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Genetic estimates of migration for white-footed mice (*Peromyscus leucopus)* at the Primmer Outdoor Learning Center

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Abstract

Peromyscus leucopus, or the white-footed mouse, is widely distributed across the eastern United States. The habitat generalists have been found to thrive in understory vegetation within small forest fragments in agricultural landscapes. Acting as reservoirs for Lyme disease, white-footed mice may actively migrate between populations potentially distributing the disease. This study estimated migration rates between two populations of mice in different habitats based on multilocus genotypes from samples collected May-August in 2016 and 2017. Field work was completed at Capital University's Primmer Outdoor Learning Center in Logan, Ohio in a secondary growth deciduous woodlot and a fencerow habitat. Mice were live-trapped, and tissues samples were collected and stored in 95% ethanol at -20℃. DNA was extracted and multilocus microsatellite PCR was performed. Sixteen samples from the woodlot and thirteen samples from the fencerow were genotyped at six loci using a 3100 Genetic Analyzer DNA sequencer. Results from GENECLASS indicated that migration was bidirectional and that five individuals likely migrated from the woodlot to the fencerow habitat with one individual in the opposite direction. Long-term gene flow was also detected in both directions. Genetic differentiation between populations was close to zero and outbreeding was also detected. Blood samples were collected this past summer along with additional tissue samples to increase sample sizes, and mice will be tested in the lab for the presence of Lyme disease in a future study.

Keywords: Migration of Mice, Population Genetics, Lyme disease

1. Introduction

Peromycus leucopus, or the white-footed mouse, is a nocturnal species widely distributed across the eastern United States. The mice act as trophic links between upper and lower trophic levels by serving as prey for hawks, eagles, and owls, and feeding on arthropods, nuts, and fruits throughout the year[1]. White-footed mice are considered habitat generalists, a species that is adaptable to a wide range of ecological conditions for survival even though it will exhibit lower populations in less than optimal conditions. Studies show that they have been found to occupy a variety of habitats including forests, forest edges, ditches, agricultural fields, and even farmsteads[2].

During summer months, *P. leucopus* tend to be found at their highest densities in forest edges followed by forest interior habitats[2]. In fact, Anderson et al. found that densities of white-footed mice were highest in the edges of small forest patches in a fragmented agricultural landscape in southwestern Ohio[3]. Studies have demonstrated a negative relationship between abundance of mice and forest patch area[1,3]. An increase in relative abundance of *P. leucopus* has been associated with an increase in structural complexity of understory habitat in the same patches between years[4]. With more vertical cover comes foraging opportunities; findings by Cameron and Klein supported this and proposed the idea that greater foraging opportunities could account for decreased space use by females in their study[5]. This environment would be found in edge habitat, where vertical cover is greater than that of the interior forest[5]. Hence,

understory vegetation may provide greater food availability and/or cover from some predators in small patches[4]. Beginning in the eighteenth century, agricultural practices have increased habitat fragmentation, thus creating more edge habitat and environments advantageous to *P. leucopus*[1,3].

The generalist attributes exhibited by *P. leucopus* have been demonstrated as a contributing factor to successful species dispersal[2,3]. Live-trapping and radio-telemetry studies of *P. leucopus* have demonstrated the potential for dispersal among populations since they inhabit a wide variety of habitats[5]. White-footed mice, a nocturnal species, have been documented in previous studies as having good dispersal abilities in fragmented landscapes[2,6]. Cummings and Vessey found in northeastern Ohio that they made the greatest number of shifts in and out of forests and edge habitats during summer[2]. They also found that mice used nonwoodland habitats, and suggested that crop fields and roadside ditches provide dispersal routes in and out of fragmented forest habitats. Research by Zollner found that the perceptual range of white-footed mice when traveling across a bare agricultural field at dusk was at its maximum at approximately 90 m[7]. This suggests that white-footed mice are capable of a 'look now and move later' strategy in relation to inter-patch dispersal in a fragmented landscape[7].

