## ABSTRACT

REED, EMILY MARGARET. Population and Landscape Genetics of the Anthropophilic Invasive Mosquito *Aedes albopictus*. (Under the direction of Dr. Martha Burford Reiskind).

Urbanization has drastically altered land use, impacted habitat quality, composition, and configuration, and fundamentally changed ecosystem function. The expanding global footprint of urban areas represents an emerging biome where fundamental ecological and evolutionary processes generate unique patterns of species response. We can observe these responses *in situ* by studying nonnative and invasive species, which dominate urban environments. We can understand the process and biology of species invasion in urban landscapes using population genomic approaches. Population and landscape genomics take advantage of powerful next-generation technology to reveal the fine-scale, complex ecological patterns of urban invasions.

The Asian tiger mosquito, *Aedes albopictus*, is a powerful study system to explore how urbanization affects the population dynamics of invasive species. *Aedes albopictus* is a cosmopolitan, anthropophilic container-breeding mosquito whose range expansion is closely associated with international trade. Consequently, this species has evolved to both travel and coexist with humans. *Aedes albopictus* is also a species is of concern because of its biting habits and capacity to transmit zoonotic disease. Therefore, understanding how urban and urbanizing landscapes shape their population genetic characteristics at fine spatial scales is important in both basic and applied scientific research.

In my dissertation, I described population genetic patterns of *A. albopictus* populations in urban and urbanizing landscapes. I first provided a review of current literature concerning urban invasion genetics. I then combined reduced-representation genomic sequencing data with ongoing mosquito surveillance efforts in southeastern United States counties to infer fine-scale population structure in *A. albopictus* populations. I found that patterns of *A. albopictus* abundance and genetic differentiation varied with location, with evidence that *A. albopictus* in urban centers have high genetic diversity relative to rural counterparts but are more genetically isolated. In the following chapter, I analyzed genetic connectivity and gene flow between adult *A. albopictus* collected across 42 sites in Wake County, North Carolina. I found little evidence of gene flow between urban locations, but significant gene flow from urban to rural regions of the county. Further, I identified percent impervious land cover and medium-intensity development as strong barriers to *A. albopictus* genetic connectivity. I then applied these finds to simulate

genetic connectivity under near-future urbanization scenarios in Wake County. To do so, I compiled future land use plans and maps from the county and local municipalities to identify priority regions of urban development. My results suggested that low-intensity, suburban development is not a strong barrier to gene flow. Finally, I evaluated the impact of sampling different *A. albopictus* life stages on population genetic inferences. I sampled five locations for both *A. albopictus* eggs and adults and found that they reveal different patterns of genetic diversity within sites and genetic differentiation between sites. My overall findings suggested that the population genetic dynamics of *A. albopictus* were closely associated with anthropogenic landscapes and are highly context dependent, requiring both further research to identify the city-specific features governing population structure and demonstrating the potential for targeted, adaptive, and place-based vector management solutions.

Population and Landscape Genetics of the Anthropophilic Invasive Mosquito *Aedes albopictus*

by
Emily Margaret Reed

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APPROVED BY:

| | |
|---|---|
| Dr. Martha Burford Reiskind<br>Committee Chair | Dr. Michael Reiskind |
| Dr. Adam Terando | Dr. Nick Haddad |

## DEDICATION

To Dad

## BIOGRAPHY

Emily Reed grew up in Pittsboro, North Carolina, with her parents Teresa & Robert and sibling Elliot. She graduated from the University of North Carolina at Asheville with a BA in French and minors in neuroscience and biology. After completing her undergraduate degree, she worked as a wildlife rehabilitation intern at the Western North Carolina Nature Center. There, she learned to care for injured and orphaned wildlife, including raptors, songbirds, and many opossums. She regularly interacted with the public to provide support and answer wildlife questions. As an intern, Emily began to consider pursuing a career in research. She returned to her alma mater part time to complete perquisite coursework before beginning her degree in biology at North Carolina State University in 2016 under the direction of Dr. Martha Burford Reiskind. During her graduate career, Emily was twice named a Global Change Fellow with the USGS Southeast Climate Adaptation Science Center. The fellowship exposed her excitement for applied, stakeholder-driven research. She intends to use her degree to promote ecosystem and community resilience and equity under ongoing global change. At the time of this writing, Emily lives in the oak-pine forests of the North Carolina piedmont with her partner, two cats Gumbo and Fry, and eighteen-week-old puppy Okra.

