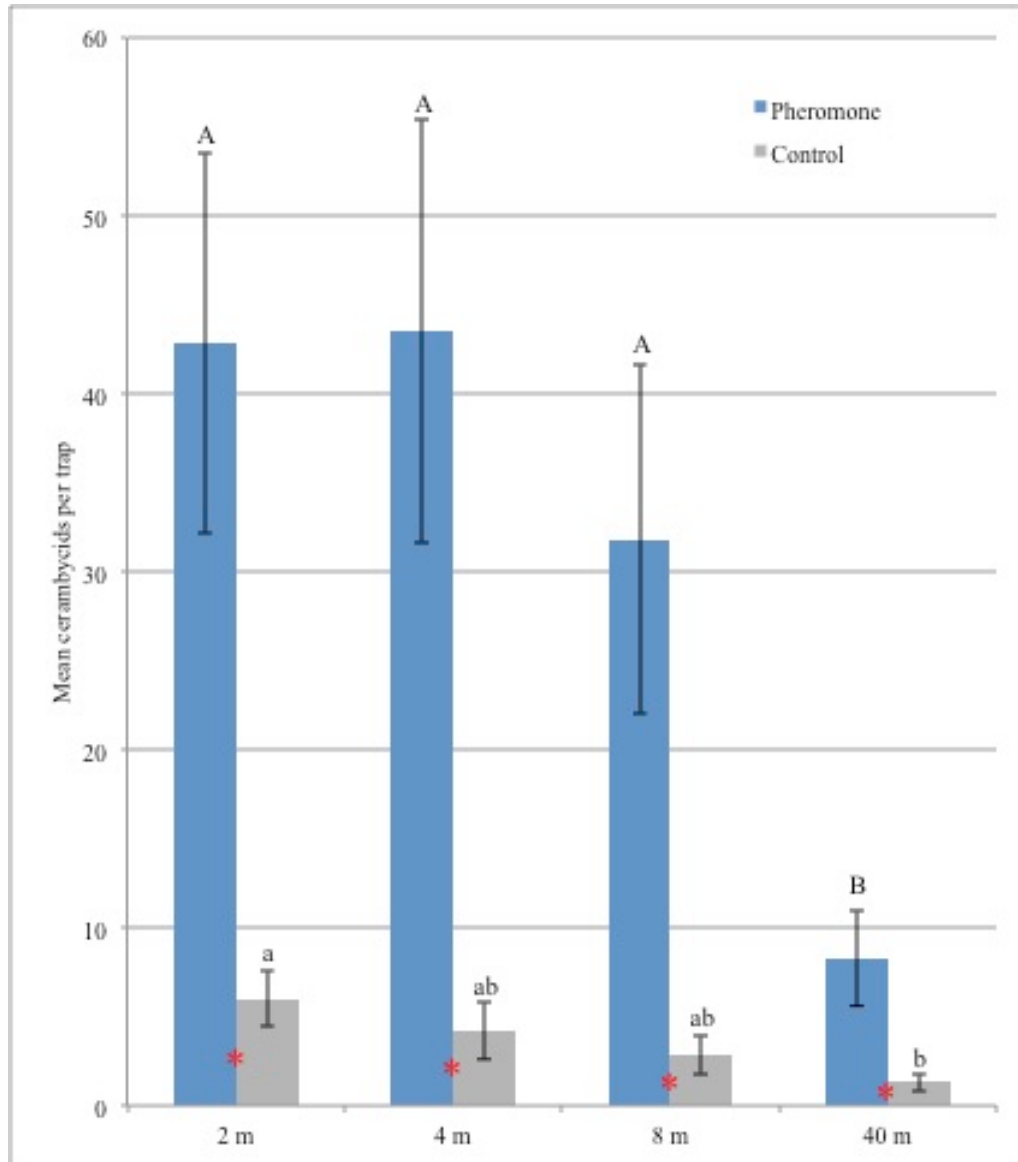


Figure 1.8: Mean  $\pm$  SE cerambycid catch per trap in the dispersal experiment in 2015, at each distance from the canopy edge. Pheromone and control traps were analyzed separately with blocked ANOVAs (pheromone:  $F = 8.7$ ,  $df = 3$ ,  $P < 0.001$ ; control:  $F = 4.4$ ,  $df = 3$ ,  $P = 0.010$ ); letters indicate significant means at the 0.05 level as determined by Tukey tests. Pheromone and control traps were compared at each distance using a paired one-tailed t-test; red asterisks indicate significance at  $P < 0.05$ .

*Xylotrechus colonus* decreased in catch with distance from the forest, with 8 m traps intermediate in catch (Fig. 1.9). Pheromone-baited traps caught significantly more *X. colonus* than their paired control traps at all distances from the forest edge (Fig. 1.9).

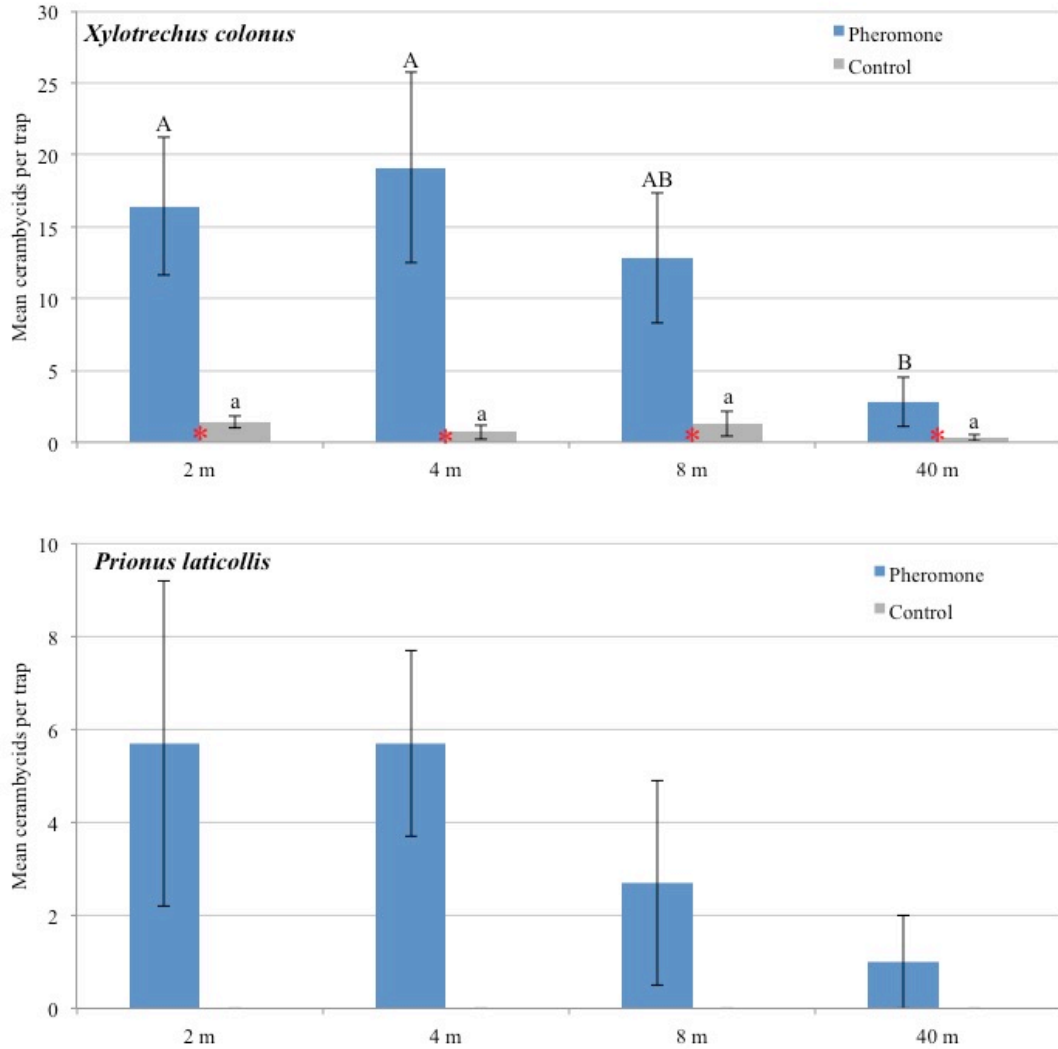


Figure 1.9: Mean  $\pm$  SE *Xylotrechus colonus* catch per trap in the dispersal experiment in 2015 at both sites, and *Prionus laticollis* at Glasgow, at each distance from the canopy edge. Pheromone and control traps were analyzed separately with blocked ANOVAs for *X. colonus* (pheromone:  $F = 5.8$ ,  $df = 3$ ,  $P = 0.002$ ; control:  $F = 0.9$ ,  $df = 3$ ,  $P = 0.472$ ) and *P. laticollis* (pheromone:  $F = 1.5$ ,  $df = 3$ ,  $P = 0.297$ ; control: 0 collected); letters indicate significant means at the 0.05 level as determined by Tukey tests. Pheromone and control traps were compared at each distance using a t-test; red asterisks indicate significance at  $P < 0.05$ .