Acting as reservoirs for Lyme disease, white-footed mice may actively migrate throughout the fragmented landscape, potentially distributing the disease if infected[8]. Caused by the spirochete *Borrelia burgdorferi*, Lyme disease is an inflammatory disorder transmitted by blacklegged ticks (*Ixodes scapularis*)[10]. Mice can carry the spirochete in their blood, and when bitten by a tick can transmit the spirochete to the tick[11]. The tick in turn can be transported by the mouse and eventually encounter a human. In turn, the human can be bitten by the infected tick and the spirochete can be transmitted to the human[11]. Ticks can attach to any part of the body and must be attached to a human for 36 to 48 hours or more before the disease can be transmitted[11]. Humans are usually infected by ticks in the nymph or immature stage that feed in the spring and summer months and are hard to see[11]. In humans, symptoms are progressive, beginning with skin lesions that eventually move into the nervous system and eventual death if left untreated; the disease is easily treatable with antibiotics[11]. A recent study in the northeastern United States showed no effect of *B. burgdorferi* infection or tick burden on the survival of mice, and that mice are asymptomatic when infected[12].

Past field work has been performed at Capital University's Primmer Outdoor Learning Center in the Hocking Hills region of Ohio[13]. This site contains a small secondary growth deciduous woodlot and an agricultural fencerow habitat with a restored prairie meadow habitat in the middle. Using small Sherman live-traps, Hanlin found that population densities of *P. leucopus* in both habitats ranged between 17.9 and 126.7 mice per 500 trap nights during the summers of 2012-2015, with densities typically double in the agricultural fencerow habitat compared to the woods[13]. These values were similar to 9.62-121.09 mice per 500 trap nights reported across 15 woodlots in another study in southern Ohio[4], and to values reported by Bope at nearby Clear Creek Metro Park[14]. Hanlin also found that recapture rates were higher in the woods compared to the fencerow in three of the four years suggesting that the woods is a higher quality habitat[13]. Higher recapture rates suggest lower losses of individuals in the population due to dispersal and/or mortality including predation. The woods also had the highest proportion of juvenile gray mice, which suggests that reproduction was occurring in that habitat or that the appearance of those younger animals could result from their dispersal from source habitats[2,13].

Building on Hanlin's previous findings, our study sought to investigate dispersal further using bioinformatic techniques for samples collected during the summers of 2016-2017. Migration of nocturnal species is difficult to measure by direct observation and live-trapping methods[15]. Hence, indirect estimates of migration and gene flow have come widely used by biologists to examine patterns of dispersal in natural populations. The goal of this study sought to assess population genetic structure of *P. leucopus* using multilocus genotypes from samples collected in the fragmented landscape at Primmer. By employing microsatellite analysis, recent migration rates can be estimated between populations based on maximum likelihood methods to determine whether mice should be assigned as residents or immigrants to the populations sampled in the landscape. The number of effective migrants based on long term gene flow estimates can also be estimated using genetic markers. Past studies utilizing molecular techniques have found them useful to test dispersal-based hypotheses and specifically whether migration of white-footed mice was inhibited from small woodlots resulting in higher densities there[3]. Based on past reports of their generalist tendencies, and dispersal abilities, I hypothesized that mice would migrate bidirectionally between habitats. The next step in this research project will be to collected small blood samples from mice during the summer of 2018 and test those samples for Lyme disease, and to collect additional tissue samples for multilocus microsatellite analyses of migration.

2. Methods

2.1 Field Site

The study was comprised of two phases; field work was completed during the summer and fall of 2016 & 2017, and laboratory analysis was conducted after sample collection during 2017-2018. The field component of the study was completed at Capital University's Primmer Outdoor Learning Center in Logan, Ohio in the Hocking Hills region (Fig. 1). The center is a 74-acre site designated for student research and community outreach. The sites contains several ecosystems including streams, a wetland with a great blue heron rookery and a bald eagle nest, prairie meadow habitats, a riparian woodlot, a fencerow next to an agricultural fencerow, and a woodlot, several of which provide habitats for native animals such as *P. leucopus* (Fig. 2).