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## TABLE OF CONTENTS

LIST OF TABLES ..... viii
LIST OF FIGURES ..... ix

**Preamble** ..... 1
References ..... 3

**Chapter 1: Gridlock and beltways: the genetic context of urban invasions** ..... 4
Introduction ..... 4
Arrival: cities as hubs for invasion ..... 6
Establishment: evolution, genetic diversity, and structure of urban invaders ..... 8
Overcoming the genetic paradox of invasion ..... 8
Genetic diversity and structure in cities ..... 9
Spread: urban areas and spread of invasive species ..... 11
Spread within the city ..... 11
Spread through the urban—rural gradient ..... 13
Considerations for spread out of urban areas ..... 14
Conclusion ..... 15
Box 1. Aquatic invasions and urbanization *in brief* ..... 16
Acknowledgements ..... 17
References ..... 18
Chapter Tables ..... 30
Chapter Figures ..... 32

**Chapter 2: Heterogeneity in *Aedes albopictus* (Diptera: Culicidae): high variation in abundance and low genetic structure across the urban-rural landscape gradient** ..... 33
Introduction ..... 33
Methods ..... 36
Study system ..... 36
Sampling ..... 37
Genomic library preparation ..... 39
Bioinformatic processing ..... 39
Abundance analyses ..... 40
Genomic analyses ..... 40
Results ..... 42
Mosquito abundance ..... 42
Population genetics ..... 44
Discussion ..... 48
Abundance ..... 48
Population genetics ..... 50
References ..... 54
Chapter Tables ..... 60
Chapter Figures ..... 65

**Chapter 3: Fine-scale landscape genetics show resistance to human development and asymmetric patterns of gene flow for the invasive mosquito *Aedes albopictus* (Diptera: Culicidae) within a local geopolitical boundary** ..... 79
Introduction ..... 79
Methods ..... 81
Study system ..... 81
Sampling ..... 81
Genomic library preparation ..... 82
Bioinformatic processing ..... 83
Genetic connectivity and gene flow ..... 84
Landscape resistance analysis ..... 85
Results ..... 87
Sampling & bioinformatic processing ..... 87
Genetic connectivity and migration ..... 87
Landscape resistances ..... 88
Discussion ..... 89
References ..... 92
Chapter Tables ..... 98
Chapter Figures ..... 100

**Chapter 4: The *Aedes albopictus* roadmap: using landscape genetics to model future gene flow in Wake County** ..... 104
Introduction ..... 104
Methods ..... 106
Genetic data ..... 106
Land use data and resistance cost optimization ..... 106
Future land use scenarios ..... 108
Results ..... 109
Resistance cost optimization ..... 109
Future land use scenarios ..... 110
Discussion ..... 111
Resistance surfaces ..... 111
Future land use scenarios ..... 112
Recommendations for Wake County Planning ..... 113
Conclusion ..... 114
References ..... 115
Chapter Tables ..... 119
Chapter Figures ..... 122

**Chapter 5: *Aedes albopictus* life stage affects population genetic patterns at fine spatial scales** ..... 129
Introduction ..... 129
Methods ..... 131
Ovitrap sampling ..... 131
Genomic bioinformatics ..... 131
Population genetic analyses ..... 132

Results ..... 133
Genomic bioinformatics ..... 133
Genetic diversity and differentiation ..... 133
Genetic structure ..... 134
Discussion ..... 135
References ..... 137
Chapter Tables ..... 140
Chapter Figures ..... 142

## LIST OF TABLES


Table 1.1 Processes and the expected genetic consequences for the stages of biological invasions and genetic tools researchers use to evaluate and test these hypotheses. Arrows indicate the directionality, when appropriate, of the process or impact. ..... 30

Table 2.1 Summary of sequenced *A. albopictus* individuals for Palm Beach County 2013, 2018, and Wake County 2016, 2018 ..... 60

Table 2.2 Summary of single nucleotide polymorphisms (SNPs) and individual filters during the bioinformatic pipeline to reach the final datasets for Wake County 2016, Wake County 2018, and Palm Beach County ..... 61

Table 2.3 Mean expected heterozygosity ($H_E$) and inbreeding coefficient ($F_{IS}$) by geographic zone for Wake County and Palm Beach County. Zones acted as categorical proxies for county-wide patterns of urbanization ..... 62

Table 2.4 Pairwise $F_{ST}$ table for sites sampled in Wake County 2016 ..... 63

Table 2.5 Pairwise $F_{ST}$ table for sites sampled in Palm Beach County 2013 and 2018 ..... 64

Table 3.1 List of landscape variables included in landscape genetic analyses ..... 98