*Prionus laticollis* showed no significant differences in catch with distance from the forest at Glasgow during trial 2, but as in the pheromone range experiment, no *P. laticollis* were caught at control traps (Fig. 1.9). A total of 61 *P. laticollis* were collected in this experiment, with the majority (54) at the Glasgow site; of these, 21 were caught within 2 m of the forest edge, and 39 were caught at 4 m of the forest edge (Fig. 1.9).

Wind patterns relative to the forest were similar at both sites, with most wind traveling either parallel to the forest edge of interest or out of the forest (Fig. 1.10). Wind patterns were similar between trials.

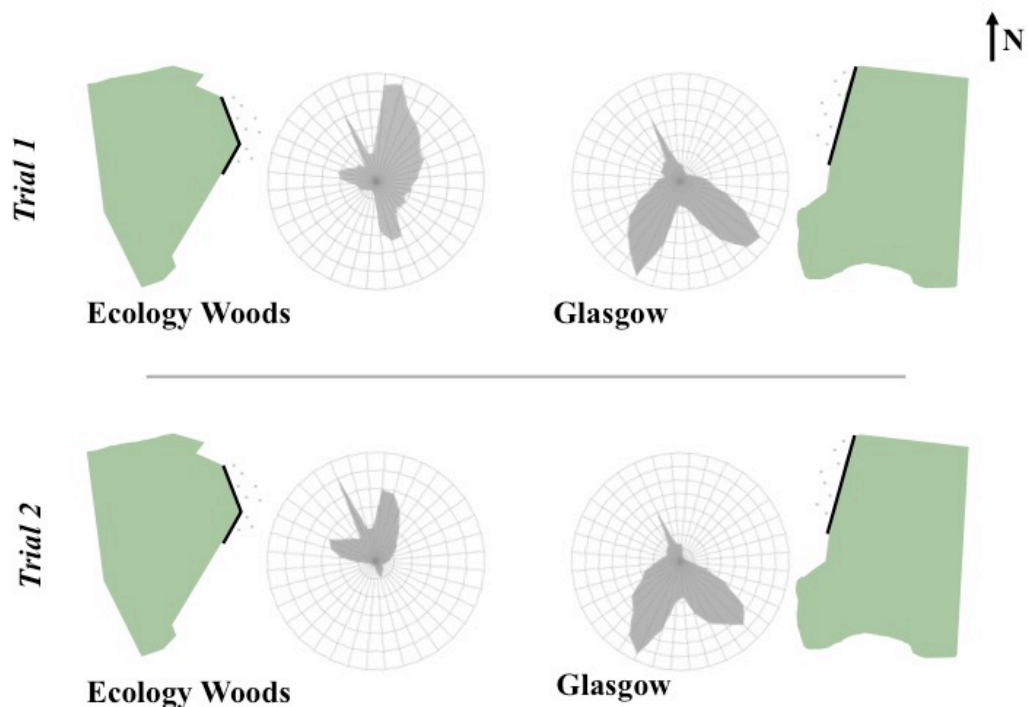


Figure 1.10: Wind direction during the field dispersal experiment for trials 1 and 2 at the Ecology Woods and Glasgow 2 sites. Black lines indicate the forest edge along which traps and anemometers were placed for each study site. Gray lobes indicate frequency for each 10° increment of the direction from which wind was traveling based on data collected every 5 min during each trial.

#### 1.4 Discussion

In the pheromone range experiment, cerambycid catch per trap was significantly greater in the pheromone arrays than in the control arrays at traps 0 or 2 m away from a pheromone lure, but not at 10 m away. These results suggest that the presence of the pheromone lure caused greater activity, possibly indicating beetles being intercepted when traveling between 10 and 2 m from the lure; however, other unknowns remain that could confound this conclusion. In these studies we did not know exactly from where each cerambycid traveled. Cerambycids may have avoided

interception by outer traps while approaching the pheromone lure, especially since the traps were somewhat farther apart at 20 m than at 10 or 2 m from the central trap; thus, the range of the pheromone's attraction may in fact be larger than 2 – 10 m. These and other unknowns could likely be clarified in follow-up studies using mark-recapture experiments (Drag et al. 2011, Etxebeste et al. 2013, Torres-Vila et al. 2015).

In the second experiment, pheromone traps caught significantly more cerambycids than their paired control trap at every distance from the forest edge, even those 40 m away. These results suggest that the range of pheromone attraction could in fact be greater than 2-10 m. As with the first experiment, however, we did not know for sure where trapped beetles came from, since some beetles may have been traveling between forest fragments seeking a new host. However, the fact that more beetles were caught near the forest edge suggests that most were coming from the forest fragment. Similar results were found by Irmeler et al. (2010), who observed a decrease in catch at 30 m from the forest edge at traps baited with decaying wood in Germany.

Individual species showed different patterns when attracted by aggregation versus sex pheromones, at least for the species found in high numbers in this study. The trend of high capture at traps 0 or 2 m away from a pheromone lure was consistent with that of the most abundant species, *X. colonus*, which is attracted by male-produced aggregation pheromones (Lacey et al. 2009). *Urographis fasciatus* showed a similar trend, and also previously has been attracted by aggregation pheromones (Lacey et al. 2009, Handley et al. 2015). Male *P. laticollis* were attracted by prionic acid, a likely female-produced sex pheromone of this species (Barbour et al. 2011), and were only caught in significant numbers at the center trap containing a pheromone lure. This pattern indicates that the mechanism of attraction for *P. laticollis* is more

precise than that of the species attracted by aggregation pheromones. For example, over shorter ranges, beetles responding to male-produced aggregation pheromones may also use host-related cues such as the visual silhouette presented by the vertical trap to assist in locating calling conspecifics. In contrast, host-related cues may be largely irrelevant to male beetles responding to a female-produced sex pheromone. The very specific attraction of *P. laticollis*, with very few intercepted by the unbaited traps at any distance, suggests that these individuals may well have arrived at the baited traps from outside of the baited arrays, and thus may disperse much longer distance than the 2-10 m estimate for cerambycids responding to the aggregation pheromone blend. A mark-recapture experiment of another prionine, *Prionus californicus* Motschulsky, estimated a maximum sampling range of a sex pheromone lure of 585 m (Maki et al. 2011); *P. laticollis* may be exhibiting a similar response in our experiment. Although catch of *P. laticollis* in the field dispersal experiment did not show any significant differences, this species was again never caught in a control trap during the entire experiment, supporting the results of the first experiment indicating high specificity of response, and suggesting that this species may be less likely to leave a forest fragment without stimulation from a pheromone source.

Most of the commonly collected cerambycid species, including *X. colonus*, were “stressed host” feeders (Hanks 1999), meaning that they oviposit on freshly felled trees or cut logs. Stressed hosts represent a more ephemeral and sporadic resource than healthy, weakened, or dead hosts, leading to intense scramble competition both within and among species that depend on this type of resource. Hanks (1999) suggested that both male and female adults of these species should be particularly mobile, because they must seek out their unpredictable and relatively rare

larval hosts. In contrast, *P. laticollis* larvae feed on living or decaying roots of a range of woody host plants. Females are sedentary and lay their eggs in the soil, while males are more mobile and seek out females for mating (Benham and Farrar 1976). For males of this species, calling females are the scarce resource, which males must locate before other males reach them. Thus, their primary if not only focus is on finding females, particularly for those species in which the adults do not feed and so have short life spans, which are dictated by the energy reserves that they have carried through from the larval and pupal stages.

Although total cerambycid catch per trap was significantly greater at traps within 2 m of the pheromone lure, cerambycids were also caught at control traps more than 100 m from a lure in the first experiment. Unbaited arrays caught 579 cerambycids compared to 1275 at the baited arrays. This result indicates active and frequent movement of cerambycids through forest fragments; however, the dark silhouette of the flight intercept traps may visually attract some species of cerambycids in the absence of pheromone lures, as indicated by significantly higher capture of cerambycids than that of less visually prominent funnel traps in British Columbia (Morewood et al. 2002). Therefore, the cerambycids caught in the control traps could have been attracted to the dark silhouette of the traps, and this result may not reflect incidental cerambycid interception. Further, pheromone-producing cerambycids and volatiles from trees and other vegetation at each site were likely present in the vicinity of the control traps. The results from these unbaited traps do still provide a measure of local abundance, as cerambycids were unlikely to respond visually to our traps from a distance.

We also consistently caught low numbers of cerambycids at control traps in the second experiment, indicating that cerambycids were moving outside of the forest fragments at some low rate; however, they may also have been lured into the vicinity due to the proximity of a baited trap 5 m away.

Our results indicate that most cerambycids were attracted to pheromone- and ethanol-baited traps over ranges of at least 2 to 10 m, and possibly up to 40 m, with *P. laticollis* showing far more specificity in its response to the pheromone than the species attracted by aggregation pheromones. Further, cerambycids were caught as far as 40 m away from the forest in traps lacking pheromone lure, suggesting that at least some cerambycid species may disperse randomly outside of forest fragments over considerable distances.

