# THE POPULATION GENETICS OF FOUR ANT SPECIES IN A FRAGMENTED URBAN ENVIRONMENT

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## ABSTRACT

Urbanization is a major feature in modern landscapes. As humans progressively develop land, a patchwork environment appears, with wide variation in the degree of modification. Different animal species can survive in each level of this urban gradient, with populations persisting in disjointed patches of suitable habitat. In this study, I evaluated how urbanization in the Philadelphia Metropolitan Area has affected the population genetics of four ant (Formicidae) species of varying urban aptitude. My focal taxa include *Aphaenogaster rudis*, a woodland ant which requires forest habitat, *Camponotus pennsylvanicus* and *Crematogaster cerasi*, two species which both can inhabit edge habitat and nest in rotting wood, and *Nylanderia flavipes*, an invasive species known for saturating urban areas. To investigate how each species was impacted, I used microsatellite genotyping with two analysis methodologies: Bayesian population assignment and landscape resistance modeling. Regarding the latter, I tested four hypotheses concerning the relationships between genetic differentiation and landscape characteristics: 1) isolation-by-distance, 2), major geographic barriers, 3) the urbanization gradient, and 4) a habitat suitability gradient. Overall, I found low genetic structure across the four species, but interspecific variation in how landscape characteristics influence genetic differentiation. My results show that genetic response to urbanization in ants varies greatly depending on species and context, with low predictability based on apparent aptitude for the urban environment.

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# LITERATURE REVIEW

Anthropogenic habitat fragmentation, a pervasive modern issue, poses a serious risk to biodiversity (Elmqvist et al., 2016; Fahrig and Merriam, 1994; Teel et al., 2018; Wilson et al., 2015). One highly common source of anthropogenic habitat fragmentation is urbanization, the transformation of land into a landscape characterized by high human population density and manmade infrastructure, including streets, parking lots, and buildings. While many forms of habitat fragmentation, such as the construction of a single road, simply create a narrow barrier to the dispersal of an animal, urbanization actively replaces large swathes of habitable land with potentially inhospitable landscape (Liu et al., 2016). In this way, the barrier can be wider and more difficult to bypass. Many natural landscape features, including rivers and mountain ranges, also pose similarly challenging obstacles for animal dispersal (Cozzi et al., 2013; Ghaezi et al., 2021). Urbanization may act more like these natural barriers than a road or piece of farmland in terms of the width and inhospitable nature of the obstacle.

The mechanisms by which habitat fragmentation impacts ecosystems are multifold, including the interruption of pollination agents (Aizen and Feinsinger, 1994), the alteration of predator-prey interactions (Kareiva, 1987), and the changing of community compositions (Gibbs and Stanton, 2001; Laurance et al., 1998). In addition to these ecosystem-wide impacts, habitat fragmentation also influences genetic diversity within species. Genetic diversity is important for the continued adaptability and evolution of a species, and low genetic variation in a population carries an increased risk of inbreeding depression and extinction (Hartl and Jones, 2005; Saccheri et al., 1998).

Compared to larger pieces of habitat, small areas of habitat are expected to contain reduced genetic diversity (Frankham, 1996; 1997). Fragmentation of habitat into smaller non-contiguous pieces results in a collection of patches with small effective population sizes. In these populations, deleterious alleles are more likely to drift to fixation, reducing heterozygosity and overall fitness (Hartl and Jones, 2005; Reed and Frankham, 2003; Saccheri et al., 1998). Thus, habitat fragmentation can lead to loss of genetic diversity within a species and potentially result in local extinction. Studies on population genetics of animal species in anthropogenically fragmented habitats consistently find a reduction in gene flow between and genetic diversity within subpopulations, including vertebrate and invertebrate taxa (Bickel et al., 200; Delaney et al, 2010; Knutsen et al., 1999; Munsho-South, 2012; Richardson et al., 2020; Vanhala et al., 2013).

The genetic effects of this fragmentation may be especially accelerated in insects, due to lower dispersal ability and shorter generation lengths (Didham et al. 1996). Moreover, social insects of the order Hymenoptera can be particularly at risk of inbreeding depression in small, fragmented populations due to sex being determined by a single locus (Chapman and Bourke, 2001; Pamilo and Crozier, 1997). Typically, in Hymenoptera, diploid individuals develop into females and haploid individuals develop into males. However, in many social Hymenoptera, diploid individuals homozygous at the sex determination locus also develop into males. However, these individuals are sterile and perform no colony function. As such, in populations with a small effective population size, as can be created in fragmented habitat, a reduction in heterozygosity may imply an increase in the production of diploid males. This squanders colony

resources on individuals which cannot work nor reproduce, thus reducing fitness of the colony overall (Chapman and Bourke, 2001).

Of the social Hymenoptera, ants may be the most successful and ubiquitous. Ants are an essential component of ecosystems, acting as significant predators and scavengers, and possessing an immense biomass presence (Chapman and Bourke, 2001; Ellison et al., 2012; Hölldobler and Wilson, 1994). Furthermore, ants are notable for their ability to create suitable environments for a wealth of other species, including via physical modification of the habitat and through interspecific interactions (Jones et al., 1994). Overall, the reduction or disappearance of ants in fragmented habitats has a high chance of impacting substantial portions of the surrounding biota (Crist, 2008).

Some ant species require undisturbed habitat to survive, while others, particularly a few notable invasive species, manage to thrive inside urbanization (Ellison et al., 2012). The ability to resist the unique challenges of an urban environment, including high heat stress, inconsistent diet, and variable nesting opportunities, allow some ant species to exhibit low population structuring within urbanization (Angilletta et al., 2007; Silverman, 2005; Tsutsui and Case, 2007; Zhang et al., 2019). Species unable to cope with an urban environment are fragmented into subpopulations in less stressful refuges, such as in urban forests and parks (Sabtu and Majid, 2017; Savage et al., 2015). If the urban environment is inhospitable for a particular species of ant, additional methods of travel become important. While worker-caste ants are flightless, males and reproductive females possess wings and thus have the potential to disperse across a barrier (Ellison et al., 2012). Flight ability in ants strongly correlates with body size, which may determine which species can

overfly urbanization to maintain gene flow between fragmented subpopulations (Helms, 2018). Additionally, external factors, such as human-mediated dispersal, may also influence the ability of a species to maintain gene flow in an urbanized habitat (Khimoun et al., 2020).

# ARTICLE

## Introduction

Over the past 200 years, urban areas worldwide have expanded at a rapid rate. Between 1800 and 2019, the percentage of the global human population living in urban areas rose from less than 10% to over 50% (Ritchie and Roser, 2019). To accommodate these populations, as of 2007, urban areas covered ~3.5 million $km^2$, or ~3% of Earth's land surface, with an increase of as much as 12.5 million $km^2$ expected by 2030 (Hooke et al., 2012; Liu et al, 2014; Seto et al., 2011). Additionally, as human populations rise, urbanization increasingly displaces areas once used for agriculture (Döös, 2002). By 2030, as much as 450,000 $km^2$ of arable land, around the size of the state of California, may be replaced by urban development worldwide.

The urban environment comprises a highly developed core surrounded by a continuous gradient of suburbia, urban fringe, and rural areas (McKinney, 2002; Pickett, 2001; Sukopp and Werner, 1982). This creates a complex mosaic of different habitats, with impervious land cover ranging widely from over 50% to less than 20%. As defined in McKinney, 2002, this patchwork can be broken into four major categories. In order of decreasing human influence, these categories are:

1. Built habitat: buildings and sealed surfaces, such as roads
2. Managed vegetation: residential, commercial, and other regularly maintained green spaces
3. Ruderal vegetation: empty lots, abandoned farmland, and other green space that is cleared but not managed

4. Natural remnant vegetation: remaining islands of original vegetation

As the proportion of land covered by impervious surfaces increases along the urban gradient, the number and size of unaltered habitat patches declines (Collins et al, 2000). Thus, the number and size of habitat patches available to a species depends upon which components of the urban mosaic that species can utilize. Applying the terminology used in Blair, 2001, animal species surviving in the urban mosaic fall into three groups: "urban avoiders", "urban adapters", and "urban exploiters". Urban avoiders are the most susceptible to habitat loss and fragmentation in an urbanized environment, due to active persecution by humans or an inability to effectively utilize anthropogenic resources. Thus, urban avoiders typically only persist in natural remnant vegetation. Notable examples of urban avoiders include large predator species, such as the cougar *(Puma concolor),* and species which rely on specific forest habitat, such as many insectivorous birds (Beissinger and Osborne, 1982; Lambert et al., 2010). Distinct from urban avoiders, urban adapters can, facultatively, utilize anthropogenic resources. Often, urban adapter species are edge-habitat specialists which thrive in natural remnant vegetation but survive in ruderal and managed vegetation. Urban adapters include small and medium-sized omnivorous mammalian scavengers, such as the Virginia opossum (*Didelphis virginiana*) and the common raccoon (*Procyon lotor*), and small omnivorous birds such as the American robin (*Turdus migratorius*) (VanDruff and Rowse, 1986). Lastly, urban exploiters are those animals that primarily or completely rely upon anthropogenic resources. These species thrive in the urban environment, typically preferring built habitat over any kind of vegetation. Often, these are well-known non-native and invasive

species which follow human development across the planet. Widespread examples of urban exploiters include brown rats (*Rattus norvegicus*) and German cockroaches (*Blattella germanica*) (Feng and Himsworth, 2013; Tang et al., 2019).