Table 3.2 Multiple regression on distance matrices model selection for landscape genetic analyses using three genetic distances and two resistance distances, least cost paths and all paths (commute distance) ..... 99

Table 4.1 Land use classification model results with resistance cost optimization ..... 119

Table 4.2 Description of future land use scenarios for Wake County ..... 120

Table 4.3 Percent cover of land use classifications in Wake County 2016 and for future land use scenarios ..... 121

Table 5.1 Genetic diversity estimates for egg and adult-caught *A. albopictus* ..... 140

Table 5.2 Pairwise genetic distance for egg and adult-caught *A. albopictus* ..... 141

## LIST OF FIGURES


Figure 1.1 Conceptual diagram of genetic characteristics of invading populations in multiple urban introduction scenarios ..... 31

Figure 2.1 Wake County geographic zones correspond to overall urbanization patterns ..... 65

Figure 2.2 Wake County sampling locations in 2016 and 2018 ..... 66

Figure 2.3 Palm Beach County sampling locations in 2013 and 2018 ..... 67

Figure 2.4 Mean *Aedes albopictus* egg abundance across locations in Wake County 2016 ..... 68

Figure 2.5 Mean *A. albopictus* adults trapped at sampling sites in Wake County 2018 ..... 69

Figure 2.6 Relative abundance of *A. albopictus* eggs and adults in Palm Beach County at sampling locations in 2013 (eggs) and 2018 (eggs and adults) ..... 70

Figure 2.7 Box plot of *A. albopictus* abundance in Palm Beach County grouped into zones based on distance from intracoastal waterway ..... 71

Figure 2.8 STRUCTURE plots for Wake County 2016, Wake County 2018, and Palm Beach County with the optimal *k* values identified by STRUCTUREharvester ..... 72

Figure 2.9 Scatterplot of sampled individuals in Wake County 2016 using discriminant analysis of principal components (DAPC) ..... 73

Figure 2.10 DAPC scatterplots of Wake County 2016 populations grouped by geographic zones (inner, outer, and outside) which correspond to county-wide patterns of urbanization ..... 74

Figure 2.11 Scatter of Wake County 2018 individuals using DAPC ..... 75

Figure 2.12 DAPC scatterplots of Wake County 2018 individuals grouped by zones ..... 76

Figure 2.13 DAPC scatterplots for Palm Beach County individuals separated by year ..... 77

Figure 2.14 DAPC scatterplots for Palm Beach County organized by year and zone ..... 78

Figure 3.1 2018 sample sites and percent impervious surface for Wake County ..... 100

Figure 3.2 Map of 2018 sample sites grouped *a priori* into 10 regions of Wake County, separated by municipalities and major roadways ..... 101

Figure 3.3 Population graph of Wake County 2018 genetic connectivity ..... 102

Figure 3.4 Circle graph of directional gene flow between regions of Wake County ..... 103

Figure 4.1 Example of development land use classifications for the USGS National Land Cover Database and satellite image of same area ..... 122

Figure 4.2 Map of Wake County municipalities..... 123

Figure 4.3 Map of high-priority areas for near-future development in Wake County ..... 124

Figure 4.4 Land cover and resistance surface after cost optimization in Wake County..... 125

Figure 4.5 Land use of high-priority development areas in Wake County in 2016 and for future land use scenarios ..... 126

Figure 4.6 Pairwise connectivity between Wake County sites sampled for *A. albopictus* for present land use and future land use scenarios..... 127

Figure 4.7 Differences in connectivity between Wake County 2016 and future land use scenarios ..... 128

Figure 5.1 Map of 2018 sampled sites with egg and adult genetic data in Wake County..... 142

Figure 5.2 STRUCTURE plots for egg and adult *A. albopictus*..... 143

Figure 5.3 Scatterplots of discriminant analysis of principal components for egg and adult *A. albopictus*..... 144

## PREAMBLE

Invasion biology has advanced scientific knowledge of species ecology and evolution in both basic and applied research. While the criteria for what makes a species "invasive" is often context-dependent and sometimes ideological (Colautti and MacIsaac 2004), the invasion process can be broadly defined as the spread and establishment of new populations outside a species' native range due to human activity (Cassey et al. 2005). This phenomenon offers opportunities to study ecological and evolutionary concepts *in situ*, such as local adaptation, metapopulation dynamics, succession, and interspecific competition (Sakai et al. 2001; With 2002; Huey et al. 2005; Prach and Walker 2011; Borden and Flory 2021). However, invasive species introductions threaten biodiversity, conservation, ecosystem services, and public health (Vitousek et al. 1996; Mack et al. 2000; Pejchar and Mooney 2009). Consequently, there is a need for applied and collaborative research between scientists and practitioners to understand the effects of invasive species on native flora and fauna, quantify ecological and economic costs, predict future invasions in the context of global change, and implement sustainable, adaptive management programs (Horan et al. 2002; Pimentel et al. 2005; Moffitt and Osteen 2006; Simberloff et al. 2013).