Dispersal, particularly for saproxylic insects, is a difficult characteristic to measure, and requires multiple methods to account for its complexity (Ranius 2006). These experiments are an important first step in understanding cerambycid movement in an urban-agricultural landscape, but future work should incorporate genetic data, mark-recapture, and other methods to complement these results.

## Chapter 2

### GENETIC POPULATION STRUCTURE OF CERAMBYCID BEETLES (*XYLOTRECHUS COLONUS*, *PRIONUS LATICOLLIS*) AND WHITE-FOOTED DEERMICE (*PEROMYSCUS LEUCOPUS*) ON A FRAGMENTED FOREST LANDSCAPE

#### 2.1 Introduction

##### 2.1.1 Urban and Suburban Ecology

Expanding human populations are constantly impacting the surrounding natural ecosystems. In fact, developed land in the United States is projected to increase to 9.2% by 2025 (Alig et al. 2004), and as early as 1992, the majority (61.8%) of forest habitat in the U.S. was within 150 m of an edge (Riitters et al. 2002). This development will certainly increase the prevalence of urban forests, which are already worth \$2.4 trillion based only on compensatory value (Nowak et al. 2002). From 2000 to 2012, global forest area has shown a 3.2% net loss, with a 9.9% net loss of global forest interior area, indicating a widespread shift to a more fragmented condition (Riitters et al. 2015). These urban forests do provide immense ecosystem services despite being heavily affected by humans. However, urban ecosystems are intrinsically different from those lacking anthropogenic influences, and therefore merit research attention (Shochat et al. 2006). Specifically, we should aim to understand the capability of wildlife to move across a landscape heavily impacted and fragmented by human development. One powerful method to assess movement of a species is the use of genetic data to determine population structure.

### **2.1.2 Genetic Population Structure Analysis**

Genetic markers allow for population-level analysis of variation within populations versus among populations, thus illuminating the degree of isolation of those populations. While a variety of markers have been used for the past several decades, single nucleotide polymorphisms (SNPs) have rapidly gained popularity and offer advantages over other markers (Morin et al. 2004). SNPs are sites in the genome at which more than one nucleotide occurs in a population. Millions of SNPs are found in both coding and noncoding regions across the entire genome, so they are representative of a large extent of genetic material (Morin et al. 2004). In addition to their abundance, SNPs tend to be higher data quality and lower cost than other comparable markers (Morin et al. 2004). They also mutate at a lower rate than other markers, particularly microsatellites, lowering the risk of homoplasy, or the convergence of two lineages on the same allele (Morin et al. 2004). Traditional methods for locating SNPs required a reference genome, but genotyping by sequencing (GBS) uses restriction enzymes to circumvent this need, and also reduces genomic complexity and decreases the prevalence of repetitive sequences (Elshire et al. 2011, Lu et al. 2013).

The relative modern ease of acquiring this genetic information allows ecologists to ask powerful questions about the population structure of wildlife across a landscape. What constitutes a barrier to movement for a species is a common inquiry, and can vary substantially both among species and even within a species' range (Montgelard et al. 2014). Further, sometimes a single barrier such as a road shows no effect, but can become a barrier when coupled with additional obstacles (Montgelard et al. 2014). Temporal scale must also be considered, as the effect of a barrier may not be immediately detected by genetic markers because of the time required for

divergence to occur (Landguth et al. 2010). Simulation studies indicate that this temporal lag depends upon the dispersal capability of the species, but that the effects of new barriers often can be detected after only 1-15 generations (Landguth et al. 2010). Knowledge of the determination of a barrier and dispersal capability of wildlife is especially crucial in cases of high habitat fragmentation, as potential barriers are even more prevalent.

### **2.1.3 Study Species**

While we are interested in how wildlife as a whole is affected by habitat fragmentation, individual species undoubtedly respond differently. We chose multiple species from different ecological niches to widen our scope of study. Our study focuses on two native species of cerambycid beetles (Coleoptera: Cerambycidae), and native white-footed deermice (*Peromyscus leucopus*).

Cerambycid beetles are prevalent in North American forest ecosystems, typically feeding on stressed or dead trees as larvae (Linsley 1959). This family therefore plays an important role in forests by aiding in decomposition and nutrient cycling. Adult cerambycid movement is typically mediated by host location via plant volatiles, and mate detection via pheromones (Ginzel and Hanks 2005). Most cerambycids use male-produced aggregation or sex pheromones (Ray et al. 2006, Hanks et al. 2007, Mitchell et al. 2013), but some, particularly in the subfamily Prioninae, use female-produced sex pheromones (Barbour et al. 2011). Our first study species, *Xylotrechus colonus* (F.), is a widespread and abundant eastern U.S. cerambycid that responds to male-produced aggregation pheromones (Lacey et al. 2009), and feeds in or under the bark of various stressed hardwood trees as larvae (Yanega 1996, Lingafelter 2007). This species was prevalent in all of our study sites

during the two years prior to this collection (Handley et al. 2015; unpublished data). The second cerambycid species, *Prionus laticollis* (Drury), is part of the subfamily Prioninae and utilizes female-produced sex pheromones (Barbour et al. 2011). *Prionus laticollis* can also be found throughout the eastern U.S., and larvae feed on living roots of a variety of trees and shrubs (Yanega 1996, Lingafelter 2007). Unlike most cerambycids, females of this species are sedentary, and only males disperse to seek mates (Benham and Farrar 1976). While still found in relatively high numbers, this species was less abundant in previous seasons and was not caught at every site (Handley et al. 2015; unpublished data). In addition to disparities in host and pheromone mechanism, these two species also differ substantially in size. *Xylotrechus colonus* adults range from 8-15 mm in length, while *P. laticollis* can reach 20-50 mm and are among the largest cerambycids in the region (Yanega 1996). Because of these physiological and behavioral differences, we anticipate that *X. colonus* and *P. laticollis* will differ in their genetic population structure on the same landscape.

Genetic analysis of cerambycids thus far is limited, and focuses on either invasive species such as the Asian longhorned beetle (*Anoplophora glabripennis* (Motschulsky)) or endangered species such as the Rosalia longicorn (*Rosalia alpina* (L.)) in Europe (Carter et al. 2009, Drag et al. 2015). Population structure varied depending on species and spatial context; on highly connected forest landscapes, little population structure has been found (Carter et al. 2009, Drag et al. 2015), but some geological barriers such as mountain ranges have been associated with genetic differentiation (Shoda-Kagaya 2007). Because we cannot easily tease apart the effects of the landscape from the variation among species in these studies, we are interested to

compare *X. colonus* and *P. laticollis* that were found on the same landscape to better identify unique species differences.

Deermice, belonging to the genus *Peromyscus*, are the most abundant mammals in North America with 56 recognized species across the continent (Bedford and Hoekstra 2015). The white-footed deermouse, *Peromyscus leucopus* (Rafinesque), occupies much of North America east of the Rocky Mountains, and can inhabit forests, croplands, and even homes in wooded areas (Reid 2006). Dispersal of white-footed deermice is male-biased and prevents local inbreeding (Wolff et al. 1988, Mossman and Waser 1999). White-footed deermice appear to be strong dispersers, with previous studies indicating individual movement across over 300 m of agricultural fields (Middleton and Merriam 1981), although in other cases individuals moved no further than 5 m outside of a forest patch (Krohne and Hoch 1999). White-footed deermouse density has been found to be higher in smaller forest fragments (Nupp and Swihart 1996, Anderson et al. 2003), particularly in those surrounded by urban or suburban development (Barko and Feldhamer 2003). Such development may impede dispersal, leading to high-density islands; however, forest edges may simply provide better habitat due to greater vegetation complexity and food diversity (Anderson et al. 2003, Barko and Feldhamer 2003). In New York City, an extreme case of an urbanized landscape, white-footed deermice showed strong population structure; urbanization appears to cause greater population divergence than natural fragmentation (Munshi-South and Kharchenko 2010). Canopy cover correlated with gene flow on this urban landscape, indicating that forest connectivity can dictate dispersal in this extreme case (Munshi-South 2012). Spatial scale can also affect the amount of genetic structure detected. In a small study in Indiana encompassing only

75 ha, genetic divergence was found between populations separated by only a power line corridor or a gorge (Krohne and Baccus 1985). In contrast, in a large-scale study encompassing a region from Ohio to Michigan, the only significant factor for genetic differentiation was proximity of agricultural land, suggesting that agriculture subtly reduces dispersal but can only be detected on a wider scale (Taylor and Hoffman 2014).

#### **2.1.4 Goals**

We aim to determine the degree to which populations of both cerambycid beetles and white-footed deermice are genetically isolated on a landscape heavily influenced by human activity. If genetic divergence is found, we are interested to determine what natural or anthropogenic features may correlate with the detected patterns.

## **2.2 Materials and Methods**

### **2.2.1 Sample Collection**

We collected adult cerambycids from 23 flight intercept traps baited with a blend of cerambycid pheromones and ethanol from 5 May to 22 August 2014. Traps were located at 17 study sites that are part of the FRAME (Forest Fragments in Managed Ecosystems) system, a collaboration between the University of Delaware and the U.S. Forest Service that aims to better understand urban and suburban forest fragment dynamics to improve management of these ecosystems (<http://sites.udel.edu/frame/>; Fig. 2.1). Most traps were located throughout Newark, DE, but we also collected from traps in Hockessin, DE, and adjacent to the port of Wilmington, DE. All traps were located within forest fragments. We caught 5,855

cerambycids, 1,633 of which were *X. colonus* and 870 of which were *P. laticollis*.