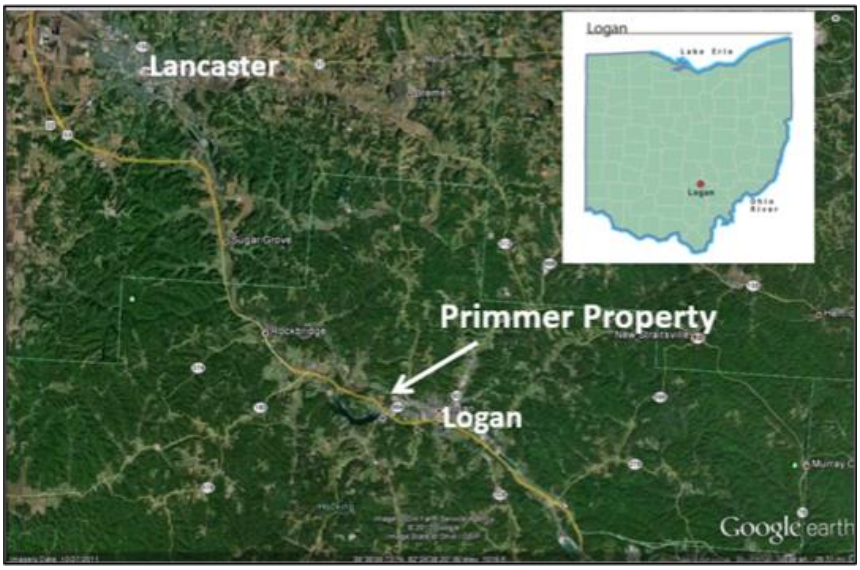

Figure. 1. Map showing the location of the Primmer Outdoor Learning Center in Logan, OH.

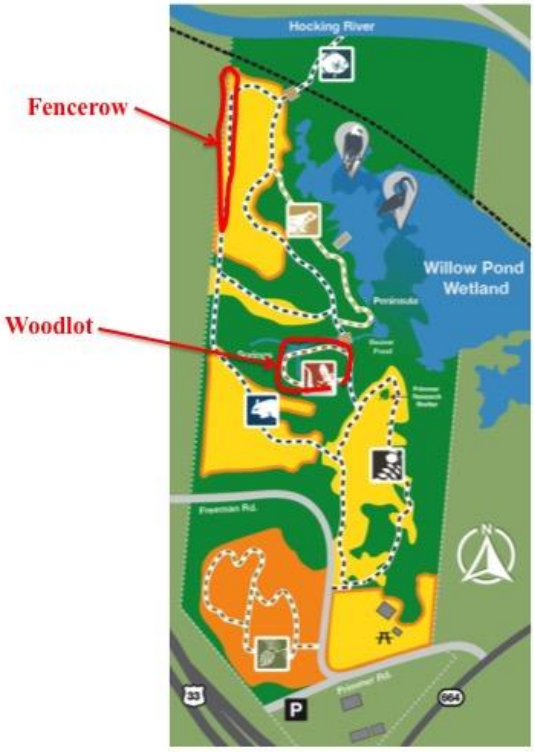

Figure. 2. Map of Capital University's Primmer Outdoor Learning Center showing the fencerow and woodlot trapping sites outlined in red separated by ~350 m with prairie habitat (colored yellow) and woods (shown as green) in between them.

2.2 Field Data Collection

Mice were live-trapped using small Sherman traps in a secondary-growth deciduous woodlot and a fencerow habitat separating an agricultural field from a recently restored prairie habitat at Capital University's Primmer Outdoor Learning Center (Fig. 2). There is a restored prairie in between the woods and the fencerow, and those two habitats are approximately 350 m apart as measured using straight-line distance (Fig. 2). There were 32 live-traps in the woods in an 8 x 4 grid all spaced 10 m apart, and there were 24 traps in a single transect along the fencerow also spaced 10 m apart. Traps were set in both habitats on six occasions during the summer between May-August in 2016 and five times during the summer in 2017. Mice were measured (including body length in mm, tail length in mm, and weight in g, and other morphological characteristics were recorded), ear tagged, and a small tail tip sample (<3mm) was collected and stored in 95% ethanol at -20℃. Twenty-three mice were live-trapped and tagged in the woods (with 24 recaptures) along with 15 mice tagged in the fencerow (with 3 recaptures) for both summers combined (Fig. 2). Tissue samples were collected from 16 mice in the woodlot and from 13 mice in the fencerow habitat for a total of 29 samples from both years combined.