Population genomics is a powerful approach to address both basic and applied questions in invasion biology (Barrett et al. 2017). High throughput sequencing combined with major scientific advances like computational power and geospatial processing permit researchers to explore invasion dynamics in increasingly complex and fine-scale systems (Chave 2013). Landscape genomic approaches have furthered the study of invasion by allowing these questions to be addressed in spatially-explicit contexts (Manel et al. 2003; Balkenhol et al. 2015). In the following dissertation, I use these approaches and tools to explore how heterogenous, urbanizing landscapes shape the population genomic dynamics of one of the fastest spreading invaders, the tiger mosquito *Aedes albopictus*.

In my first chapter, I reviewed current literature on the population and landscape genetics of invasion in urban environments. I explored how urbanization aids the invasion process from the introduction of a population to a novel environment through its establishment within urban settings and eventual spread into rural landscapes. I focused on the genetic patterns observed during each stage of an urban invasion and emphasize the utility of genomic tools to study these events. This review provides a framework for my original research on *A. albopictus* and

acquaints readers with the terminology and population genetic concepts that I explored extensively in subsequent chapters.

In my second chapter, I explored patterns of *A. albopictus* population abundance, genetic diversity, and genetic structure over two years along urban—rural networks in Wake County, North Carolina, and Palm Beach County, Florida. I compared population characteristics of *A. albopictus* larvae and adults between time periods, counties, and levels of urbanization and provided a descriptive analysis of population trends. In my third chapter, I use landscape genomic approaches to further elucidate the broad patterns I observed across counties by modelling the effects of urban features on directional gene flow and genetic connectivity in adult populations of *A. albopictus* sampled extensively across Wake County. I apply these model results in chapter four by optimizing parameter estimates for the dispersal costs of developed land use classes. I used these parameter estimates to hindcast *A. albopictus* genetic connectivity in the recent past and predict connectivity in the near future. To generate future land use change scenarios in Wake County, I used comprehensive development plans available for each of Wake County's ten municipalities to identify regions of urban and suburban growth. In doing so, I connected the invasion biology and population genetic research from chapters 1-3 with applied methods and place-based knowledge to make data-driven recommendations for mosquito control at a scale appropriate for local management. In a brief fifth chapter, I consider how sampling different life stages of *A. albopictus* affects population genetic results and conclusions. I compare levels of genetic diversity and differentiation between *A. albopictus* individuals collected as eggs and as adults from a set of sites in Wake County and discuss whether observed differences would result in different management recommendations.

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# CHAPTER 1

## Gridlock and beltways: the genetic context of urban invasions

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### Introduction

Invasive species can cause dramatic alterations to biotic and abiotic components of the environment (Dukes and Mooney 1999; Ehrenfeld 2003; Salo et al. 2007; Molnar et al. 2008; Vila et al. 2011). In urban landscapes, the effects of invasion are compounded with habitat fragmentation, pollution, and disturbance, intensifying strain on ecosystems (Munns 2006; Boone et al. 2007; Holmstrup et al. 2010). While biologists acknowledge the increased presence of invasive species within cities (Pyšek 1998; Bolger et al. 2000; Alberti 2005; Lambdon et al. 2008; Cavin and Kull 2017), the influence of the urban environment on the ecology and evolution of invaders has been largely overlooked. It is important to understand this relationship given the threats of both urbanization and invasive spread to biodiversity. These threats are ongoing and may also be expected to accelerate given the increasing extent of urban land cover and the synergistic relationship between urbanization and invasion (Seto et al. 2012).

One consequence of urban expansion is an increase in invasive species presence, abundance, and diversity in cities (Gaertner et al. 2017). Urbanization is becoming a dominant global land cover and habitat type. There were 28 megacities globally in 2014 with a projected 13 more by 2030 (United Nations Statistics Division 2017). Megacities, defined as metropolitan areas with >10 million population, are the result of cities expanding and fusing (Terando et al. 2014). These may represent an emerging, novel biome where fundamental ecological and evolutionary processes may generate unique patterns of species response. To address threats posed by invasive species and urbanization, we must understand the fundamental ecological and evolutionary processes at play and how they interact to produce patterns of species distribution and composition in cities. This can be assisted by using genetic tools to understand the driving forces of invasive species dispersal, connectivity, and adaptation within cities.