Specimens were collected alive and stored frozen at  $-62.2^{\circ}\text{C}$ .

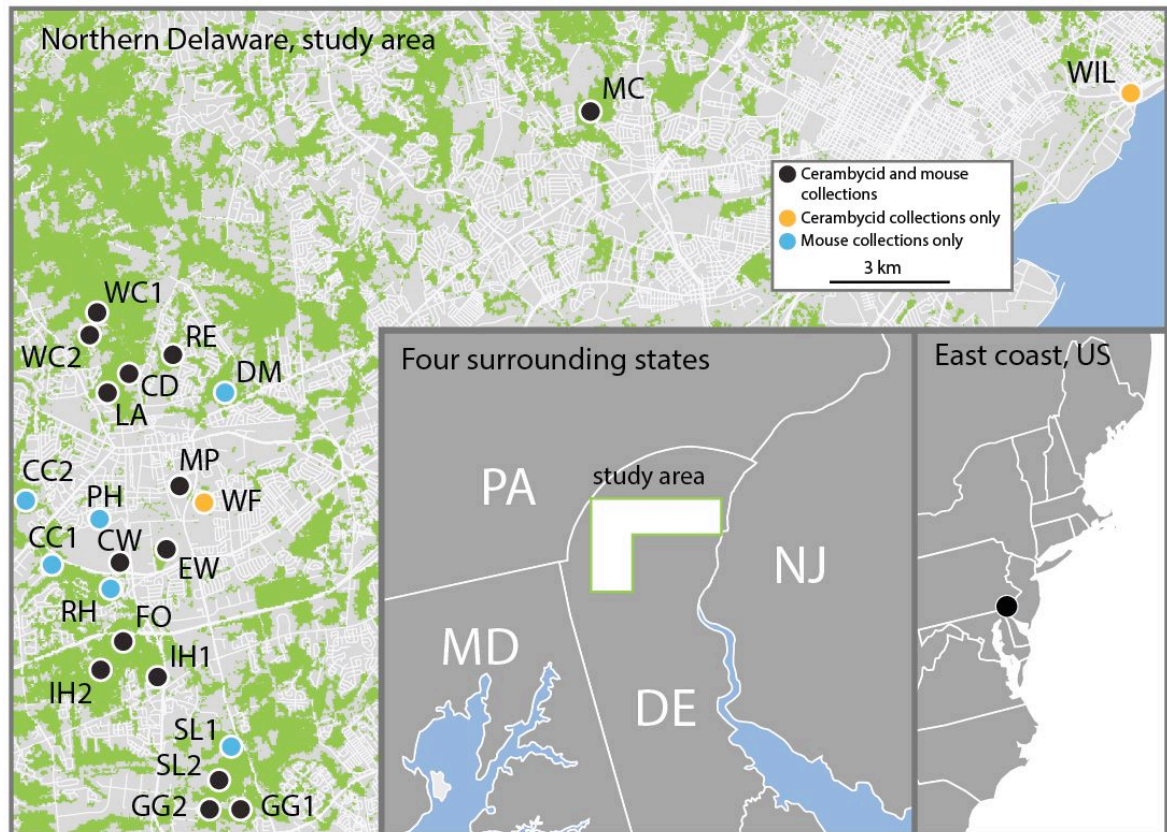


Figure 2.1: Locations of FRAME study sites where cerambycids and mice were collected in New Castle County, DE in 2014. Abbreviations as in Table 2.1.

We collected ear biopsy tissue samples from 458 white-footed deermice at 164 nest boxes from 20 June to 12 November 2014, according to IACUC protocol 1249-2014-A. Traps were located at 21 study sites, once again part of the FRAME system

(Fig. 2.1). Most samples again were collected throughout Newark, DE, in addition to Hockessin, DE. Tissue samples were stored frozen at  $-62.2^{\circ}\text{C}$ .

### 2.2.2 SNP Sequencing and Analysis

We extracted DNA from the thorax of 285 *X. colonus* specimens and from the antennae of 190 *P. laticollis* specimens using DNeasy tissue kits (QIAGEN, Valencia, CA). All sequencing took place at the Cornell University Biotechnology Resource Center (BRC). Genotyping-by-sequencing libraries were constructed for both species using a protocol modified from Elshire et al. 2011. Libraries that passed quality control were quantified, diluted, and sequenced on the Illumina HiSeq 2500 (Illumina, Inc., San Diego, CA; NIH grant 1S10OD010693-01). Raw sequence data was analyzed and filtered for missingness using the UNEAK non-reference pipeline in TASSEL (Lu et al. 2013).

Analysis of *X. colonus* in TASSEL produced 876 SNP loci. Because of high levels of missing data still remaining at many loci according to GenAEx (Peakall and Smouse 2006, 2012), we further filtered our data to 485 loci (based on a minimum 70% site count and a minimum allele frequency of 0.05) and pooled individuals from sites in close geographic proximity and forest continuity (Table 2.1; Fig. 2.1). We used the program *structure* (Pritchard et al. 2000) to infer population structure, employing a Bayesian method to determine the probability of assigning each individual to  $K$  hypothetical clusters, when  $K$  is initially unknown (admixture model, correlated allele frequencies). We ran 3 runs for each value of  $K$  from  $K = 1$  to  $K = 9$ , with 50,000 burn-ins and 100,000 replications after burn-in. We estimated the optimal  $K$  value using the  $\Delta K$  method (Evanno et al. 2005) in STRUCTURE HARVESTER (Earl and vonHoldt 2012). We used the software GenAEx 6.5 (Peakall and Smouse 2006, 2012)

to calculate an analysis of molecular variance (AMOVA) within versus among populations (Excoffier et al. 1992), and pairwise population values of  $\Phi_{PT}$ , a metric analogous to  $F_{ST}$  that gauges genetic divergence, along with their  $P$ -values for significant divergence based on 999 permutations. We also used GenAlEx 6.5 to produce a principal coordinate analysis (PCoA) based on pairwise population  $\Phi_{PT}$  values.

Table 2.1: Sample sizes for all species by population in northern Delaware.

Population	Pop. ID	Sites	Fragment Size (ha)	<i>X. colonus</i>	<i>P. laticollis</i>	<i>P. leucopus</i>
Christina Creek 1	CC1	CC1	50.8	0	0	11
Christina Creek 2	CC2	CC2	12.7	0	0	13
Chrysler Woods	CW	CW	5.5	21	16	5
Dorothy Miller	DM	DM	68.7	0	0	3
Ecology Woods	EW	EW	16.6	21	7	10
Glasgow	GG	GG1, GG2, SL1, SL2	233.4	57	48	31
Iron Hill	IH	FO, IH1, IH2	150.1	22	19	28
Mount Cuba	MC	MC	62.1	20	16	15
Motor Pool	MP	MP	5.9	22	16	3
Phillips	PH	PH	4.6	0	0	13
Rittenhouse	RH	RH	50.8	0	0	5
White Clay	WC	CD, LA, RE, WC1, WC2	609.5	80	50	53
Webb Farm	WF	WF	11.2	21	18	0
Wilmington	WIL	WIL	1.0	21	0	0
<i>Total</i>				285	190	190

We used the same filters and pooling methods for *P. laticollis* as for *X. colonus*, resulting in 1398 SNP loci and 8 pooled populations (Table 2.1; Fig. 2.1). We again used *structure* (Pritchard et al. 2000) to infer population structure, using the same parameters from  $K = 1$  to  $K = 8$ , and determined the optimal  $K$  value using the  $\Delta K$  method in STRUCTURE HARVESTER (Evanno et al. 2005, Earl and vonHoldt

2012). We again used GenAlEx 6.5 (Peakall and Smouse 2006, 2012) to calculate an AMOVA, pairwise population values of  $\Phi_{PT}$ , and a PCoA.

We extracted DNA from ear biopsy tissue samples of 190 *P. leucopus* using DNeasy tissue kits (QIAGEN, Valencia, CA). All sequencing took place at the Cornell University Biotechnology Resource Center (BRC). A genotyping-by-sequencing library was constructed using a protocol modified from Elshire et al. 2011. Libraries that passed quality control were quantified, diluted, and sequenced on the Illumina HiSeq 2500 (Illumina, Inc., San Diego, CA; NIH grant 1S10OD010693-01). Raw sequence data was analyzed against *Peromyscus maniculatus* (Wagner) reference genome sequences using the GBS pipeline in TASSEL (Lu et al. 2013).

Due to a high volume of SNP calls for *P. leucopus*, we filtered for 100% site count and a minimum allele frequency of 0.05, resulting in 8878 loci and 12 pooled populations (Table 2.1). We again used *structure* (Pritchard et al. 2000) to infer population structure, using the same parameters from  $K = 1$  to  $K = 12$ , and estimated the optimal  $K$  value using the  $\Delta K$  method in STRUCTURE HARVESTER (Evanno et al. 2005, Earl and vonHoldt 2012). We generated bar plots for  $K = 2$  to  $K = 6$  in order to visually present possible substructures. We again used GenAlEx 6.5 (Peakall and Smouse 2006, 2012) to calculate an AMOVA, pairwise population values of  $\Phi_{PT}$ , and a PCoA.