2.3 Laboratory Techniques

DNA was extracted using the Qiagen DNeasy blood and tissue extraction kit with with two modifications: (1) the samples were lysed overnight at 65℃, and (2) the samples were eluted in 100 µl of AE buffer after an incubation period of 5 minutes. DNA concentration and the purity ratio was measured using the Nanodrop One spectrophotometer before polymerase chain reaction (PCR) was employed to amplify six microsatellite loci (Pml12, Pml04, PLGT67, PLGT15, Schmidt66, Bw4-200; Table 1)[16,17,18] Loci were multiplexed into two different multiplex mixes for a total of 35 cycles in the PCR with Qiagen multiplex master mix (Table 1). PCR products were genotyped on a 3100 Genetic Analyzer DNA Sequencer in the Gibbs lab at The Ohio State University. Following genotyping, allele calls were made

using PeakScanner. Multilocus genotypes were recorded along with sample ID so that individuals could be assigned to their respective habitats during data analysis.

Table 1. The multilocus pairs of primers used in the study after literature review[16,17,18]. Columns indicate the publication source of the primers, the fluorescent dye tag on the forward primer (FAM = blue, HEX = green, and NED = yellow/black), the multiplex mix, the annealing temperature, and the size range of alleles in base pairs (bp).

Primer	Publication	Fluorescent dye on forward primer	Multiplex mix	Annealing temperature (˚C)	Allele size range (bp)
Pml12	Chirhart et al. 2000	FAM	1	55	144-169
Pml04	Chirhart et al. 2000	HEX	1	55	187-241
PLGT67	Schmidt 1999	FAM	1	55	262-292
PLGT15	Schmidt 1999	NED	2	59	238-270
Bw4-200	Mullen et al. 2006	FAM	2	59	297-341
Schmidt66	Schmidt 1999	FAM	2	59	85-120

2.4 Data Analysis

GENECLASS2 was used to estimate recent migration between the two habitats[19]. This program allows for the detection of migrants and assignment of individuals through a Bayesian approach described in Rannala and Mountain[20]. The program MIGRATE was used to provide historical gene flow estimates through use of a coalescent-based maximum likelihood model[21]. The program was used to run a microsatellite data stepwise mutation model. In the simulation, both long and short Markov chains were run, with the number of chains being set to 1 and 10 respectively, the number of burn-ins was set at 10,000 for both long and short chains. In MIGRATE, long term gene flow is estimated from F_{ST} calculations and can provide both emigration and immigration estimates[22].

Genetic differentiation between populations (F_{ST}) was estimated with FSTAT. Genetic variation within populations, based on expected (H_E) and observed (H_O) heterozygosity was estimated using option 5 in GENEPOP version 4.2[23]. FSTAT uses the procedures of Weir and Cockerham[24] and Nei[25] to provide multilocus estimates. Inbreeding within populations (F_{IS}) was estimated with FSTAT[26]. Confidence intervals for F_{IS} (and F_{ST}) were measured at the 95% level and estimated by bootstrapping for 12,000 and 1,000 randomizations respectively. Values for genetic diversity and inbreeding were checked using the HIERFSTAT and Pegas packages in the programming language R[27,28]. Genetic diversity measures using procedures developed by Nei[25] were estimated with the basic.stats function in HIERFSTAT[27]. Another tool used to analyze population structure was the computer program STRUCTURE[29]. It is a model-based clustering program that uses multilocus genotype data[29].

3. Results

Twenty-nine samples were analyzed in this study at six microsatellite loci (Table 1). Results from GENECLASS2 suggest that six mice likely migrated between populations, with five migrating from the woods to the fencerow and one individual migrating from the fencerow to the woods (Fig. 3). In the analysis, only individuals that were estimated to have migrated with a confidence level over 95% were accepted.