Despite the immense body of literature on the genetics of invasives, the population genetic characteristics of invasive species in urban environments has remained unreviewed. We

discuss how urban landscapes exacerbate the risks posed by invasive species by facilitating introduction and establishment while simultaneously providing a source for outward spread. We examine the influence of urban landscapes on invasion genetics in three parts that mirror the stages of invasion: (1) cities as hubs for invasion [arrival], (2) genetic diversity and structure within cities [establishment], and (3) movement within and out of cities [spread]. Throughout, we demonstrate the utility of population genetic studies to inform invasive species management. The invasion history of a species may be highly relevant to management if, for instance, certain genotypes are more damaging to ecosystems or travel through the landscape in unique ways. In these cases, studies that look at gene flow or rapid evolution of invasive species in urban systems are valuable.

Discussion concerning invasion requires deliberate and precise language given the dubious and often political nature of the term (Pyšek 1995; Larson 2005; Larson 2007). Here, we define invasive as nonnative species whose introductions are linked to anthropogenic activity and which have negative effects on local ecosystems. We describe urban areas according to United Nations guidelines, which recognizes national differences in definitions of urban and rural that may not be reflected by purely quantitative measures such as population density (United Nations Statistics Division 2017). Generally, urban areas are more densely populated and contain higher proportions of impervious surface and human infrastructure than rural or unsettled area. Socioeconomic structure differs between urban and rural communities; specifically, urban economies are not as agriculturally focused and urban areas offer more human services and higher qualities of life (United Nations Statistics Division 2017). Ecologically, urban areas have higher levels of pollution, are more fragmented, and experience higher levels of anthropogenic disturbance (Miles et al. 2019). When we discuss spread of invasive species out of urban landscapes, we focus on spread into areas that are not already degraded and fragmented directly by urban features. We avoid the term "natural" because it connotes an area unaffected by anthropogenic activity, an unrealistic suggestion in the face of pressure from human-induced climate and land use change (McIntyre et al. 2000).

All species are affected in some way by anthropogenic changes, but while some struggle and perish, others thrive. Urban-associated invaders, such as rats, pigeons, and mosquitoes, have adapted to be city specialists and are mainstays of urban areas globally (Luniak 2004; Francis and Chadwick 2012; Hulme-Beaman et al. 2016; Johnson and Munshi-South 2017). Other

invasive species are adapted to specific plant or animal hosts that are commonly found in cities, such as the Asian long-horned beetle *Anoplophora glabripennis*, the birch leaf-mining weevil *Orchestes fagi*, and the Asian longhorned tick *Haemaphysalis longicornis* (Kirichenko et al. 2018; Javal et al. 2019; Tufts et al. 2019). These species were introduced to cities unintentionally. However, most invasions in cities start as deliberate introductions (Padayachee et al. 2017). For example, ornamental plants like the glossy buckthorn *Frangula alnus* are imported to beautify cities and subsequently proliferate out of control (Lambdon et al. 2008; Milbau and Stout 2008; Hulme 2011; Dlugosch et al. 2015). Some invasive animals, such as Burmese pythons *Python bivittatus* and monk parakeets *Myiopsitta monachus*, are intentionally released as unwanted pets (Russello et al. 2008; Willson et al. 2010). These pathways also apply to aquatic invaders, which are also impacted by urbanization (**Box 1)**. One explanation for the apparent proclivity for invasion success (invasiveness) is that many of these organisms have evolved in cities for centuries (Hulme-Beaman et al. 2016). However, this hypothesis does not account for why some species are able to survive and spread in both urban and undeveloped environments (e.g., black rats *Rattus rattus*, found in both cities and uninhabited islands), or why other species that have been exposed to urban settings for similar lengths of time did not also evolve to overcome the obstacles of city living.

Population genetics can help us understand why certain species thrive in urban systems while others do not. The genetic characteristics of invaders allow researchers to test hypotheses about invasion origins, spread, and adaptation. Genetic comparisons between native and introduced populations can identify the source of the invasive species (Quinn et al. 2012; Burford Reiskind et al. 2019). Landscape genetics can reveal fine-scale patterns of invasive dispersal and spread (Balkenhol et al. 2015), and population and quantitative genomics can identify putative regions of selection within the genome highlight how invasive species adapt to novel environments (Burford Reiskind et al. 2018; Burford Reiskind et al. 2019).