## 2.3 Results

### 2.3.1 *Xylotrechus colonus*

Sample sizes are reported in Table 2.1. According to the  $\Delta K$  method (Evanno et al. 2005), the optimal number of clusters for *X. colonus* was 3; however, no

substructure was found in *structure* for any value of  $K$ . Pairwise values of  $\Phi_{PT}$  indicated little genetic divergence, although a few pairs did show significant values at the  $P = 0.05$  level after 999 permutations (Table 2.2). The  $\Phi_{PT}$  divergence of the Wilmington population was statistically significant at the  $P = 0.05$  level from half of the remaining populations: Chrysler Woods, Ecology Woods, Glasgow, and Motorpool. White Clay was significantly divergent from Glasgow and Motorpool, and Motorpool was also significantly divergent from Ecology Woods (Table 2.2). The Iron Hill, Mount Cuba, and Webb Farm populations did not show significant genetic divergence from any other population, based on  $\Phi_{PT}$  values (Table 2.2). The AMOVA based on these  $\Phi_{PT}$  values indicated that 0.126% of the total genetic variation was among populations (Table 2.3). The principal coordinates analysis (PCoA) based on pairwise population  $\Phi_{PT}$  values indicated that, within the small amount of genetic distance present among populations of *X. colonus*, two populations appeared to be more divergent from the majority: Wilmington and Motorpool (Fig. 2.2). The first two axes of this multidimensional analysis (which are those shown in Fig. 2.2) explained 87.0% of the total variation in pairwise population  $\Phi_{PT}$  values.

Table 2.2: Pairwise population matrix of  $\Phi_{PT}$  values (below diagonal) and probability values based on 999 permutations (above diagonal) for *X. colonus*. Significant probabilities for population differentiation at the 0.05 level and their corresponding  $\Phi_{PT}$  values are shown in bold; at the 0.01 level, with an asterisk.

Population	CW	EW	GG	IH	MC	MP	WC	WF	WIL
CW	–	0.501	0.480	0.471	0.449	0.120	0.266	0.495	<b>0.032</b>
EW	0.000	–	0.494	0.504	0.208	<b>0.033</b>	0.468	0.474	<b>0.037</b>
GG	0.000	0.000	–	0.513	0.117	0.108	<b>0.033</b>	0.477	<b>0.003*</b>
IH	0.000	0.000	0.000	–	0.486	0.405	0.489	0.493	0.431
MC	0.000	0.003	0.003	0.000	–	0.495	0.450	0.344	0.462
MP	0.004	<b>0.006</b>	0.003	0.001	0.000	–	<b>0.034</b>	0.423	<b>0.011</b>
WC	0.001	0.000	<b>0.002</b>	0.000	0.000	<b>0.004</b>	–	0.319	0.114
WF	0.000	0.000	0.000	0.000	0.001	0.000	0.001	–	0.288
WIL	<b>0.006</b>	<b>0.007</b>	<b>0.007*</b>	0.000	0.000	<b>0.009</b>	0.003	0.002	–

Table 2.3: Analysis of molecular variance (AMOVA) based on 485 SNP loci for *X. colonus*.

Source	df	SS	MS	Variation	% of Total Variation
Among Populations	8	691.911	86.489	0.105	0.126%
Within Populations	276	23000.963	83.337	83.337	99.874%
Total	284	23692.874		83.442	100%

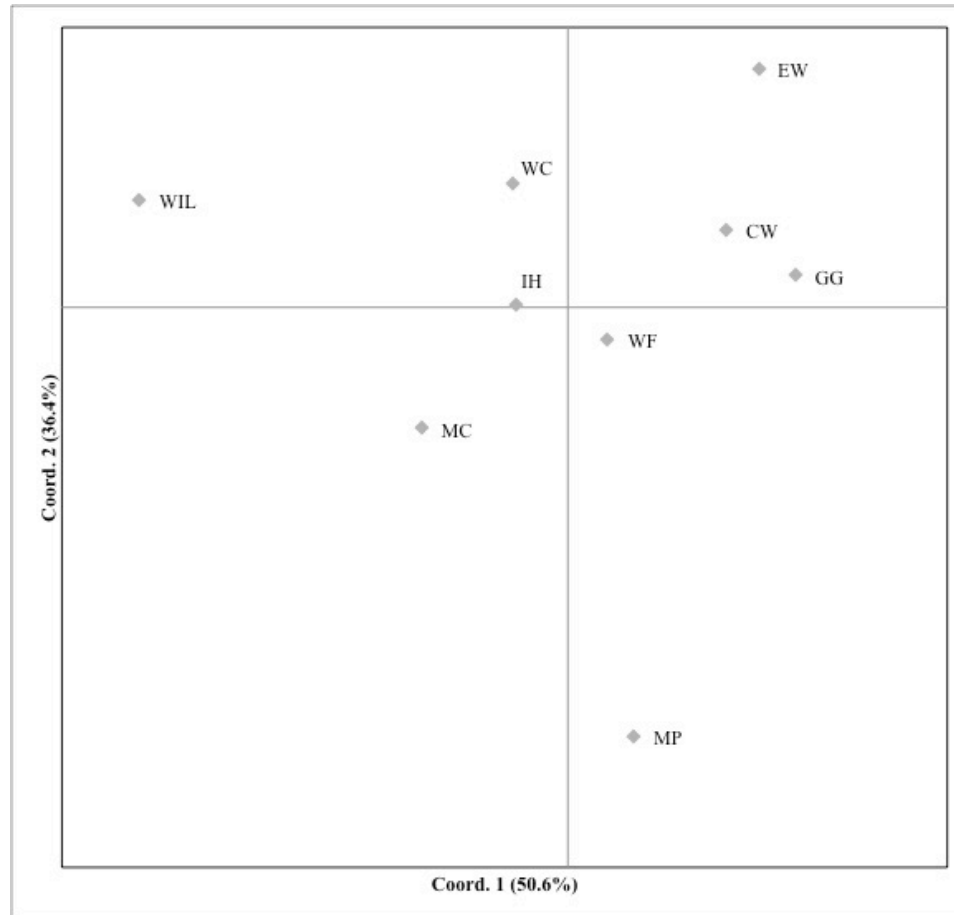


Figure 2.2: Principal coordinate analysis (PCoA) of pairwise population  $\Phi_{PT}$  values among 9 populations of *X. colonus*. The first three axes explained 50.6%, 36.4%, and 8.8% of the total variation.

### 2.3.2 *Prionus laticollis*

Sample sizes are reported in Table 2.1. According to the  $\Delta K$  method (Evanno et al. 2005), the optimal number of clusters for *P. laticollis* was 2; however, this analysis revealed little pattern in genetic structure (Fig. 2.3). Subsequent values of  $K$  did not reveal any substructure in *structure*. Pairwise values of  $\Phi_{PT}$  indicated more population divergence than those of *X. colonus*, particularly for Motorpool, Chrysler Woods, and Webb Farm (Table 2.4). Motorpool showed statistically significant

genetic divergence at the  $P = 0.05$  level from every remaining population. Chrysler Woods showed significant divergence at the  $P = 0.01$  level for every other population except Ecology Woods and White Clay. Webb Farm showed significant divergence at the  $P = 0.05$  level for every population except Ecology Woods. The AMOVA indicated that 0.801% of the total genetic variation was among populations (Table 2.5); while still a small percentage, this is a greater amount than that of *X. colonus*. The PCoA indicated that Motorpool, Chrysler Woods, and Webb Farm were most divergent from the remaining populations (Fig. 2.4). The first two axes of this multidimensional analysis (which are those shown in Fig. 2.4) explained 74.3% of the total variation in pairwise population  $\Phi_{PT}$  values.

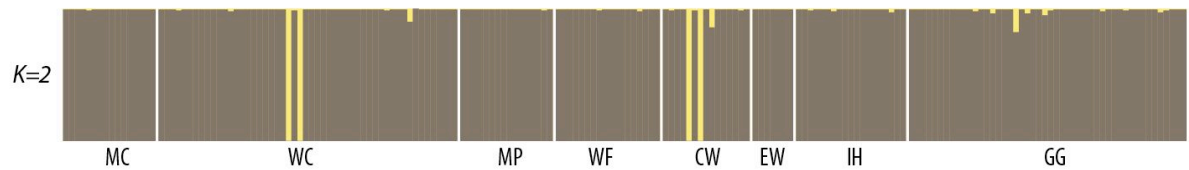


Figure 2.3: Summary plot of the *structure* analysis of 1398 SNP markers of *P. laticollis* with  $K = 2$  hypothetical clusters. Each individual *P. laticollis* is represented by a single vertical line, with the length of each segment proportional to the membership coefficient in each of the  $K$  inferred clusters. The bottom labels indicate the region from which each individual was collected.