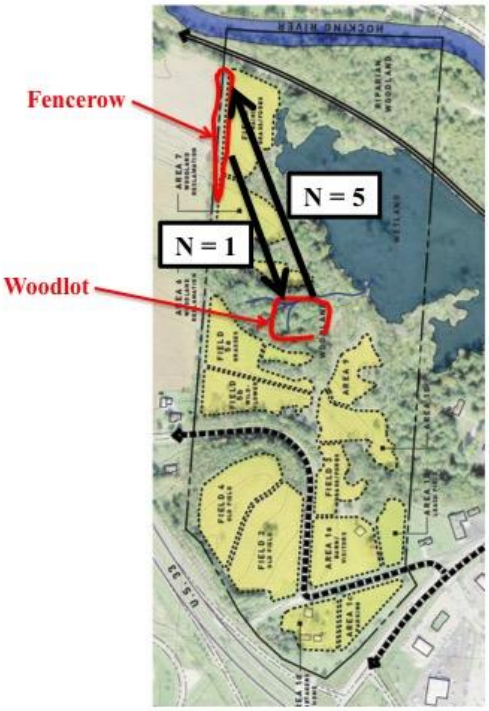

Figure 3. A map of the Primmer Outdoor Learning center that indicates migration estimates provided by GENECLASS2. Arrows represent movement of *P. leucopus* from one habitat to another. Five individuals are estimated to have migrated from the woods to the fencerow and one individual is estimated to have migrated from the fencerow to the woods. The distance between the research sites is approximately 350 m.

Long term gene flow estimates by MIGRATE estimated the number of effective migrants (*Nm)* between populations[21]. The program estimated that 2.47 individuals migrated from the fencerow habitat to the woods habitat, and that 2.35 individuals migrated from the woods habitat to the fencerow habitat (Table 2).

Table 2. Historical gene flow estimates as the number of effective migrants (*Nm*) from population *i* into population *j* as estimated using MIGRATE.

Population *i*	**Population *j***	
	Fencerow	**Woods**
Fencerow	---	2.47
Woods	2.35	---

Genetic differentiation between populations was close to zero (F_{ST} = 0.024; Table 3). Population structure analysis performed by the program STRUCTURE also indicated only one population genetically. Analysis with STRUCTURE was performed on multiple occasions, each providing the same estimation.

Table 3. Summary of sample size (N), average number of alleles per locus, genetic variation within populations (expected heterozygosity (H_E) and observed heterozygosity (H_O)), inbreeding within populations (F_{IS}), and differentiation between populations (F_{ST}). Values were also estimated for the total of both populations combined.

Population	Sample Size	Average number of alleles per locus	H_E	H_O	F_{IS}	F_{ST}
Fencerow	13	9.2	0.74	0.83	0.022	---
Woods	16	8.8	0.74	0.91	-0.510	---
Total	29	9.0	0.74	0.87	-0.035	0.024

Results from GENEPOP estimated genetic variation within populations to be moderate to high based on expected heterozygosity (H_E) and observed heterozygosity (H_O) values, which ranged from 0.74 to 0.91 (Table 3). Inbreeding (F_{IS}) in the fencerow was close to zero and outbreeding was detected in the woods (F_{IS} = -0.51). The overall value for both populations also suggested outbreeding (F_{IS} = -0.035; Table 3). The p-value at the 95% confidence level for F_{IS} within samples over 1,000 randomizations was 0.005. The confidence interval at the 95% level was (-0.114 - 0.061) for F_{IS} and (0.017 - 0.034) for F_{ST}; Table 3).

4. Discussion

The migration estimates from both GENECLASS2 and MIGRATE support the hypothesis that mice are moving between trapping sites at the Primmer Outdoor Learning Center. While our sample size may be limited, results suggest that bidirectional migration is present both within the past few generations and also more historical on site. These estimations are further supported by results from STRUCTURE and pairwise F_{ST}. After analyzing data in STRUCTURE multiple times, the results showed repeatedly that the mice in both sites at Primmer should be classified as one genetic cluster. In addition, genetic differentiation between populations was low and close to zero, and outbreeding based on F_{IS} was also detected. Altogether, these results indicate that mice are likely migrating between trapping sites.