Urban avoiders, adapters, and exploiters are found across a wide range of taxa. Examples of all three groups can be found amongst species of Polish bees, Californian butterflies, Arizonan lizards, and Ohioan mammals (Banaszak-Cibicka and Żmihorski, 2012; Blair and Launer, 1997; Germaine and Wakeling, 2001; Riem et al., 2012). However, a good example of species-dependent urban survival ability can be found in ants (Formicidae). Following a similar pattern to other taxa, most ant species avoid highly developed areas of the urban gradient, while a prominent minority thrive in human infrastructure (Silverman, 2005). The urban mosaic presents a unique set of pressures for ants, including high heat stress, inconsistent diet, and variable nesting opportunities, to which ant species have a wide range of responses (Angilletta et al., 2007; Silverman, 2005). Many of the most well-known invasive and pest ant species, such as *Linepithema humile*, *Monomorium pharoensis*, and *Solenopsis invicta,* regularly take advantage of anthropogenic resources in urban areas, and could be considered urban exploiters (Santos, 2016). Urban exploiter ants typically share a few key characteristics that allow them to take advantage of an urban environment, including omnivory, polydomy, polygyny, and the ability to disperse via human activity (Blumenfeld et al., 2021; Silverman, 2005). Depending on the region, species, and timescale involved, the effects of urbanization on overall ant biodiversity can range from an increase in species richness due to the introduction of non-native species, to a slight reduction in species richness, to

the permanent loss of 85% of species (Abdel-Dayem et al., 2022; Buczkowski and Richmond, 2012; Rocha-Ortega and Castaño-Meneses, 2015; Perez and Diamond, 2019). Overall, the effects of urbanization on ant biodiversity appear to be highly variable, thus emphasizing the importance of continued research.

If an ant species cannot survive in built habitat, any remaining patches of managed, ruderal, and natural remnant vegetation in the area may provide refuges (McKinney, 2002; Savage et al., 2015). These plots of vegetated land constitute a highly fragmented habitat, with many small habitat patches containing relatively low effective population sizes (Hartl and Jones, 2005). Genetically, this implies an increased likelihood of alleles drifting to fixation, reducing population heterozygosity. As such, compared to larger pieces of habitat, small patches are expected to contain reduced genetic diversity (Frankham, 1996; 1997). Genetic diversity allows the continued adaptability and evolution of a species, and low genetic variation in a population carries an increased risk of reduced fitness due to deleterious alleles becoming fixed (Hartl and Jones, 2005; Reed and Frankham, 2003; Saccheri et al., 1998). This can result in local extinction. Many social Hymenoptera, including ants, face particular risk of inbreeding depression in these scenarios due to sex being determined by a single locus (Chapman and Bourke, 2001; Pamilo and Crozier, 1997). In Hymenopterans, diploid individuals typically develop into females and haploid individuals into males. However, in many social Hymenoptera, diploid individuals homozygous at the sex determination locus also develop into sterile males serving no colony function. As such, a reduction in heterozygosity may imply an increase in the production of diploid males. This squanders colony resources on

individuals which cannot work nor reproduce, thus reducing fitness of the overall colony (Chapman and Bourke, 2001).

The ability of some urban exploiter ant species to survive in built habitat, including *Tapinoma sessile*, *Linepithema humile,* and *Tetramorium immigrans*, allows these species to exhibit low population structuring within urbanization, even across large distances (Blumenfeld et al., 2021; Tsutsui and Case, 2001; Zhang et al., 2019). Urban adapter species, such as *Formica japonica*, survive in vegetated areas in cities but still maintain genetic connectivity, possibly due to high dispersal ability (Yamamoto et al., 2013). Meanwhile, other ant species which have more specific habitat requirements, for example the arboreal-nesting *Tetraponera rufonigra*, may not be able to travel between vegetated habitat patches and thus show higher levels of population differentiation (Sabtu and Majid, 2017). Depending on the species, human-mediated dispersal may also play a large role in maintaining genetic connectivity (Khimoun et al., 2020).

In this study, I examined the population genetics of four ant species, including urban avoider, adapter, and exploiter species. I evaluated how the aptitude of a species for the urban environment influences genetic diversity and structure, and assessed the influence of landscape characteristics on genetic patterns. As ant urban aptitude decreases, I expect decreasing genetic diversity, increasing genetic structure, and increasing association between genetic patterns and the urban landscape, due to an inability of some ant species to disperse effectively across urbanized environments. I evaluated population genetic patterns based on microsatellite genotyping of ants collected

from the fragmented urban environment in and around Philadelphia, PA, and unfragmented forest environment in Lancaster County, PA.

## Methodology

### Study species

Four focal ant species were selected, representing a range of urban tolerances (Figure 1). *Aphaenogaster rudis*, a woodland ant which requires ample leaf litter and woody debris in which to forage and nest, exemplifies an urban avoider (Lubertazzi, 2011; Pećarević et al., 2010; Savage et al., 2015). Representing urban adapters are *Camponotus pennsylvanicus* and *Crematogaster cerasi.* These species are more tolerant of edge habitat and nest in rotting wood, thus enabling them to inhabit wooden buildings and survive in moderate urbanization (Ellison et al., 2012; Enzmann, 1946; Pećarević et al., 2010). Finally, *Nylanderia flavipes*, an invasive species known for saturating urban areas, serves as our urban exploiter species (Pećarević et al., 2010; Savage et al., 2015; Smith, 1874).


**Figure 1:**
*From left to right:* Aphaenogaster rudis, Camponotus pennsylvanicus, Crematogaster cerasi, *and* Nylanderia flavipes.

## Study regions and sampling

This study was conducted during the summer of 2022 and 2023 in two major regions: the fragmented urban and suburban landscape of the Philadelphia metropolitan area in Pennsylvania and New Jersey, and the relatively contiguous forest habitat of Pennsylvania Game Lands Number 156 and surrounding land in Lancaster County, PA (Table 1; Figure 2). Ants were collected at 14 study locations, which ranged in size from 0.06 to 1.15 $km^2$. At each study location, 19-48 bait stations were established with a spacing of 100-200 m, to avoid multiple bait stations attracting ants from the same colony (Ellison et al., 2012). Each bait station consisted of a 3" × 5" notecard with an eighth of a crushed Keebler® Sandies® Pecan Shortbread cookie and ~2 g wet cat food. This combination of baits was chosen to attract a range of ant species by providing a variety of proteins, fats, and sugars (Ellison et al., 2012). After one hour, any ants attending the bait were collected via aspiration and stored in 95% ethanol. Ant genera and species were identified via Ellison et al. 2012 dichotomous keys.

**Table 1:**
*Study location names, ID numbers, sampling parameters, and sampling permissions contacts.*

| Study location | Location ID | Sampled area (km^2) | Number of bait stations | Location contact |
|---|---|---|---|---|
| Amico Island Park | 1 | 0.10 | 31 | Jennifer Bulava (jbulava@co.burlington.nj.us) |
| Boundary Creek Natural Resource Area | 2 | 0.07 | 21 | Jennifer Bulava (jbulava@co.burlington.nj.us) |
| FDR Park | 3 | 0.45 | 30 | Susan Buck (Susan.Buck@phila.gov) |
| Mount Vernon Cemetery | 4 | 0.06 | 21 | Dustin Houck (dustin@phlcdc.org) |
| Palmyra Cove Nature Park | 5 | 0.31 | 20 | John Moore (jmoore@bcbridges.org) |
| Pennypack Ecological Restoration Trust | 6 | 1.15 | 48 | Maria Mugnani (MPMugnani@pennypacktrust.org) |
| Pennypack Park | 7 | 0.86 | 41 | Susan Buck (Susan.Buck@phila.gov) |
| Tacony Creek Park | 8 | 0.13 | 30 | Susan Buck (Susan.Buck@phila.gov) |
| West Deptford Nature Trail | 9 | 0.13 | 19 | Bill Gigliotti (bgigliotti@westdeptford.com) |
| Pennsylvania State Game Lands 145 | 10 | 0.71 | 30 | Alexander Murray (alemurray@pa.gov) |
| Pennsylvania State Game Lands 156, Location A | 11 | 0.43 | 29 | Alexander Murray (alemurray@pa.gov) |
| Pennsylvania State Game Lands 156, Location B | 12 | 0.99 | 30 | Alexander Murray (alemurray@pa.gov) |
| Pennsylvania State Game Lands 156, Location C | 13 | 0.50 | 30 | Alexander Murray (alemurray@pa.gov) |
| Pennsylvania State Game Lands 156, Location D | 14 | 0.20 | 30 | Alexander Murray (alemurray@pa.gov) |