Table 2.4: Pairwise population matrix of  $\Phi_{PT}$  values (below diagonal) and probability values based on 999 permutations (above diagonal) for *P. laticollis*. Significant probabilities for population differentiation at the 0.05 level and their corresponding  $\Phi_{PT}$  values are shown in bold; at the 0.01 level, with an asterisk.

Population	CW	EW	GG	IH	MC	MP	WC	WF
CW	–	0.053	<b>0.001*</b>	<b>0.001*</b>	<b>0.001*</b>	<b>0.001*</b>	0.147	<b>0.001*</b>
EW	0.019	–	0.194	0.172	0.158	<b>0.048</b>	0.107	0.194
GG	<b>0.014*</b>	0.002	–	0.135	0.133	<b>0.013</b>	<b>0.032</b>	<b>0.012</b>
IH	<b>0.019*</b>	0.004	0.002	–	0.113	<b>0.001*</b>	0.059	<b>0.027</b>
MC	<b>0.023*</b>	0.005	0.003	0.003	–	<b>0.004*</b>	0.052	<b>0.050</b>
MP	<b>0.021*</b>	<b>0.011</b>	<b>0.008</b>	<b>0.011*</b>	<b>0.011*</b>	–	<b>0.004*</b>	<b>0.009*</b>
WC	0.004	0.005	<b>0.003</b>	0.004	0.005	<b>0.009*</b>	–	<b>0.003*</b>
WF	<b>0.029*</b>	0.005	<b>0.011</b>	<b>0.009</b>	<b>0.008</b>	<b>0.017*</b>	<b>0.016*</b>	–

Table 2.5: Analysis of molecular variance (AMOVA) based on 1398 SNP loci for *P. laticollis*.

Source	df	SS	MS	Variation	% of Total Variation
Among Populations	7	2748.677	392.668	2.685	0.801%
Within Populations	182	60518.418	332.519	332.519	99.199%
Total	189	63267.095		335.204	100%

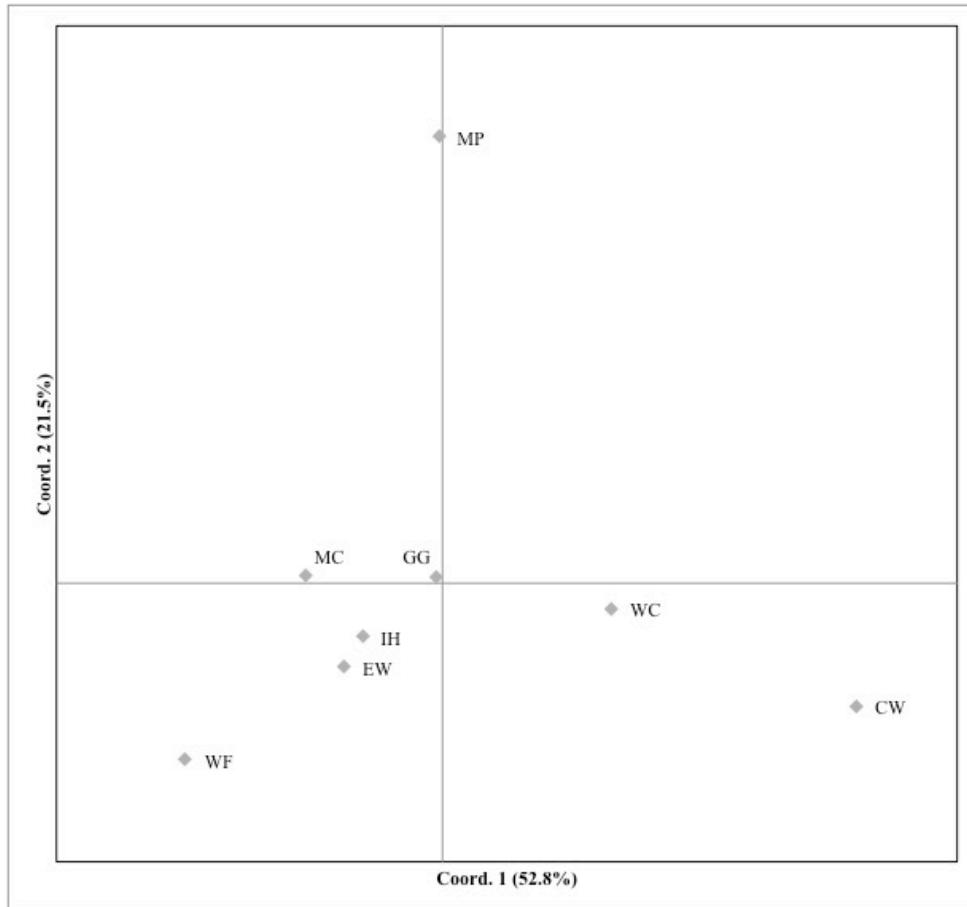


Figure 2.4: Principal coordinate analysis (PCoA) of pairwise population  $\Phi_{PT}$  values among 8 populations of *P. laticollis*. The first three axes explained 52.8%, 21.5%, and 15.4% of the total variation.

### 2.3.3 *Peromyscus leucopus*

Sample sizes are reported in Table 2.1. According to the  $\Delta K$  method (Evanno et al. 2005), the optimal number of clusters for *P. leucopus* was 2; subsequent values of  $K$  did reveal further substructure in *structure* (Fig. 2.5). Pairwise values of  $\Phi_{PT}$  indicated statistically significant genetic divergence at the  $P = 0.05$  or  $P = 0.01$  level for nearly every pair of populations of *P. leucopus* (Table 2.6). Six of the 12 populations showed significant divergence from every other population (Table 2.6).

The Dorothy Miller population showed the least divergence, with no significant difference from Chrysler Woods, Motorpool, Rittenhouse, or White Clay populations (Table 2.6). The Rittenhouse population also showed no significant divergence from Christina Creek 1 or Chrysler Woods populations (Table 2.6). The AMOVA based on these  $\Phi_{PT}$  values indicated that 3.504% of the total genetic variation in *P. leucopus* was among populations (Table 2.7). The principal coordinates analysis (PCoA) based on pairwise population  $\Phi_{PT}$  values indicated that the Motorpool, Chrysler Woods, and Phillips populations of *P. leucopus* were most divergent from the majority (Fig. 2.6). The first two axes of this multidimensional analysis (which are those shown in Fig. 2.6) explained 38.1% of the total variation in pairwise population  $\Phi_{PT}$  values.

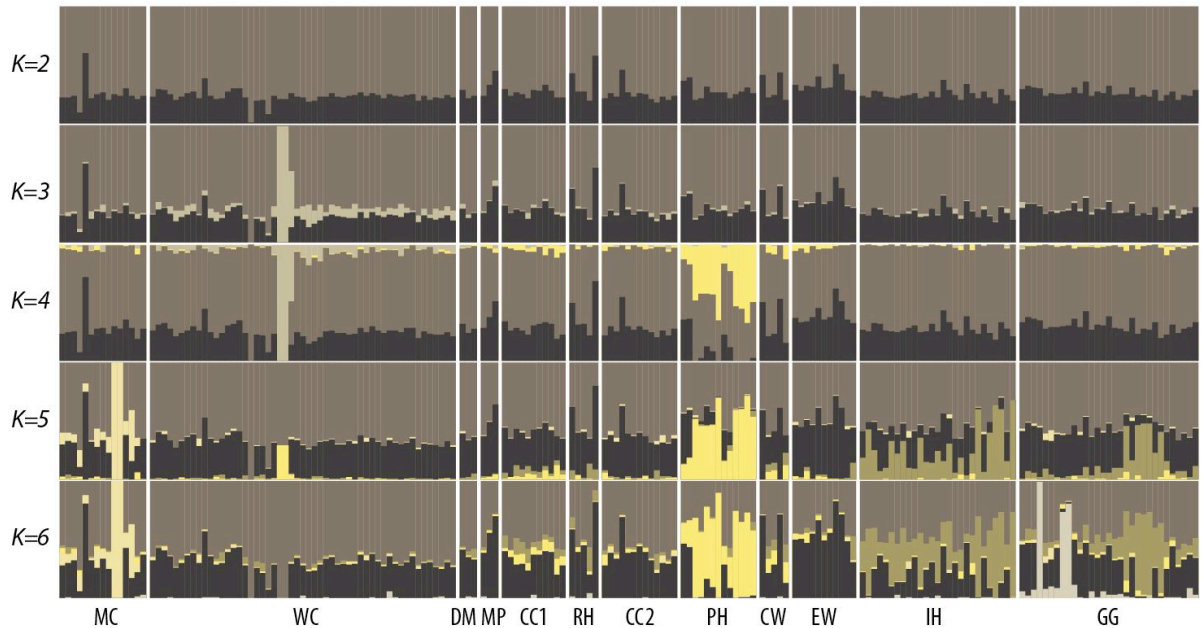


Figure 2.5: Summary plot of the *structure* analysis of 8878 SNP markers of *P. leucopus* with  $K = 2$  to  $K = 6$  hypothetical clusters. Each individual *P. leucopus* is represented by a single vertical line, with the length of each segment proportional to the membership coefficient in each of the  $K$  inferred clusters. The bottom labels indicate the region from which each individual was collected.