MIGRATE provides historical gene flow estimates and found that 2.47 individuals likely migrated from the fencerow to the woods and that 2.35 individuals migrated from the woods to the fencerow. GENECLASS2 provides estimates of recent migration (e.g., within the last one to three generations) and found that 5 likely individuals migrated from the woods to the fencerow and that 1 individual migrated from the fencerow to the woods. These values are similar and within the same order of magnitude of one another. When interpreting these results, it is important to note that MIGRATE provides historical estimates of long-term gene flow employing a coalescent-based likelihood method, and has been shown to be precise with even a few loci[21]. It has also been recommended for studies where nonsymmetrical migration is suspected and for populations of different sizes. The program relies on the user to choose between a variety models and allows for estimation of asymmetric migration with as few as two parameters with a standard error of 0.00007[21]. The methodology has also been tested using a real-world data set and was supported by similar findings of another study that did not use MIGRATE[21].

Estimations by the computer programs were also supported by population demographic data calculated from live-trapping surveys by Hanlin that suggested that migration is present and bidirectional at the research site[13]. In the study, Hanlin trapped *P. leucopus* at the Primmer Outdoor Learning Center during the summer months of 2012-2015 to investigate the distribution of white-footed mice between the fencerow and woods habitats[13]. Hanlin's findings suggest that the woods habitat could be a higher quality habitat throughout the entire year as the recapture rate of individuals was higher in the woods habitat[13]. Migration to the fencerow habitat could be explain by the availability of resources in a nearby crop field[13]. Likewise, Nupp and Swihart identified resource availability as a potential driver for migration[1]. Cummings and Vessey found that crop fields can facilitate movement of white-footed mice in summer months as the crops provide overhead cover from predators[2]. In the past studies have found that population densities of *P. leucopus* are negatively correlated with habitat size[1,25]. Nupp and Swihart suggest that this relationship can be affected by the habitat type that surrounds the habitat that the mice occupy[1]. Habitats such as crop fields can act as both barriers and facilitators of dispersal as they provide overhead cover in the summer but do not provide the same protections in the winter and spring[1]. Anderson and Meikle attributed the negative relationship between population size and habitat size to the amount of edge habitat and complexity of understory vegetation[3]. In their study, Anderson

and Meikle suggest that greater understory vegetation complexity provides cover from predators and food resources for the mice[3].

The evidence of bidirectional migration between habitats at Primmer supported our prediction since *P. leucopus* have been shown to disperse relatively well throughout fragmented habitats[30]. The distance between the two trapping grids in my study was approximately 350 m, although it is likely further if the mice are using dispersal routes and not following a straight-line distance. Maier noted that two female mice were found over 6,000 m from where they were originally tagged in a study conducted in Massachusetts[30]. Other studies have also found that mice are capable of migrating between habitats using various dispersal methods[1,2,6]. As supported by Nupp and Swihart, Cummings and Vessey found that agricultural fields can facilitate dispersal of mice by providing cover from predators and food resources[1,2]. Zollner found that the mice have a perceptual ability and are able to migrate nocturnally using a behavior Zollner refers to as "look now move later"[7]. Additionally, the mice are generalists and can survive in a variety of habitats[22]. These studies demonstrate that *P. leucopus* thrive in fragmented landscapes under correct conditions and have the potential to disperse year-round.

Not all genetic or field studies have found that mice are excellent dispersers. Cummings and Vessey suggest that the effects of these landscapes may be seasonal[2]. For example, agricultural fields may act as a barrier to dispersal for much of the year, as they do not provide protection for mice from predation. Though, when crops are planted and begin to grow the mice would be protected from predation by the overhead cover provided by the crops[2]. Rogic noted that highways and bodies of water can act as barriers for dispersal[31]. Recognizing that these barriers are present at the Learning Center, future research should focus on the effect of these landscapes as barriers and as facilitators of dispersal.

Heterozygosity values were comparable to those found in other studies and are indicative of moderate to high genetic variation. Munshi-South found heterozygosity values ranging from 0.625 to 0.819 at fifteen fragmented sites in New York[32]. Like the findings of Munshi-South, the observed heterozygosity values of 0.83 and 0.91 demonstrate a diverse white-footed mouse population that is not in danger of local extinction or inbreeding depression. Rogic also found moderate to high genetic variation in a study of eleven sites in Quebec, reporting observed heterozygosity values ranging from 0.83 to 0.86[31]. Paired with the work of these studies and the work of Anderson and Meikle[3,4,22] our study supports a diverse *P. leucopus* population.