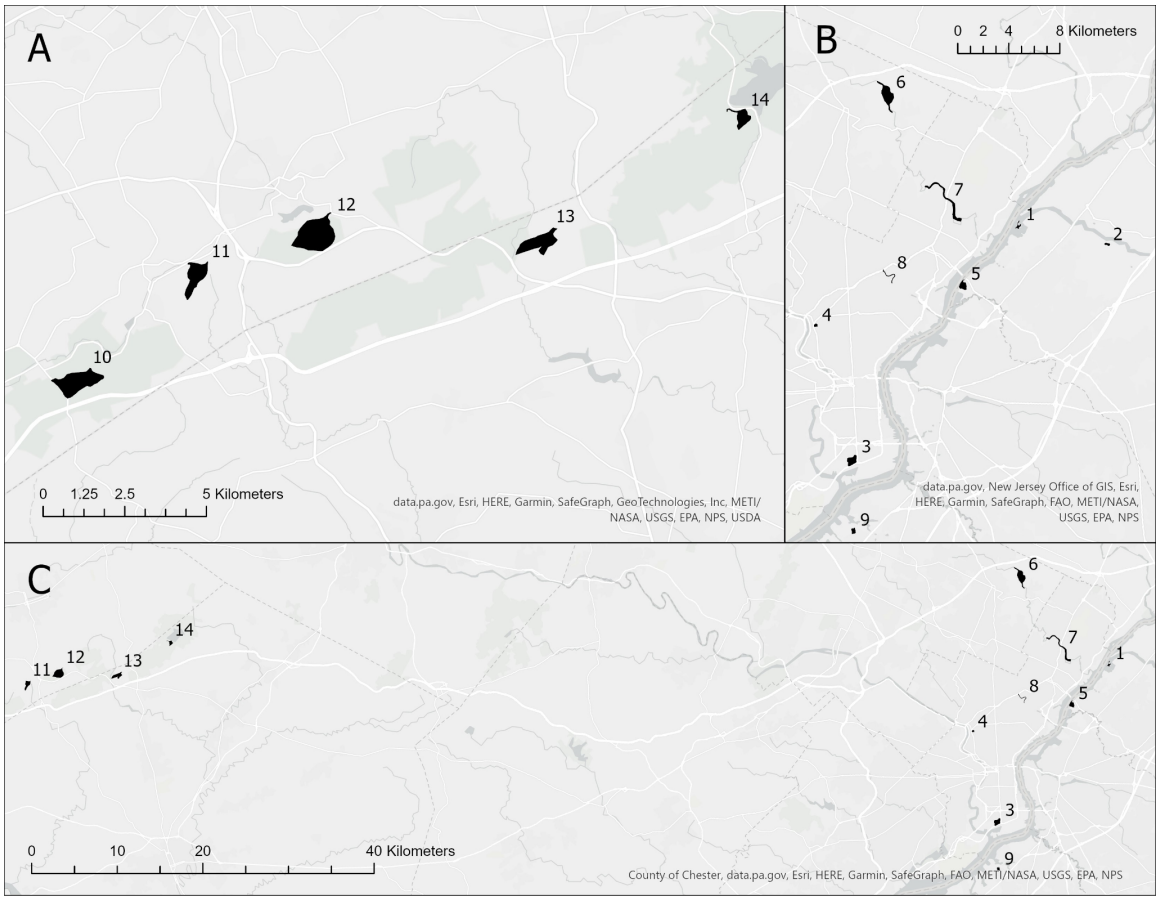


**Figure 2:**
*Study locations in A) contiguous forest habitat, B) the Philadelphia metropolitan area, and C) the relative positions of the two study regions. The location ID number key can be found in Table 1.*

## Genotyping

Nuclear DNA was extracted from one whole ant per focal species per bait station using a Chelex 100 chelating resin methodology (Walsh et al., 1991). Genotypes were assembled using four microsatellite loci for *A. rudis*, *C. pennsylvanicus*, and *N. flavipes,* and five for *C. cerasi* (Table 2). Multiplex polymerase chain reactions (PCRs) were performed with a final volume of 10 μL, containing 4.84 μL $H_2O$, 1.00 μL 10 x reaction buffer, 0.75 μL deoxynucleotide triphosphate mixture, 0.4 μL $MgCl_2$, 0.5 μL of 10 μM

| Species | Locus | Tag | Forward primer sequence (5'-3') | Reverse primer sequence (5'-3') | Number of individuals | Allele size range (bp) | Number of alleles | Ho | He | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| *Aphaenogaster rudis* | L18 | VIC | TGA ATT TGG ATG GCG GTA GAC | ACC TAA TGC ACG CTT TAG AAT | 157 | 132-179 | 25 | 0.70 | 0.92 | Foitzik et al., 1997 |
| *Aphaenogaster rudis* | Myrt3 | NED | GAG CAC GTC GCT ATC TCT GAA TAT G | GAG TAC ATA CAT ACG CTA CGG GCA | 161 | 189-249 | 24 | 0.72 | 0.90 | Bourke et al., 1997 |
| *Aphaenogaster rudis* | Pb8 | VIC | GAG ATG GGC AAG GAA CAG GAC | GGA AGA ATC TGC GGA GTG C | 148 | 284-298 | 8 | 0.67 | 0.77 | Volny and Gordon, 2002 |
| *Aphaenogaster rudis* | C20 | NED | GCA CAC ACG CAT ACG AGA CAC | GAC AGT CAA CAA TCG ACA ATC | 146 | 187-192 | 6 | 0.90 | 0.75 | Heinze et al., 2000 |
| *Camponotus pennsylvanicus* | Cru01 | PET | AGG CAA AAT CGA TGG TGT A | TGT TTT CAT GCT TAT TCA AGG | 36 | 238-249 | 6 | 0.78 | 0.73 | Azevedo-Silva et al., 2015 |
| *Camponotus pennsylvanicus* | LF02 | VIC | AAC CCG ATT TAA TGA CGC | CTT CGA GTA TGA TAG TTC ATC G | 42 | 104-108 | 3 | 0.50 | 0.56 | Falk et al., 1997 |
| *Camponotus pennsylvanicus* | Cgg01 | NED | CAT AAA TAA TTG ACG GAG AA | AAT GTG GCT TTA ATC TGT CT | 36 | 178-234 | 14 | 0.50 | 0.77 | Azevedo-Silva et al., 2015 |
| *Camponotus pennsylvanicus* | Cru02 | PET | GTA TCG GGG ACG TCA CCT TTT G | ACG CGT TGC CGG AAG AGA A | 44 | 154-170 | 3 | 0.39 | 0.43 | Azevedo-Silva et al., 2015 |
| *Crematogaster cerasi* | LxAGT1 | 6-FAM | GTG GCG ACC AAT TCT GCA AG | GCA GGA CCA GCA TCA AAT GAC AG | 36 | 141-177 | 17 | 0.86 | 0.93 | Bourke et al., 1997 |
| *Crematogaster cerasi* | RRB476 | PET | CAT TGA TGG TGG AAC ATT TCA C | TGT TGG ATC AAT CGG CAT TC | 33 | 265-284 | 9 | 0.38 | 0.76 | Rubin et al., 2009 |
| *Crematogaster cerasi* | Myrt3 | NED | GAG CAC GTC GCT ATC TCT GAA TAT G | GAG TAC ATA CAT ACG CTA CGG GCA | 36 | 161-167 | 5 | 0.61 | 0.65 | Bourke et al., 1997 |
| *Crematogaster cerasi* | RRB470 | 6-FAM | GGA ACG AAC GAG CCG ATG | CGA GAG CGC GAA CCA AG | 36 | 196-201 | 6 | 0.47 | 0.60 | Rubin et al., 2009 |
| *Crematogaster cerasi* | L5 | PET | CCC ACG GTA ACC CTC GAG AAC | AGC GGA GAA ACA GGC GAG AAA | 36 | 111-146 | 18 | 0.89 | 0.91 | Foitzik et al., 1997 |
| *Nylanderia flavipes* | Po3 | 6-FAM | ATT GCT TTC CCA CTT GAC C | TGA AGA GCG ATA GGC AGA GT | 172 | 181-218 | 16 | 0.85 | 0.90 | Wiernasz et al., 2004 |
| *Nylanderia flavipes* | LF02 | VIC | AAC CCG ATT TAA TGA CGC | CTT CGA GTA TGA TAG TTC ATC G | 172 | 115-121 | 4 | 0.10 | 0.13 | Falk et al., 1997 |
| *Nylanderia flavipes* | P22 | 6-FAM | TTG CTG GAA CAC GTA TCA CAC | GTC GAC TCG GAT AGT GGG AG | 170 | 142-148 | 4 | 0.35 | 0.36 | Trontti and Sundström, 2003 |
| *Nylanderia flavipes* | Po8 | VIC | ACC ACC AAC CTC ATT ACG A | GCT CAG CAT ACT GTT CTC CA | 169 | 198-204 | 3 | 0.40 | 0.37 | Wiernasz et al., 2004 |
| *Aphaenogaster* population 1 | L18 | VIC | TGA ATT TGG ATG GCG GTA GAC | ACC TAA TGC ACG CTT TAG AAT | 53 | 135-157 | 11 | 0.77 | 0.88 | Foitzik et al., 1997 |
| *Aphaenogaster* population 1 | Myrt3 | NED | GAG CAC GTC GCT ATC TCT GAA TAT G | GAG TAC ATA CAT ACG CTA CGG GCA | 53 | 198-241 | 15 | 0.64 | 0.80 | Bourke et al., 1997 |
| *Aphaenogaster* population 1 | Pb8 | VIC | GAG ATG GGC AAG GAA CAG GAC | GGA AGA ATC TGC GGA GTG C | 47 | 288-292 | 3 | 0.57 | 0.63 | Volny and Gordon, 2002 |
| *Aphaenogaster* population 1 | C20 | NED | GCA CAC ACG CAT ACG AGA CAC | GAC AGT CAA CAA TCG ACA ATC | 47 | 188-192 | 3 | 1.00 | 0.52 | Heinze et al., 2000 |
| *Aphaenogaster* population 2 | L18 | VIC | TGA ATT TGG ATG GCG GTA GAC | ACC TAA TGC ACG CTT TAG AAT | 47 | 132-141 | 7 | 0.66 | 0.72 | Foitzik et al., 1997 |
| *Aphaenogaster* population 2 | Myrt3 | NED | GAG CAC GTC GCT ATC TCT GAA TAT G | GAG TAC ATA CAT ACG CTA CGG GCA | 50 | 200-225 | 9 | 0.68 | 0.78 | Bourke et al., 1997 |
| *Aphaenogaster* population 2 | Pb8 | VIC | GAG ATG GGC AAG GAA CAG GAC | GGA AGA ATC TGC GGA GTG C | 46 | 284-292 | 6 | 0.72 | 0.79 | Volny and Gordon, 2002 |
| *Aphaenogaster* population 2 | C20 | NED | GCA CAC ACG CAT ACG AGA CAC | GAC AGT CAA CAA TCG ACA ATC | 46 | 188-192 | 5 | 0.96 | 0.62 | Heinze et al., 2000 |
| *Aphaenogaster* population 3 | L18 | VIC | TGA ATT TGG ATG GCG GTA GAC | ACC TAA TGC ACG CTT TAG AAT | 57 | 133-179 | 23 | 0.68 | 0.93 | Foitzik et al., 1997 |
| *Aphaenogaster* population 3 | Myrt3 | NED | GAG CAC GTC GCT ATC TCT GAA TAT G | GAG TAC ATA CAT ACG CTA CGG GCA | 58 | 189-249 | 17 | 0.83 | 0.89 | Bourke et al., 1997 |
| *Aphaenogaster* population 3 | Pb8 | VIC | GAG ATG GGC AAG GAA CAG GAC | GGA AGA ATC TGC GGA GTG C | 55 | 284-298 | 7 | 0.66 | 0.71 | Volny and Gordon, 2002 |
| *Aphaenogaster* population 3 | C20 | NED | GCA CAC ACG CAT ACG AGA CAC | GAC AGT CAA CAA TCG ACA ATC | 53 | 187-192 | 6 | 0.72 | 0.63 | Heinze et al., 2000 |