**Arrival: Cities as hubs for invasion**

Humans mediate spread of invasive species. Therefore, cities, as nexi of anthropogenic settlement and movement, are hotspots for invasion (Gaertner et al. 2017; Kühn et al. 2017; Seebens et al. 2018). Historically, most unintentional introductions into cities occurred via water-based trade, where organisms were stowaways on ships or in ballast water (**Box 1**). Many

invasive species, including rodents and shellfish, still arrive in this manner (Kühn et al. 2017; Tingley et al. 2018). Invasive species also enter urban environments via land-based trade pathways, as escapees from botanical gardens, the aquarium and pet trades, or through intentional introductions (Hulme et al. 2015; Padayachee et al. 2017; Yanai et al. 2017). Multiple pathways of introduction increase the likelihood of multiple invasions from novel source locations.

Frequent introduction events, which are likely to occur in urban areas with trade and long-distance transportation, can increase a population's propagule pressure and subsequently their probability of successful establishment (**Table 1.1**; Lockwood et al. 2005; Simberloff 2005; Signorile et al. 2014; Blackburn et al. 2015). Multiple introductions of the same non-native species to an area are more frequent in urban areas, and therefore there is a higher likelihood of two genetically distinct invasive populations meeting and interbreeding. Cities can therefore be hotspots for genetic admixture (e.g., Lindholm et al. 2005; Kolbe et al. 2008; Chapple et al. 2013; Vargo et al. 2014; Fischer et al. 2017; Qiao et al. 2019).

When multiple introductions do occur, admixture may occur that would be unlikely in the native range. This can lead to an increase in genetic diversity and novel allelic combinations between genes that may enhance evolutionary and adaptive potential (Verhoeven et al. 2011). When investigating the role of multiple introductions in urban invasions, we can use genetic tools to quickly identify signatures of multiple invasions and subsequent admixture. Observed linkage disequilibrium in cities within the invasive range of the monk parakeet *Myiopsitta mochacus,* but not the native range, showed that high propagule pressure from multiple introductions was important in their establishment in the United States (Da Silva et al. 2010). If researchers conduct robust sampling in the native range, then mitochondrial DNA (mtDNA) or genomic markers can be highly effective at detecting multiple introductions in an invasive area. In urban areas of Lord Howe Island, Australia, populations of the invasive delicate skink *Lampropholis delicata* contained mtDNA haplotypes from all five source locations examined, indicating extensive admixture. More rural populations of the skink on the same island did not show the same degree of hybridization, demonstrating that multiple introductions occurred in cities (Chapple et al. 2013). We can better understand the consequences of increased propagule pressure in urban areas by measuring genetic structure and genetic diversity of invasive populations within a city.

## Establishment: Evolution, genetic diversity, and structure of urban invaders

### Overcoming the genetic paradox of invasion

Cities can be stressful environments for organisms to invade and establish. Urban areas are highly disturbed, stochastic, and have limited areas of suitable habitats for many species (Airoldi et al. 2015; Kühn et al. 2017). For example, urban centers have high proportions of impervious surfaces, fragmented green spaces, and elevated levels of pollution (Munns 2006; Boone et al. 2007; Holmstrup et al. 2010). Paired with founder effects, this should make establishment unlikely (see **Table 1.1**). However, many introduced populations successfully proliferate in cities despite genetic and ecosystem obstacles (Frankham 2005; Allendorf et al. 2014; Estoup et al. 2016). There are several explanations for why cities may be nurseries for invasions. There is less competition from native species, as biodiversity is lower in urban areas, and consequently cities may have unfilled ecological niches (Moles et al. 2007; Schrieber and Lachmuth 2017). Cities offer a variety of natural and anthropogenic resources and habitats for new populations to exploit and settle (McDonnell and Hahs 2015; Kühn et al. 2017) and many routes for natural and human-mediated dispersal (discussed further in the next section; Padayachee et al. 2017). Cities also have unique climatological features (e.g., the urban heat island effect) that may be more suitable to the invading population than surrounding areas (Leniaud et al. 2009; Menke et al. 2011; Groeneveld et al. 2014). For instance, subterranean termites *Reticulitermes* have been found in urban dwellings in Hamburg, Germany, Paris, France, and Toronto, Canada, while the nearby forest habitats are too cool to support termites (Leniaud et al. 2009).