Table 2.6: Pairwise population matrix of  $\Phi_{PT}$  values (below diagonal) and probability values based on 999 permutations (above diagonal) for *P. leucopus*. Significant probabilities for population differentiation at the 0.05 level and their corresponding  $\Phi_{PT}$  values are shown in bold; at the 0.01 level, with an asterisk.

	CC1	CC2	CW	DM	EW	GG	IH	MC	MP	PH	RH	WC
CC1	–	<b>0.001*</b>	<b>0.003*</b>	<b>0.035</b>	<b>0.001*</b>	<b>0.001*</b>	<b>0.001*</b>	<b>0.001*</b>	<b>0.019</b>	<b>0.001*</b>	0.070	<b>0.001*</b>
CC2	<b>0.018*</b>	–	<b>0.001*</b>	<b>0.042</b>	<b>0.001*</b>	<b>0.001*</b>	<b>0.001*</b>	<b>0.001*</b>	<b>0.006*</b>	<b>0.001*</b>	<b>0.002*</b>	<b>0.001*</b>
CW	<b>0.043*</b>	<b>0.061*</b>	–	0.135	<b>0.001*</b>	<b>0.001*</b>	<b>0.001*</b>	<b>0.001*</b>	<b>0.016</b>	<b>0.001*</b>	0.137	<b>0.001*</b>
DM	<b>0.017</b>	<b>0.025</b>	0.067	–	<b>0.003*</b>	<b>0.038</b>	<b>0.035</b>	<b>0.046</b>	0.109	<b>0.004*</b>	0.191	0.071
EW	<b>0.047*</b>	<b>0.058*</b>	<b>0.084*</b>	<b>0.052*</b>	–	<b>0.001*</b>	<b>0.001*</b>	<b>0.001*</b>	<b>0.004*</b>	<b>0.001*</b>	<b>0.001*</b>	<b>0.001*</b>
GG	<b>0.021*</b>	<b>0.027*</b>	<b>0.055*</b>	<b>0.021</b>	<b>0.049*</b>	–	<b>0.001*</b>	<b>0.001*</b>	<b>0.006*</b>	<b>0.001*</b>	<b>0.007*</b>	<b>0.001*</b>
IH	<b>0.024*</b>	<b>0.031*</b>	<b>0.057*</b>	<b>0.028</b>	<b>0.054*</b>	<b>0.023*</b>	–	<b>0.001*</b>	<b>0.002*</b>	<b>0.001*</b>	<b>0.014</b>	<b>0.001*</b>
MC	<b>0.034*</b>	<b>0.040*</b>	<b>0.069*</b>	<b>0.030</b>	<b>0.064*</b>	<b>0.036*</b>	<b>0.041*</b>	–	<b>0.022</b>	<b>0.001*</b>	<b>0.025</b>	<b>0.001*</b>
MP	<b>0.052</b>	<b>0.060*</b>	<b>0.104</b>	0.065	<b>0.074*</b>	<b>0.049*</b>	<b>0.057*</b>	<b>0.068</b>	–	<b>0.008*</b>	<b>0.033</b>	<b>0.009*</b>
PH	<b>0.036*</b>	<b>0.048*</b>	<b>0.082*</b>	<b>0.053*</b>	<b>0.070*</b>	<b>0.045*</b>	<b>0.051*</b>	<b>0.059*</b>	<b>0.078*</b>	–	<b>0.001*</b>	<b>0.001*</b>
RH	0.016	<b>0.026*</b>	0.035	0.012	<b>0.048*</b>	<b>0.021*</b>	<b>0.021</b>	<b>0.035</b>	<b>0.043</b>	<b>0.047*</b>	–	<b>0.017</b>
WC	<b>0.019*</b>	<b>0.024*</b>	<b>0.055*</b>	0.013	<b>0.050*</b>	<b>0.022*</b>	<b>0.026*</b>	<b>0.030*</b>	<b>0.051*</b>	<b>0.043*</b>	<b>0.022</b>	–

Table 2.7: Analysis of molecular variance (AMOVA) based on 8878 SNP loci for *P. leucopus*.

Source	df	SS	MS	Variation	% of Total Variation
Among Populations	11	31890.052	2899.096	68.665	3.504%
Within Populations	178	336540.685	1890.678	1890.678	96.496%
Total	189	368430.737		1959.343	100%

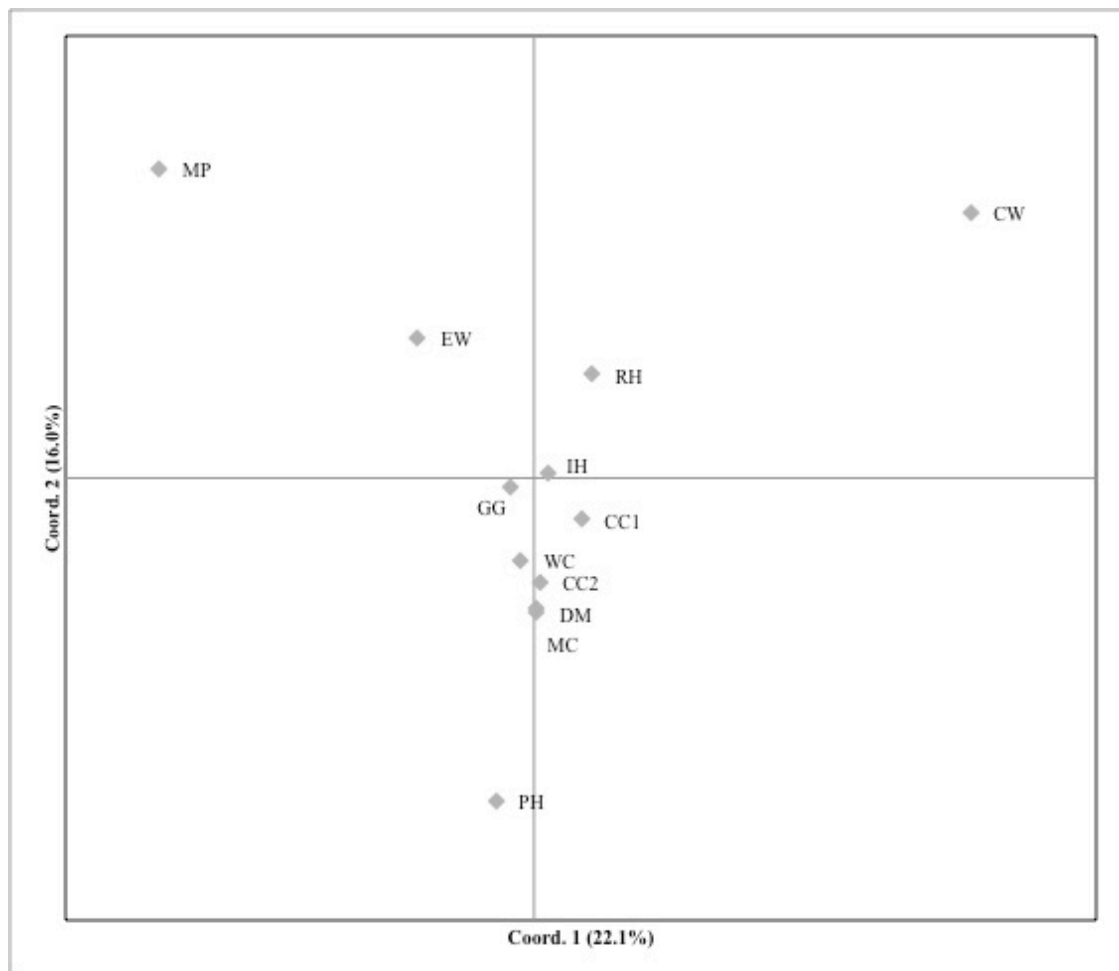


Figure 2.6: Principal coordinate analysis (PCoA) of pairwise population  $\Phi_{PT}$  values among 12 populations of *P. leucopus*. The first three axes explained 22.1%, 16.0%, and 15.2% of the total variation.

## 2.4 Discussion

### 2.4.1 Cerambycids

*Xylotrechus colonus* showed limited genetic structure among populations in northern Delaware, based upon  $\Phi_{PT}$  values. The Wilmington population, one of the most northern populations, most frequently showed significant genetic divergence from the remaining populations, including the Glasgow population, which is the furthest south. Ecology Woods and Motorpool showed statistically significant genetic divergence, but are within 2 km of each other. These two sites are separated by agricultural land and train tracks; one or both of these features may serve as an impediment to dispersal for this species. The Webb Farm population showed no significant genetic divergence from any other population, but is immediately adjacent to the Motorpool site and is only separated by a two-lane road and train tracks. Small-scale features may dictate cerambycid movement on this landscape, as these two adjacent populations show very different levels of genetic structure. The Mount Cuba and Iron Hill populations also showed no significant genetic divergence from any other populations. Mount Cuba is particularly surprising, as this site is one of the most geographically separated from the others; however, it also is in an area of higher forest connectivity, which may facilitate dispersal.