fluorescently tagged forward microsatellite primer mixture, 0.5 μL of 10 μM reverse microsatellite primer mixture, and 0.05 μL *Taq* DNA polymerase (5 units/μL). Thermal cycling started with a 6-minute denaturization at 96°C, followed by 37 annealing cycles (94°C for 30 seconds, 50°C for 30 seconds, 72°C for one minute and 30 seconds), and finishing with 10 minutes at 72°C. A microliter of the amplified product was mixed with 10.45 μL of HIDI formamide buffer and 0.55 μL of 600 LIZ and analyzed by an Agilant Bioanalyzer 2100 at the Vermont Integrative Genomics Resource at the University of Vermont. Alleles were scored manually using GeneMarker® V3.0.1.

**Hardy–Weinberg equilibrium, genetic diversity, and STRUCTURE analyses**

For each species, locus, and study location, conformity with Hardy-Weinberg equilibrium (HWE) was evaluated using Markov chain approximation exact tests in Genepop 4.7.5., with 1000 dememorizations, 100 batches, and 1000 iterations per batch (Raymond and Rousset, 1995; Rousset, 2008). I used GenAIEx 6.503 and the R package 'mmod' 1.3.3 to determine observed allelic richness, observed heterozygosity, and expected heterozygosity (Peakall and Smouse, 2006; 2012; Winter et al., 2022). Differences in genetic diversity between urban and forested areas were assessed at both regional and locational scales with Welch's t-tests, using allelic richness values estimated with rarefaction in the program HP-Rare (Kalinowski, 2004; 2005). Only study regions or locations with a sample size ≥4 were included in rarefaction. Estimated allelic richness values averaged across loci were also applied to test the relationship between genetic diversity and habitat area, excluding study locations in the unfragmented region, using simple linear regressions (Table 3).

**Table 3:**
*Total geographic areas of study locations, excluding the unfragmented region.*

| Study Location | Location area (km^2) | Reference |
|---|---|---|
| Amico Island Park | 0.22 | Burlington County Parks System, 2020a |
| Boundary Creek Natural Resource Area | 0.14 | Burlington County Parks System, 2020b |
| FDR Park | 1.41 | Fairmount Park Conservancy, 2023 |
| Mount Vernon Cemetery | 0.09 | Mt. Vernon Cemetery Conservation Company, Inc., n.d. |
| Palmyra Cove Nature Park | 1.01 | Palmyra Cove Nature Park, 2019 |
| Pennypack Ecological Restoration Trust | 3.45 | Pennypack Ecological Restoration Trust, 2023 |
| Pennypack Park | 7.08 | Natural Lands Restoration and Environmental Education Program, 1999b |
| Tacony Creek Park | 1.22 | Natural Lands Restoration and Environmental Education Program, 1999a |
| West Deptford Nature Trail | 0.36 | Rails-to-Trails Conservancy. n.d. |

Inference of population structure was performed using Bayesian population assignment in the program STRUCTURE 2.3.4 (Pritchard et al., 2000; Falush et al., 2003, 2007; Hubisz et al., 2009). The most likely number of clusters (K) was determined using an ad hoc quantity based on the second order rate of change of the likelihood (ΔK), as described in Evanno et al., 2005. For all focal species, K-values from 1-14 were run 20 times each using the admixed ancestry model, correlated allele frequencies, a burn-in period of 10,000, and 50,000 Markov chain Monte Carlo (MCMC) iterations. Once the most likely value of K was determined, a final run was conducted using the same parameters.

**Genetic differentiation and landscape resistance**

Genetic differentiation between study locations was assessed using Nei's GST, a multiallelic extension of Wright's fixation index (FST), calculated in the R package 'mmod' 1.3.3 (Nei, 1973; Winter et al., 2022). For each species, GST was estimated pairwise across all loci between all combinations of locations.

I tested four models concerning the relationship between genetic differentiation and geographical characteristics (Cushman et al., 2006):

1. Isolation-by-distance: Assumes that genetic differentiation is purely a function of geographic distance.
2. Putative major barrier: Assumes that genetic differentiation is determined by the interaction of geographic distance and a small number of highly significant physical barriers.
3. Urbanization gradient: Assumes that genetic differentiation is determined by the interaction of geographic distance and the magnitude of urbanization.
4. Habitat gradient: Assumes that genetic differentiation is determined by the interaction of geographic distance and the varying suitability of land cover types as habitat.

Thus, for each of the four focal species, four pairwise resistance-distance values were calculated for all combinations of locations using the program CIRCUITSCAPE 4.0.5 (McRae et al., 2008). Calculation of resistance-distance values utilizes circuit theory to generate least-cost paths on a map of landscape features with varying resistance values McRae, 2006). This allowed pairwise resistance-distance values to be compared with pairwise Nei's GST values to evaluate my four models.