Additionally, estimated inbreeding values of 0.022 suggest that little inbreeding is taking place in the fencerow habitat; an overall inbreeding estimation (F_{IS}) of -0.035 suggests outbreeding. Moreover, a genetic relatedness value (F_{ST}) of 0.024 would suggest that mice in both sites are acting as one genetic population. Munshi-South also found *P. leucopus* populations to be diverse and reported F_{ST} values of 0.071[32]. Rogic also found similar pairwise F_{ST} values ranging from 0 to 0.054[31]. Based on this work, it is expected that the *P. leucopus* populations at the Primmer Outdoor Learning Center will continue to dispersal relatively easily through the prairie and wooded habitats into the future.

Between the habitats are hiking trails open to the researchers on site. Knowing that the mice actively migrate between the two habitats, the trails act as potential sites of interaction. Ticks have also been found on site at Primmer. The interaction could be direct or indirect, as ticks could be transported to the trails by mice where they could then transmit the spirochete to humans. As hosts, mice continue to move asymptomatically when infected, contributing to the spread of infected ticks[12,32]. The literature demonstrates that *P. leucopus* is the most competent reservoir for Lyme disease, and that the potential interactions may pose a threat to human health[33]. White-footed mice act as the most competent reservoir for Lyme disease, and that the prevalence of Lyme disease increases in habitats with low species diversity[8,34].

Often times, habitat fragmentation can be a driver in the loss of species diversity[2]. Though unlike other species, *P. leucopus* has been found to thrive in fragmented habitats and can exhibit a negative population density to area relationship[4]. It is thought that the mice thrive in these fragmented due to the amount of edge habitat; for *P. leucopus*, edge habitat can provide protection and foraging opportunities[5]. Though, where *P. leucopus* thrives, other organisms do not and will leave a fragmented habitat, thus increasing Lyme disease prevalence[8]. Prevalence increases due to the emigration of other species that could carry ticks without becoming reservoirs for Lyme disease[8]. In the absences of these species, *P. leucopus* remain in the habitat as reservoirs for Lyme disease[8]. Rogic indicates that this could cause issues regarding the dispersal of Lyme disease[31]. The migrating mice could carry the disease, thus expanding the geographic range of the disease[8].

Knowing that migration is present at the Primmer Outdoor Learning Center, future work should continue to investigate the significance of the migration between populations. Currently, a study at the Primmer Outdoor Learning Center is testing for Lyme disease in *P. leucopus*. Based on results from GENECLASS2, MIGRATE, GENEPOP, and FSTAT, our study recognizes that migration is present and bidirectional between habitats. These findings support our predictions and demonstrate that future work should focus on studying the migration of *P. leucopus* between other habitats on the property, and expand this work to other sites outside of Primmer in the fragmented agricultural landscape in the Hocking Hills region. Work to expand the dataset should also include investigating other loci for

analysis. Additional samples should be collected at both fencerow and woods sites to increase sample size, and testing for Lyme disease or the presence of ticks on the mice would provide a better prospective into the potential impact of Lyme disease on the site and surrounding community.

5. Conclusion

Ultimately, our findings supported our initial predictions. Based on indirect estimates of dispersal, we found that mice were migrating between two habitats at the Primmer Outdoor Learning Center. Estimates of recent migration and long-term gene flow were the same order of magnitude and both suggest that mice are traveling between habitats. Population differentiation and estimates of genetic structure between populations also support this finding; they showed that the mice are acting as one genetic population at Primmer likely due to the high rates of dispersal between sites. There was also no evidence of inbreeding, and in fact outbreeding seems more likely, and genetic variation within populations indicated no barriers to dispersal. This supports the literature that has found in the past that mice are excellent dispersers in fragmented agricultural landscapes in the Midwest USA. Future work includes additional field work in Summer 2018 to increase the number of samples analyzed to estimate migration between populations and overall population structure, and to test those mice to determine whether they are reservoirs for Lyme disease at the Primmer Outdoor Learning Center.

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