The AMOVA analysis indicated that approximately 99.874% of the total variation present in the genetic data could be attributed to variation within populations; thus, populations contained so much more intrinsic variability that any differences among populations could not easily be detected. However, a PCoA built from the  $\Phi_{PT}$  values of divergence among the populations does reveal some patterns; populations from Wilmington and Motorpool separate from the remaining sites.

Wilmington is one of the most geographically distant sites included in our study, roughly 21 km from the closest trap in the Newark region, and 13 km from the Mount Cuba site. The genetic separation of Wilmington may be attributable to its geographic separation from the remaining sites. Motorpool, however, is in close proximity to several other sites. We are therefore surprised that this site in particular appears to separate from the rest.

*Prionus laticollis* showed more genetic divergence among populations in northern Delaware than *X. colonus* based on  $\Phi_{PT}$  values, with every population significantly divergent from at least one other population. The Motorpool population showed statistically significant divergence from every other population, including Webb Farm, which as noted before is immediately adjacent to the Motorpool site. Chrysler Woods and Webb Farm also showed significant divergence from nearly every other population; all three of these sites are found in forest patches less than 12 ha in area and are adjacent to urbanized areas and roads. *Prionus laticollis* may be less able to readily migrate across these urbanized zones. The Mount Cuba population again showed relatively little divergence compared to other populations, suggesting that geographic separation does not necessarily lead to genetic separation. The Ecology Woods population of *P. laticollis* showed very little divergence from other populations, despite the fact that this forest fragment is isolated in an agricultural matrix and has been for more than a century (unpublished data). The AMOVA analysis for *P. laticollis* once again indicated a high level of variation within populations, with only 0.801% attributable to among-population variation. The PCoA indicated that Motorpool, Chrysler Woods, and Webb Farm separate the most from the remaining sites, in agreement with the  $\Phi_{PT}$  results.

Our findings on cerambycid population structure agree with those found on other cerambycid species, in which little divergence occurs among populations on a regional scale (Carter et al. 2009, Drag et al. 2015). We report lower levels of among-population variance (less than 1% of the total variance for both species) than previous genetic analyses of cerambycids, which found as much as 8% for *Monochamus alternatus* Hope in northeast Asia, 12.1% for *R. alpina* in Europe, and 13% for *A. glabripennis* in China (Kawai et al. 2006, Carter et al. 2009, Drag et al. 2015). However, all of these studies were conducted on much larger spatial scales, which could account for the greater population divergence. Our study does afford the opportunity to compare relative amounts of genetic divergence for different cerambycid species on the same forest landscape, to determine the degree to which these species differ in movement. Based on our analysis of SNP loci for both species, *X. colonus* may disperse more readily than *P. laticollis*, resulting in less genetic divergence among populations. Multiple factors could account for this difference in genetic isolation. *Prionus laticollis* larvae feed on living or decaying roots of woody host plants, a relatively constant resource (Benham and Farrar 1976), while *X. colonus* utilize stressed hosts (freshly felled trees) that are a more unreliable resource (Linsley 1964, Hanks 1999). *Xylotrechus colonus* therefore may need to disperse more frequently and at further distances in order to locate hosts. Further, female *P. laticollis* are known to be sedentary, with only males dispersing to locate mates (Benham and Farrar 1976, Yanega 1996), while both male and female *X. colonus* disperse (Lacey et al. 2009). In field experiments, adult *X. colonus* traveled further and at a higher rate out of a forest fragment in response to a pheromone lure than adult *P. laticollis* (Chapter 1), indicating a greater propensity for dispersal. Our findings contradict

previous studies indicating that larger beetles respond to forest habitat at a larger scale (Holland et al. 2005), although both inter- and intraspecific morphological differences such as wing loading should also be taken into consideration (Wainwright 1994, Bouget et al. 2014).

The Motorpool populations of both cerambycid species showed among the most genetic divergence from other populations, despite close geographic proximity to other sites. This forest fragment is surrounded by human development on three sides and by train tracks on the fourth side, and together these features may serve as significant barriers to movement for both cerambycid species. In contrast, the Webb Farm population, in close proximity to Motorpool, showed significant genetic divergence for *P. laticollis* but not for *X. colonus*. This site is surrounded by a mix of agriculture and human development, which may serve as more of a barrier to *P. laticollis* than to *X. colonus*. The Mount Cuba populations showed no genetic divergence for *X. colonus* and only moderate divergence for *P. laticollis*, despite significant geographic distance from the remaining sites. Physical barriers may play a larger role than spatial separation in the divergence of populations of cerambycids.

#### **2.4.2 White-Footed Deermice**

*Peromyscus leucopus* showed more genetic structure among populations in northern Delaware than either cerambycid species on the same landscape, according to  $\Phi_{PT}$  values and AMOVA results. Half of the populations of *P. leucopus* showed statistically significant divergence from every other population. The AMOVA analysis indicated that 3.504% of the genetic variation could be attributed to among-population differences. This value is less than half that which was previously reported for the same species in New York City (Munshi-South and Kharchenko 2010); thus, this

suburban and agricultural landscape appears to provide weaker impediments to movement than a highly urban matrix. Based on the PCoA, the *P. leucopus* populations from Motorpool, Chrysler Woods, and Phillips separate the most from the remaining sites. All three of these sites are less than 6 ha in area and are surrounded by roads and human development, suggesting that these features may serve as barriers to dispersal for white-footed deermice.

While the  $\Delta K$  method (Evanno et al. 2005) estimated the optimal value of  $K$  to be 2, plots of subsequent  $K$  values in *structure* revealed patterns of substructure. Evaluation of the  $\Delta K$  method has indicated that it may only be effective in cases of extremely strong genetic differentiation (Waples and Gaggiotti 2006). Further, *structure* can only be considered an approximate method for determining substructure, and biological context of that structure should be taken into account (Pritchard et al. 2000). Here we present successive values of  $K$  that, in conjunction with the PCoA analysis, can provide some insight into the population structure of white-footed deermice in this region.

Results in *structure* at  $K = 2$  and  $K = 3$  indicate very little substructure, with most individuals assigned to each subpopulation with relatively similar probability with the exception of a few divergent White Clay individuals. Beginning at  $K = 4$ , Phillips emerges as a subpopulation, and select individuals from Glasgow and Mount Cuba Interior also diverge. Based on a qualitative evaluation of these results, we find the clearest and most simplified structure at  $K = 4$ , in which most individuals share similar assignment probabilities with the exception of the Phillips population. This population did also separate in the PCoA analysis and was significantly divergent from every remaining population according to  $\Phi_{PT}$  values. This site is bordered by

train tracks on one side, and residential areas on the remaining three sides; as white-footed deermice are known to easily inhabit residential areas (Reid 2006), the train tracks appear to be the only potential barrier to movement from this site.

### 2.4.3 Conclusions

*Peromyscus leucopus* showed much more population structure than either cerambycid species, and *P. laticollis* showed much more structure than *X. colonus*, on the same landscape of forest fragments. We hypothesize that generation time and dispersal rates both could explain, at least in part, these differences. *Peromyscus leucopus* are capable of several generations per year (Krohne et al. 1984, Keane 1990, Nupp and Swihart 1996), while *X. colonus* completes one life cycle every two years (Gardiner 1960) and *P. laticollis* completes one life cycle every three to four years (Benham and Farrar 1976). Therefore, *P. leucopus* has completed more generations than either cerambycid species since forest fragmentation occurred, allowing more evolutionary time for barriers to become detectable (Landguth et al. 2010). Cerambycid populations may be more functionally isolated than reflected in our results, but could require more generations to account for the lag effect of barrier detection.

In terms of dispersal, *P. leucopus* has been documented as capable of rapid movements across hundreds of meters, particularly across agricultural areas (Middleton and Merriam 1981). Highways and roads significantly decreased recapture of *P. leucopus*, particularly in high traffic areas (Clark et al. 2001, McGregor et al. 2003, McLaren et al. 2011), and detailed tracking of individuals indicated that *P. leucopus* actively avoid crossing roads (Merriam et al. 1989). The high density of roads and train tracks around our sites, which maintain relatively high traffic volume,

therefore may be responsible for isolating populations of this otherwise capable disperser. While cerambycid dispersal requires more research and likely varies tremendously by species, field experiments have indicated that *X. colonus* travels further and at a higher frequency out of a forest fragment in response to pheromones and plant volatiles than *P. laticollis* (Chapter 1). Populations of *X. colonus* may therefore mix and interbreed at a higher rate than those of *P. laticollis*. Cerambycid use of aggregation and sex pheromones for host and mate location may also play a role in their dispersal among forest fragments; however, previous research has found that cerambycid attraction to a pheromone lure significantly decreases just 40 m away from the canopy edge (Chapter 1).

Understanding wildlife movement, particularly in the face of anthropogenic influences, is critical for maintaining ecological functioning on human-dominated landscapes. Our results demonstrate that wildlife of dissimilar life histories and ecological niches respond to forest fragmentation and isolation in unique ways, even on the same landscape. Management strategies to enhance habitat connectivity and wildlife movement must take into account the range of responses of wildlife to urban and suburban landscapes.

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