Seven maps were created to generate resistance-distance values: an isolation-by-distance map, a putative major barrier map, an urbanization map, and a habitat gradient map for each of the four focal species. Resistance maps were created in ArcGIS Pro 2.6.0 using 30 m resolution 2021 land cover data from the National Land

Cover Database (NLCD; Dewitz, 2023). For my study regions, 15 of the 20 NLCD land cover classes were present and utilized: 1) “Open Water”, 2) “Developed, Open Space” (> 20% impervious cover), 3) “Developed, Low Intensity” (20-49% impervious cover), 4) “Developed, Medium Intensity” (50-79% impervious cover), 5) “Developed, High Intensity” (80-100% impervious cover), 6) “Barren Land” (Rock/Sand/Clay), 7) “Deciduous Forest”, 8) “Evergreen Forest”, 9) “Mixed Forest”, 10) “Shrub/Scrub”, 11) “Grassland/Herbaceous”, 12) “Pasture/Hay”, 13) “Cultivated Crops”, 14) “Woody Wetlands”, and 15) “Emergent Herbaceous Wetlands”. The original NLCD raster data was converted to resistance maps by coding a resistance value to each land cover class on a 1-9 scale. The isolation-by-distance map was created by assigning all land cover classes the minimum resistance value, thus allowing distance to be the sole determining variable (Table 4). For the putative major barrier map, I assumed the Delaware River and associated tributaries as the most distinct barriers. Thus, “Open Water” was assigned the maximum resistance value while all other classes were given minimum resistance. The urbanization gradient map was created by coding the land cover classes “Developed, Open Space”, “Developed, Low Intensity”, “Developed, Medium Intensity”, and “Developed, High Intensity” resistance values of 3, 5, 7, and 9, respectively, and all other classes the minimum value. For the habitat gradient maps, land cover classes were assigned to four discrete categories based on suitability as a nesting site; 1= no nesting resistance, 3 = weak nesting resistance, 7 = medium nesting resistance, and 9 = high nesting resistance (Jha and Kremen, 2013). Categorization of land cover classes into the

four levels of nesting resistance was based on the specific nesting requirements of the species in question.

**Table 4:**
*Land cover class resistance values in each of the seven resistance maps.*

| Land cover class | Isolation-by-distance | Putative major barrier | Urbanization gradient | *A. rudis* habitat gradient | *C. pennsylvanicus* habitat gradient | *C. cerasi* habitat gradient | *N. flavipes* habitat gradient |
|---|---|---|---|---|---|---|---|
| Open Water | 1 | 9 | 1 | 9 | 9 | 9 | 9 |
| Developed, Open Space | 1 | 1 | 3 | 3 | 1 | 1 | 1 |
| Developed, Low Intensity | 1 | 1 | 5 | 7 | 3 | 3 | 1 |
| Developed, Medium Intensity | 1 | 1 | 7 | 9 | 7 | 7 | 3 |
| Developed, High Intensity | 1 | 1 | 9 | 9 | 9 | 9 | 7 |
| Barren Land | 1 | 1 | 1 | 9 | 9 | 9 | 9 |
| Deciduous Forest | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Evergreen Forest | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Mixed Forest | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Shrub/Scrub | 1 | 1 | 1 | 3 | 3 | 1 | 1 |
| Grassland/Herbaceous | 1 | 1 | 1 | 7 | 7 | 3 | 1 |
| Pasture/Hay | 1 | 1 | 1 | 9 | 7 | 7 | 1 |
| Cultivated Crops | 1 | 1 | 1 | 9 | 7 | 7 | 1 |
| Woody Wetlands | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Emergent Herbaceous Wetlands | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

Mantel tests, calculated using the R package ‘ecodist’ 2.0.9, were used to evaluate the relationship between Nei’s GST and resistance distance for each model (Goslee and Urban, 2022). In determining the relative support for each of the comparisons, I utilized Pearson correlation coefficients ($r$), with the 95% confidence limits estimated using 500 bootstrap repetitions and a resampling level of 0.9.

## Results

### Hardy-Weinberg equilibrium, genetic diversity, and STRUCTURE analyses

All *A. rudis* loci were significantly out of HWE at six (L18), five (Myrt3), three (Pb8), or nine (C20) study locations. One *C. cerasi* locus showed no significant deviation

from HWE at any location (LxAGT1), with all other loci significantly out of HWE at one but separate locations (RRB476, Myrt3, RRB570, and L5). Loci for *C. pennsylvanicus* mostly exhibited no significant deviation from HWE at any location (Cru01, LF02, and Cru02), with one locus deviating from HWE significantly at one location (Cgg01). Two *N. flavipes* loci were not significantly out of HWE at any location (LF02 and P22), and two showed significant deviance at one (P08) or two (Po3) locations. For *A. rudis*, *C. pennsylvanicus*, *C. cerasi*, and *N. flavipes*, respectively, average expected heterozygosity across all loci was 0.84, 0.62, 0.77, and 0.44, and average observed allelic richness was 15.75, 6.5, 11, and 6.75 (Table 2). No species exhibited significant differences in estimated allelic richness between urban and forested areas at regional or locational scales, or a significant correlation between estimated allelic richness and study location area (Table 5; 6; 7).

**Table 5:**
*T-test results, assessing differences in allelic richness between urban and forested locations for each focal species and* Aphaenogaster *subpopulation, including the t-statistic (t) degrees of freedom (Df), and P-value. Species and* Aphaenogaster *subpopulations lacking any study locations with a sample size ≥4 for either the urban or forested categories were excluded from analysis.*

| Species | *t* | Df | P-value |
|---|---|---|---|
| *Nylanderia flavipes* | 0.597 | 34.561 | 0.554 |
| *Aphaenogaster rudis* | 0.318 | 33.444 | 0.752 |
| *Camponotus pennsylvanicus* | -0.236 | 16.508 | 0.816 |
| *Aphaenogaster* population 2 | 1.416 | 9.317 | 0.189 |

**Table 6:**
*T-test results, assessing differences in allelic richness between urban and forested regions for each focal species and* Aphaenogaster *subpopulation, including the t-statistic (t) degrees of freedom (Df), and P-value. Species and* Aphaenogaster *subpopulations possessing a sample size <4 for either the urban or forested regions were excluded from analysis.*

| Species | *t* | Df | P-value |
|---|---|---|---|
| *Nylanderia flavipes* | -0.168 | 5.996 | 0.872 |
| *Aphaenogaster rudis* | 0.060 | 5.994 | 0.954 |
| *Crematogaster cerasi* | 0.426 | 5.676 | 0.686 |
| *Camponotus pennsylvanicus* | -0.212 | 4.838 | 0.841 |
| *Aphaenogaster* population 2 | -0.178 | 5.978 | 0.865 |

**Table 7:**
*Linear regression results testing allelic richness as a function of habitat area for each focal species and* Aphaenogaster *subpopulation, including the coefficient of determination (R^2), degrees of freedom (Df), F-statistic (F), and P-value.*

| Species | R^2 | Df | *F* | P-value |
|---|---|---|---|---|
| *Nylanderia flavipes* | 0.014 | 1, 7 | 0.101 | 0.760 |
| *Aphaenogaster rudis* | 0.112 | 1, 7 | 0.881 | 0.379 |
| *Crematogaster cerasi* | 0.001 | 1, 5 | 0.003 | 0.957 |
| *Camponotus pennsylvanicus* | 0.185 | 1, 4 | 0.906 | 0.395 |
| *Aphaenogaster* population 1 | 0.034 | 1, 7 | 0.249 | 0.633 |
| *Aphaenogaster* population 2 | 0.179 | 1, 6 | 1.309 | 0.296 |
| *Aphaenogaster* population 3 | 0.727 | 1, 1 | 2.667 | 0.350 |

Analysis using STRUCTURE and ΔK calculations suggested a most-likely K-value of 2, 4, and 3 for *C. pennsylvanicus*, *C. cerasi*, and *N. flavipes*, respectively. However, all individuals of these species were assigned approximately equal likelihoods for all clusters. As per Pritchard et al. 2010, this indicates a lack of genetic structuring present across the study regions. For *A. rudis*, ΔK calculations suggested 3 distinct clusters, with most individuals assigned over 90% likelihood to one cluster (Figure 3). The first cluster centers on the fragmented urban environment in and around Philadelphia, the third on the unfragmented forest habitat to the west, predominantly

Pennsylvania State Game Lands 156 Locations A-D, and the second cluster was distributed across both regions (Figure 4).

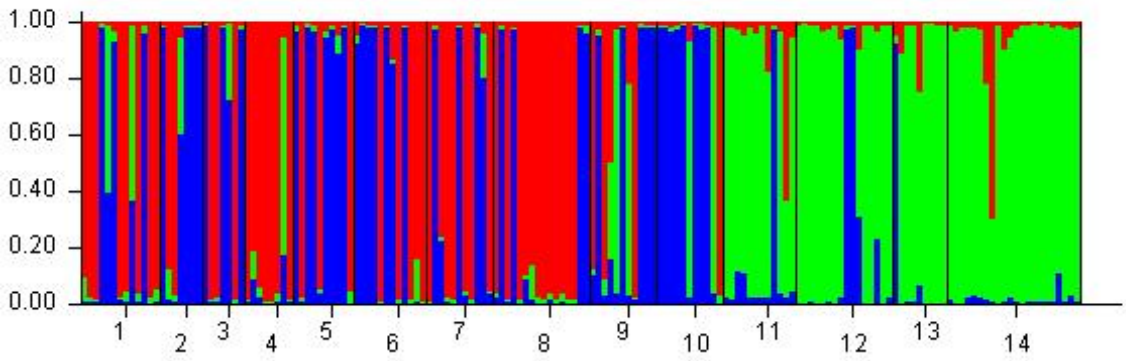


**Figure 3:**
*Population structure of* Aphaenogaster rudis*, assessed using Bayesian population assignment at K=3 in the program STRUCTURE. Each vertical bar represents an individual, with colors illustrating the relative likelihood of each cluster. Red = population 1, blue = population 2, and green = population 3. X-axis values correspond to location ID numbers found in Table 1.*