Cities present unique habitat features for invading organisms, but recent work suggests that it may also be important to consider the recent evolutionary trajectory of the invader. There are numerous cases of invasive species establishing in urban areas after a single introduction. European starlings *Sturnus vulgaris* in New York, USA and black rats in the Mediterranean basin had low founding population sizes and low genetic diversity, but nevertheless successfully established and spread (Linz et al. 2007; Colangelo et al. 2015). These populations may have had a particular advantage: anthropogenically-induced adaptation to invade (AIAI; Hufbauer et al. 2012). AIAI benefits synurbic species because they are pre-adapted to metropolitan conditions in their native ranges, and these adaptations contribute to invasion success (Hufbauer et al. 2012; Duncan 2016; MacDougall et al. 2018). This theory posits that urban habitats and the associated selection pressures are similar despite geographic distance. Therefore, a city environment in Asia

may be more similar to a city environment in North America than either of those cities are to their adjacent, less urban areas, both in terms of biotic communities and abiotic landscape and environmental properties (McKinney 2006; Pauchard et al. 2006; Pickett et al. 2011; Polsky et al. 2014). Adaptations to cities include increased aggression and risk-taking behaviors, faster growth and reproductive rates, pesticide/insecticide resistance, and changes in thermal tolerances (Duckworth and Badyaev 2007; Romero et al. 2007; Dalla Bona et al. 2012; Koch et al. 2016; Brans et al. 2017a; Brans et al. 2017b; Diamond et al. 2017; Kamdem et al. 2017; Miranda 2017; Wang and Hung 2019; see Johnson and Munshi-South 2017 for a review on evolution in cities). The degree to which these adaptations may increase invasiveness is debated, and research on the evolutionary ecology of invasive populations in cities is still developing (Hufbauer et al. 2012). If adaptation to urban environments enhances invasiveness, we expect to see cities as major sources for invasive populations, new species becoming globally invasive following their adaptation to urban areas, and traits evolving multiple times in invasive urban populations.

Probability of successful establishment may also increase if populations undergo rapid evolution in the invaded area. Rapid evolution can be facilitated by increased genetic diversity following multiple introductions. For example, greenfinch *Carduelis chloris* populations in New Zealand, house finch *Carpodacus mexicanus* populations in Eastern North America, and the brushtail possum *Trichosurus vulpecula* in New Zealand all experienced multiple introductions and exhibited comparable or elevated genetic diversity compared to populations within their native ranges (Triggs and Green 1989; Merilä et al. 1996; Wang et al. 2003; Dlugosch and Parker 2008). Research on rapid evolution in urban invasions is limited. However, laboratory experiments conducted by Li et al. (2018) showed that admixture from multiple populations increased establishment success via increased reproductive ability in the invasive ladybird *Cryptolaemus montrouzieri*.

Genetic diversity and structure in cities

Several patterns of genetic structure may emerge following establishment (**Figure 1.1**). An introduced population with high genetic diversity but low genetic structure throughout a city suggests a recent, rapidly spreading invasion and/or high rates of movement and genetic exchange across the urban range. Patterns of high diversity and low structure are common in synurbic invasive invertebrate and rodent populations (Gardner-Santana et al. 2009; Mangombi

et al. 2016; Rutkowski et al. 2017; Sherpa et al. 2018). However, the cause of this pattern varies between invasions. The harlequin ladybird *Harmonia axyridis* invasion in Poland showed high genetic diversity and low genetic structure initially, which was attributed to a large founding population and rapid spread (Rutkowski et al. 2017). In contrast, longer established urban populations of the Asian Tiger Mosquito *Aedes albopictus* in Reunion Island, France, had higher genetic diversity and were more genetically structured than newly-introduced European populations, implying the accumulation of genetic variation over time (Sherpa et al. 2018).

High genetic diversity and gene flow could facilitate rapid evolution and the spread of adaptations beneficial to the population, respectively, which is worrisome because pesticide resistance and diseases may proliferate rapidly under these conditions (Caprio and Tabashnik 1992; Miller and Sappington 2017; Collins and Schlipalius 2018). Conversely, high gene flow may be a benefit for management when using genetic control techniques (Webber et al. 2015; National Academies of Sciences and Medicine 2016). High migration will allow the altered gene to propagate across populations, so fewer releases of modified individuals are required for effective control (Prowse et al. 2017). Population and landscape genetic research can inform strategic management regimes by enhancing demography data for the invasive population and identifying areas of elevated gene flow to optimize control strategies.