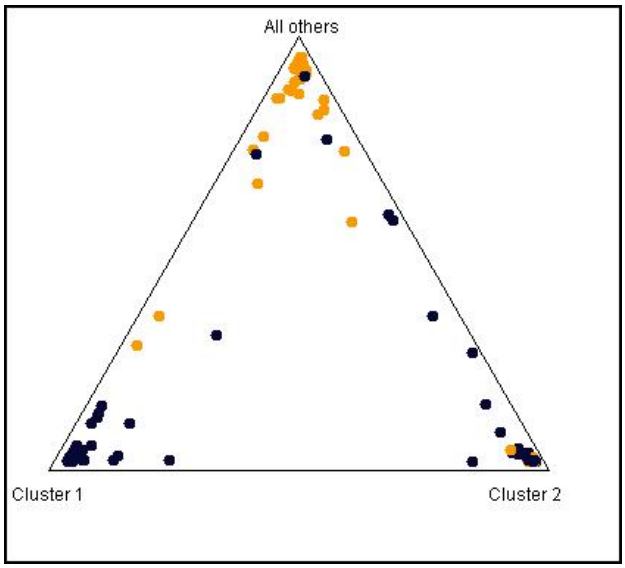


**Figure 4:**
*Population structure of* Aphaenogaster rudis*, assessed using Bayesian population assignment at K=3 in the program STRUCTURE. Each point represents an individual, with proximity to each vertex representing proportions of admixture (Cluster 1 =* Aphaenogaster *population 1, Cluster 2 =* Aphaenogaster *population 2, All others =* Aphaenogaster *population 3). Black points represent individuals sourced from an urban study location, while orange points represent individuals sourced from unfragmented forest study locations.*

To further evaluate the effects of urbanization on *Aphaenogaster rudis*, I split the genotype dataset into three subsets, with individuals sorted based on the most likely cluster assigned by STRUCTURE. Only individuals possessing ≥90% assignment to one of the three populations were sorted. Henceforth, these subsets will be identified as *Aphaenogaster* population 1, 2 and 3. All previously described Hardy–Weinberg equilibrium, genetic diversity, STRUCTURE, genetic differentiation, and landscape resistance analyses conducted upon the original *Aphaenogaster* dataset were reapplied to *Aphaenogaster* populations 1, 2 and 3.

*Aphaenogaster* population 1 loci were significantly out of HWE at one (L18, Myrt3, and C20) or no (Pb8) study locations. Loci of *Aphaenogaster* population *2* mostly exhibited no significant deviation from HWE at any location (L18, Myrt3, and Pb8), with one locus deviating significantly at one location (C20). One locus of *Aphaenogaster* population 3 was significantly out of HWE at three locations (C20), one locus at two locations (L18), one at one, (Pb8), and the final at none (Myrt3). For *Aphaenogaster* populations 1, 2, and 3, respectively, average expected heterozygosity across all loci was 0.71, 0.73, and 0.79, and average observed allelic richness was 8, 6.75, and 13.25 (Table 2). No population exhibited significant differences in estimated allelic richness between urban and forested study locations, or a significant correlation between estimated allelic richness and study location area, (Table 5; Table 6).

**Genetic differentiation and landscape resistance**

In *A. rudis*, GST values correlated significantly with the resistance-distance values generated for all four models (Table 8; Figure 5). However, isolation-by-distance

carried the strongest correlation (Mantel test: $r = 0.476$ (0.333 to 0.666) and $P = 0.004$). I found no support for the isolation-by-distance, putative major barrier, or habitat gradient model in *C. pennsylvanicus*, but significant

**Table 8:**
*Mantel test results for each focal species and* Aphaenogaster *population against each model, including the Pearson correlation coefficients (r), P-values for r > 1 (P1), r < 1 (P2), and r =1 (P3), and the upper and lower 95% confidence intervals (CL).*

| Species | Model | *r* | P1 | P2 | P3 | Lower CL | Upper CL |
|---|---|---|---|---|---|---|---|
| *Aphaenogaster rudis* | Isolation-by-distance | 0.476 | 0.004 | 0.997 | 0.004 | 0.333 | 0.666 |
| *Aphaenogaster rudis* | Putative major barrier | 0.400 | 0.006 | 0.995 | 0.006 | 0.281 | 0.590 |
| *Aphaenogaster rudis* | Urbanization gradient | 0.321 | 0.004 | 0.997 | 0.004 | 0.196 | 0.542 |
| *Aphaenogaster rudis* | Habitat gradient | 0.353 | 0.009 | 0.992 | 0.009 | 0.204 | 0.568 |
| *Camponotus pennsylvanicus* | Isolation-by-distance | 0.203 | 0.100 | 0.901 | 0.151 | 0.011 | 0.369 |
| *Camponotus pennsylvanicus* | Putative major barrier | 0.205 | 0.101 | 0.900 | 0.157 | -0.049 | 0.343 |
| *Camponotus pennsylvanicus* | Urbanization gradient | 0.256 | 0.044 | 0.957 | 0.047 | 0.073 | 0.418 |
| *Camponotus pennsylvanicus* | Habitat gradient | 0.254 | 0.054 | 0.947 | 0.060 | 0.039 | 0.449 |
| *Crematogaster cerasi* | Isolation-by-distance | 0.008 | 0.436 | 0.565 | 0.965 | -0.380 | 0.170 |
| *Crematogaster cerasi* | Putative major barrier | -0.006 | 0.508 | 0.493 | 0.977 | -0.337 | 0.143 |
| *Crematogaster cerasi* | Urbanization gradient | -0.022 | 0.531 | 0.470 | 0.899 | -0.445 | 0.168 |
| *Crematogaster cerasi* | Habitat gradient | -0.053 | 0.564 | 0.437 | 0.797 | -0.395 | 0.134 |
| *Nylanderia flavipes* | Isolation-by-distance | 0.245 | 0.013 | 0.988 | 0.016 | 0.147 | 0.468 |
| *Nylanderia flavipes* | Putative major barrier | 0.200 | 0.040 | 0.961 | 0.080 | 0.099 | 0.422 |
| *Nylanderia flavipes* | Urbanization gradient | 0.129 | 0.067 | 0.934 | 0.139 | 0.023 | 0.409 |
| *Nylanderia flavipes* | Habitat gradient | 0.133 | 0.113 | 0.888 | 0.215 | 0.039 | 0.381 |
| *Aphaenogaster* population 1 | Isolation-by-distance | 0.373 | 0.030 | 0.971 | 0.030 | -0.017 | 0.541 |
| *Aphaenogaster* population 1 | Putative major barrier | 0.344 | 0.023 | 0.978 | 0.030 | 0.007 | 0.495 |
| *Aphaenogaster* population 1 | Urbanization gradient | 0.293 | 0.022 | 0.979 | 0.033 | -0.091 | 0.447 |
| *Aphaenogaster* population 1 | Habitat gradient | 0.328 | 0.023 | 0.978 | 0.027 | -0.028 | 0.533 |
| *Aphaenogaster* population 2 | Isolation-by-distance | 0.134 | 0.181 | 0.820 | 0.381 | -0.038 | 0.325 |
| *Aphaenogaster* population 2 | Putative major barrier | 0.080 | 0.296 | 0.705 | 0.570 | -0.079 | 0.383 |
| *Aphaenogaster* population 2 | Urbanization gradient | 0.067 | 0.310 | 0.691 | 0.568 | -0.068 | 0.281 |
| *Aphaenogaster* population 2 | Habitat gradient | 0.046 | 0.336 | 0.665 | 0.659 | -0.108 | 0.258 |
| *Aphaenogaster* population 3 | Isolation-by-distance | 0.163 | 0.269 | 0.732 | 0.524 | -0.094 | 0.544 |
| *Aphaenogaster* population 3 | Putative major barrier | 0.061 | 0.382 | 0.619 | 0.851 | -0.165 | 0.418 |
| *Aphaenogaster* population 3 | Urbanization gradient | 0.212 | 0.257 | 0.744 | 0.496 | -0.127 | 0.508 |
| *Aphaenogaster* population 3 | Habitat gradient | 0.160 | 0.305 | 0.696 | 0.643 | -0.114 | 0.476 |

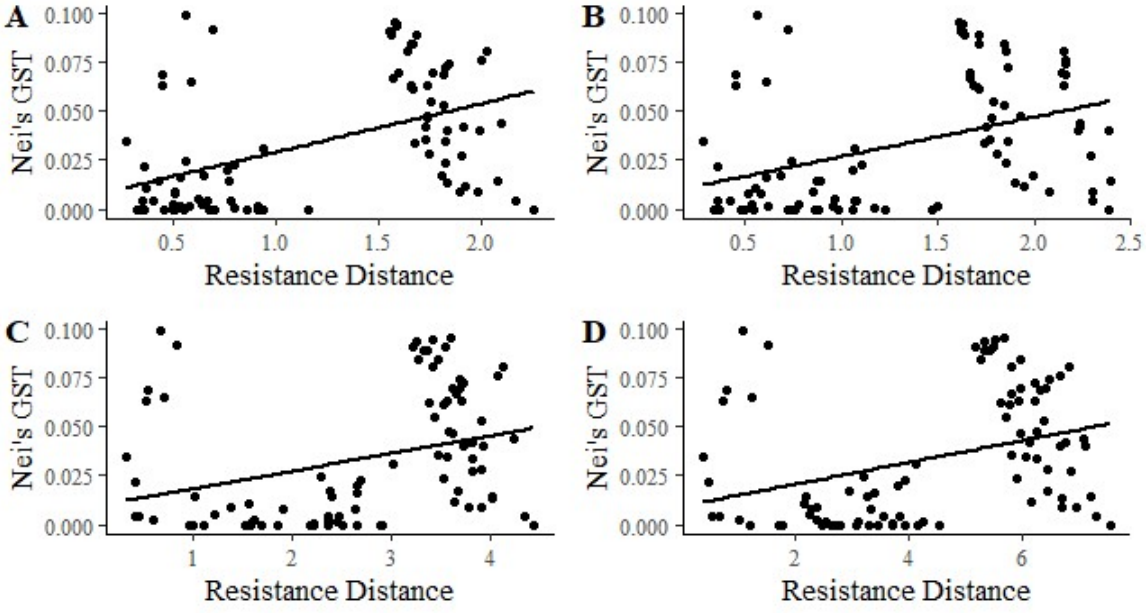


**Figure 5:**
Aphaenogaster rudis *correlation of pairwise GST values with the resistance distance values of A) the isolation-by-distance model, B) the putative major barrier model, C) the urbanization gradient model, and D) the habitat gradient model.*

support for the urbanization gradient model (Figure 6; Mantel test: $r = 0.256$ (0.073 to 0.418) and $P = 0.044$). Neither the isolation-by-distance, putative major barrier, habitat gradient, or urbanization gradient model showed a significant correlation with the GST values generated for *C. cerasi*, and *Aphaenogaster* populations 1, 2, 3. In *N. flavipes*, I found significant support only for the isolation-by-distance model (Figure 7; Mantel test: $r = 0.245$ (0.147 to 0.468) and $P = 0.013$).

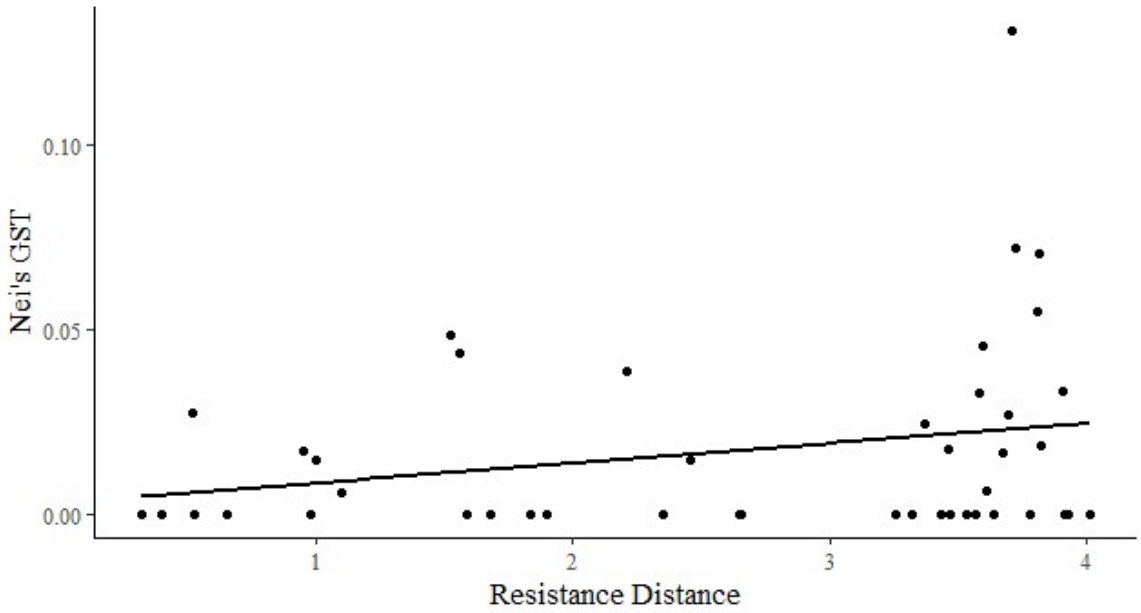


**Figure 6:**
Camponotus pennsylvanicus *correlation of pairwise GST values with the resistance distance values of the urbanization gradient model.*

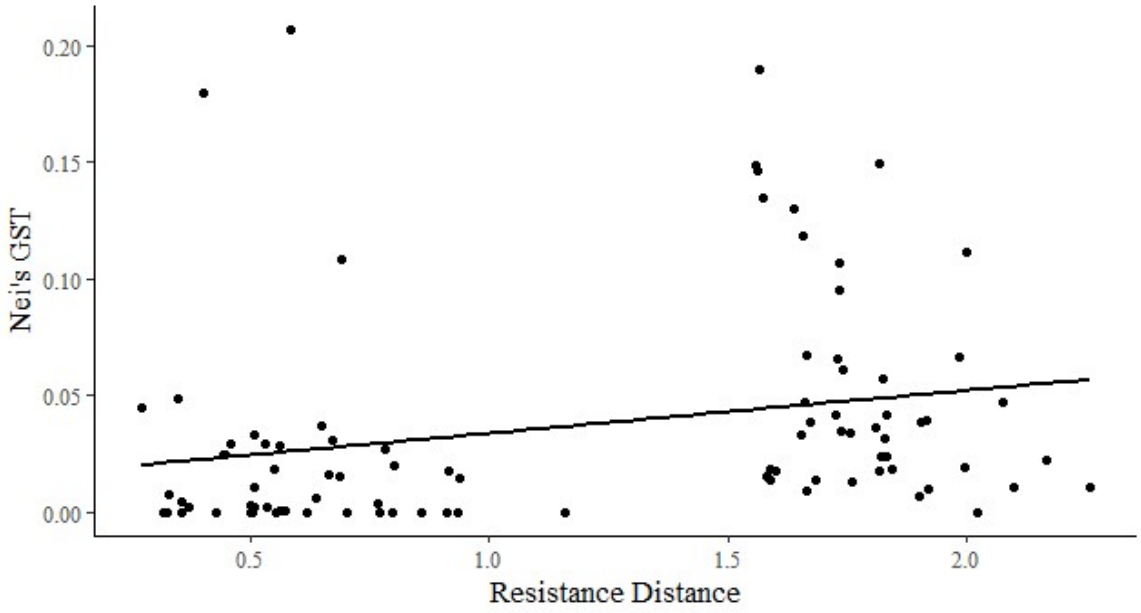


**Figure 7:**
Nylanderia flavipes *correlation of pairwise GST values with the resistance distance values of the isolation-by-distance model.*

## Discussion

In this study, I characterized the population genetics of four ant species in a heterogenous urban environment using microsatellite genotyping. Species were chosen to represent three major behavioral categories animals exhibit in response to urbanization: urban avoider species, urban adapter species, and urban exploiter species (Blair, 2001). However, my results show that ant population genetic patterns do not consistently fit with perceived aptitude for urbanization. Some *Aphaenogaster* species maintain connectivity in the urban environment, while others are notably absent in urbanized areas. Likewise, for the urban adapter *Crematogaster cerasi*, neither distance nor characteristics of the physical landscape pose a barrier to dispersal. However, for *Camponotus pennsylvanicus*, known for exploiting human infrastructure and presumed at least a match to *C. cerasi* in terms of urban proclivity, urbanization posed a significant problem for connectivity. The invasive species, *Nylanderia flavipes*, an urban exploiter species, also defies expectations. Though not affected by urbanization, *N. flavipes* differs from other invasive ant species by facing a significant isolation-by-distance effect. Overall, this study demonstrates that the specific biology and context of an ant species can greatly influence population genetics in an urban environment, regardless of whether the species thrives on a pavement-dominated landscape or stays in remnant vegetation.

My *A. rudis* genotype dataset likely represents three cryptic but distinct *Aphaenogaster* species, based on the high incidence of loci out of HWE and the low frequency of mixed-ancestry individuals identified by STRUCTURE, even in study locations where multiple clusters co-occur. Specifically, these species would fall under

the *Aphaenogaster fulva-rudis-texana* species complex, which includes several undescribed species morphologically similar or indistinguishable from *A. rudis* (Umphrey, 1992). Based on distributions and morphological characteristics, and applying the terminology of Umphrey, 1992, my *Aphaenogaster* sampling likely includes *Aphaenogaster rudis*, the similar but undescribed species N22b, and *Aphaenogaster picea.* All three species occur in my study regions and possess clear morphological characteristics which separate them from other co-occurring members of the *fulva-rudis-texana* species group, such as *A. fulva*, including short propodeal spines and a non-peaked mesonotum (Ellison et al., 2012; Umphrey, 1992; 1996). Morphologically distinguishing *A. picea* from *A. rudis* and N22b hinges on slight differences in antennal segment shading and propodeal spine angle, while inspection under light microscopy cannot distinguish *A. rudis* from N22b. Positive species identification would require morphometric and karyotype information, but individuals of *Aphaenogaster* population 3 generally match *A. picea* morphological characteristics, while individuals of population 1 and 2 more closely match descriptions of *A. rudis* or N22b. Based on this identification scheme, a clear exclusion of *A. picea* from urbanized regions becomes apparent, with a near-complete absence of *Aphaenogaster* population 3 in urban study locations.

Ant species can face extirpation when land becomes urbanized (Rocha-Ortega and Castaño-Meneses, 2015; Perez and Diamond, 2019). The exact factor which makes the urban landscape inhospitable varies species to species, but can include the unavailability of suitable nutritional resources or habitat substrates (Silverman, 2005). Since *A. picea, A. rudis*, and N22b share similar diets and nesting requirements, these factors alone

cannot fully explain why only *A. picea* faces exclusion from urban areas (Ellison et al., 2012). However, another environmental variable often influences which ant species or populations persist in the urban mosaic: heat tolerance (Angilletta et al., 2007; Diamond et al., 2017). The increase in average surface temperature in urban areas compared to adjacent non-urban areas, also known as the urban heat island effect, has been well-documented in cities across the planet (Qian et al., 2022). This includes the city of Camden, NJ, a component of the Philadelphia metropolitan area (Rosenzweig et al., 2005). A higher thermal tolerance has been noted in *A. rudis* over *A. picea*, often resulting in the former replacing the latter in warming environments (Warren et al., 2013; 2016). Therefore, the urban heat island effect may exclude *A. picea* from urbanized environments and allow the proliferation of more heat-tolerant species, such as *A. rudis* and N22b.

For those species which persist in the urban mosaic, the construction of impervious infrastructure transforms and fragments suitable habitat (McKinney, 2002). However, not all aspects of urbanization are universally impenetrable. Several courses exist by which species can travel through or circumvent an inhospitable built environment, thus maintaining connectivity across suitable habitat patches. Over even very long distances, humans themselves serve as one means of travel (Meurisse et al., 2019). Human-mediated dispersal of many species occurs via a variety of vectors, including during leaf litter removal, the transport of plant species, and within firewood and lumber (Hulme, 2009; Mazzi and Dorn, 2012; Solano et al., 2021). In ants, this permits the establishment of new populations in novel locations and enables connectivity

between populations (King et al., 2009). Human-mediated dispersal may explain some of the genetic patterns seen in my results, specifically for *C. cerasi* and *Aphaenogaster* species 1 and 2, urban adapter and avoider species, which both possess limitations as far as colonizing urbanization. These species exhibited a lack of population structure based on Bayesian population assignment, and any genetic differentiation observed between study locations had no significant correlation to any of my four geographic models, including isolation-by-distance. Both *C. cerasi* and relevant members of the *A. fulva-rudis-texana* species complex nest in rotting wood, thus creating a feasible human-mediated method of dispersal via the transportation of woody debris or building materials (Ellison et al., 2012; Lubertazzi, 2012; Morgan and Mackay, 2017). Similar patterns have been observed in *Formica japonica*, Temnothorax *nylanderi*, and *Tetraponera rufonigra* with populations in these species exhibiting low genetic differentiation in an urban environment, also possibly due to human interference (Khimoun et al., 2020; Sabtu and Majid, 2017; Yamamoto et al., 2013). Alternatively, or additionally, genetic connectivity could be maintained by overflying obstacles during nuptial flights. While nuptial flight behavior has not been described for *C. cerasi* or any member of the *A. fulva-rudis-texana* species complex, the congeners *C. captiosa* and *A. treatae* conduct nuptial flights involving large numbers of alates and fully winged males dispersing away from natal colonies (Fiala et al., 2017; Talbot, 1966). Studies examining the flight mechanics of other Myrmecine ants of the genera *Solenopsis* and *Atta* estimate a maximum nuptial flight range of 5-10 km for both alates and males (Jutsom and Quinlan, 1978; Markin et al., 1971; Moser, 1967; Vogt et al., 2000). Assuming these

metrics approximate the nuptial flight ranges for *C. cerasi* and members of the *A. fulva-rudis-texana* species complex, flight-based connectivity may persist for these species between my study locations.

While urban avoiders and adapters may opportunistically evade heavily urbanized areas via human-mediated dispersal and flight, urban exploiters lack this necessity, especially invasive species such as *N. flavipes*. Invasive ant species characteristically exhibit no population structure and low genetic differentiation in any environment, including urbanization, even over long distances (Eyer et al., 2018; Tsutsui and Case, 2001; Zhang et al., 2019). Several invasive ants, including *Nylanderia fulva* and *Linepithema humile*, exhibit supercoloniality in non-native ranges, implying an especially low isolation-by-distance effect as polygynous colonies spread by nest fission across the landscape, mate within the nest, and avoid nuptial flights. In partial contrast to this pattern, the invasive *N. flavipes* population examined in this study similarly lacked structure but exhibited a significant correlation between genetic differentiation and distance. Unlike *N. fulva* and *L. humile*, *N. flavipes* is monogynous and lacks supercoloniality, with nest fission a rare event (Ichinose, 1987; 1991). As such, despite being an urban exploiter and an invasive species, dispersion of *N. flavipes* is not dissimilar to *C. cerasi* and members of the *A. fulva-rudis-texana* complex. Dispersal methods may include flight and human-mediated methods, with the latter probably a common occurrence. The first records of *N. flavipes* presence in the USA originate from Philadelphia, in 1939, likely facilitated by potted plants and mushroom-growing logs carried by Chinese immigrants in the early 20$^{th}$ century (Williams and Lucky, 2020;

Trager, 1984). *Nylanderia flavipes* probably continue to locally disperse in a similar fashion today. As invasive *N. flavipes* populations spread from points of arrival over the last century, dispersal was apparently limited enough and genetic diversity high enough for the development of isolation-by-distance effects.

Overall, due to human-mediated dispersal, nuptial flights, or otherwise, less-than-significant genetic differentiation and isolation-by-distance appear to be the predominant paradigms for ants in an urban environment. Of the handful of studies examining the effects of urbanization on ant population genetics, only one, examining *Tapinoma sessile,* has categorically found urbanization driving genetic differentiation (Blumenfeld et al., 2021; Khimoun et al., 2020; Sabtu and Majid, 2017; Yamamoto et al., 2019). Despite the effect lacking enough power to drive measurable population divergence, my results show a significant correlation between genetic differentiation and urbanization in *C. pennsylvanicus*, notably without a significant isolation-by-distance effect. Significant changes in colony structure have been recorded in urban populations of *C. pennsylvanicus*, with a reduction in suitable nesting sites causing shifts from polydomy to monodomy and drops in worker counts (Buczkowski, 2011). These changes, in addition to the physical barriers posed by urban infrastructure, may be reducing the long-distance dispersal capacity of *C. pennsylvanicus*. Furthermore, while the wood-nesting *C. pennsylvanicus* possesses the potential for human-mediated dispersal, the propensity of this species for nesting in and damaging wood-framed buildings means homeowners commonly consider it a pest (Ellison et al., 2012; Dukes, 1982). As such, humans may be more likely to utilize pest control measures, including chemical killing

agents, on *C. pennsylvanicus* and other *Camponotus* species than with other ant genera (Sutton, 2018). Utility companies also routinely treat electrical and telephone poles with the goal of avoiding infrastructure damage by *Camponotus* (Shields et al., 2000). This factor may prevent the consistent spread of *C. pennsylvanicus* into urban environments, confining populations within wooded habitat fragments and further reducing genetic connectivity.

In analyzing the results of my study, I must note that sample size, especially for *C. cerasi* and *C. pennsylvanicus*, was low, with some study locations represented by as few as one individual. The division of the *Aphaenogaster* dataset into three populations also created similar sample size issues. Further sampling and the addition of more study locations could clarify the effects of urbanization, or the lack thereof, in my focal species. Additionally, statistical power could increase if individuals were genotyped at more microsatellite loci.

Overall, my research emphasizes the importance of perceiving different species as unique cases. Although species can be functionally classified as urban avoiders, adapters, and exploiters, these behavioral categories, while useful, do not necessarily reflect genetic patterns as expected. Research examining the population genetics of ants in urban environments, including this study, has generally focused on common or invasive species, with little investigation being made of rare and cryptic species. (Blumenfeld et al., 2021; Eyer et al., 2018; Khimoun et al., 2020; Sabtu and Majid, 2017; Tsutsui and Case, 2001; Yamamoto et al., 2019; Zhang et al., 2019). As such, the general patterns of ant population genetics in urban environments have yet to be established, and further

research on less obvious species with different biological characteristics would help elucidate these trends.

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