Some invasive populations show both high genetic diversity and genetic structure within cities. In such cases, the invasive population is predicted to exhibit metapopulation dynamics because there are discrete, fragmented patches connected by limited migration (**Table 1.1**; **Figure 1.1**; Facon and David 2006). This pattern is frequently observed in synurbic arthropod species with limited natural dispersal distances, such as termites, bedbugs, and cockroaches (Crissman et al. 2010; Vargo et al. 2014; Akhoundi et al. 2015; Baudouin et al. 2018). Baudouin et al. (2018) identified four differentiated subpopulations composed of multiple colonies of termites *Reticulitermes flavipes* in Paris using microsatellite loci. In a study of bedbug populations in a single suburb of Paris, France, Akhoundi et al. (2015) found that populations were highly differentiated from one another and within-population genetic diversity was low, potentially established from a single fertilized female. Similarly, German cockroach *Blattella germanica* populations showed signals of panmixia within, but not between, infested buildings in two separate studies (Crissman et al. 2010; Vargo et al. 2014). This scenario with high genetic diversity across the metapopulation paired with isolation between subpopulations suggests a

need for spatiotemporally comprehensive sampling, including investigation of genetic structure when conducting population genetics studies on urban invasions.

In a genetically differentiated metapopulation scenario, a beneficial mutation in a single population is not as likely to spread unless that mutation increases an individual's ability to disperse or reproduce. However, management of invasive species in one location is unlikely to have large-scale effects on overall population size, requiring separate local applications. The exception may be if populations exhibit a core-satellite pattern, in which subpopulations arise from a single source within the city (Hanski 1982). In this case, management would be most effective when targeting the source population, and high-resolution genetic sampling would assist in locating said source and identifying avenues of spread (**Table 1.1**).

**Spread: Urban areas and spread of invasive species**

Measuring genetic diversity and structure of invasive species is necessary, but not sufficient, to understand the ecological and evolutionary feedbacks between populations and urban environments. It is also essential to understand the mechanisms of persistence and dispersal of the invading population and how these mechanisms are impacted by the urban environment. Studying these features allow researchers to address one of the most pressing questions following an urban invasion event: whether the species will spread within the city and outward into rural areas less affected by human settlement. The ability for an invasive species to spread within the city has economic, ecologic, and public health consequences. Spread into rural areas may threaten rare and vulnerable ecosystems and native species, risk spreading diseases and parasites to other locations, and make invasion management more difficult as the invasive species becomes regionally distributed. Again, we can use genetic tools to investigate the influence of landscape on species dispersal and gene flow within the system, especially in urban areas where human activity and infrastructure have pervasive yet disparate effects on different species (**Table 1.1**).

<u>Spread within the city</u>

Anthropogenic features (e.g. roads, buildings) and resources (e.g. food waste, artificial water supplies) can facilitate gene flow and connectivity in invasive populations as they spread within cities. Just as the distribution of humans and their refuse influence the presence and abundance of

invasive species, human density directly mediates genetic connectivity between some invasive populations. For example, Combs et al. (2018) showed that gene flow in New York City brown rats (*Rattus rattus*) decreased in midtown Manhattan, which is less residential than neighboring up- and downtown. Close associations between invasive species and human density can lead to more opportunities for invasive dispersal via human-mediated transport within a city. Landscape genetics studies provide evidence that transportation networks facilitate gene flow for metropolitan populations of many invasive species (Rosenfeld et al. 2016; Baudouin et al. 2018; Combs et al. 2018). For instance, Baudouin et al. (2018) compared termite colonies along railroads in Paris, France to colonies located away from railroads. They found that proximity to a railway led to more genetically similar populations, even when separated by greater geographic (Euclidean) distance. Rosenfeld et al. (2016) found a similar pattern with bed bug populations and distance to the subway system in New York City, USA. This pattern also arises for species that are not typically human associated. Railways facilitated gene flow in wall lizard *Podarcis muralis* individuals that were admixed from invasive and native populations, but not in native populations without admixture (Beninde et al. 2018).

In addition to anthropogenic structures and transportation networks, invasive species exploit urban green spaces. This is an important consideration given the growing emphasis on "greening" cities to improve biodiversity and offer ecosystem services to residents (Li et al. 2005; Goddard et al. 2010; Kong et al. 2010; Francis and Lorimer 2011; Lovell and Taylor 2013). Numerous studies have found that urban green spaces harbor a plethora of invasive species (Pauchard et al. 2006; Ishii et al. 2016; Mayer et al. 2017; Moricca et al. 2018; Riley et al. 2018; Sasaki et al. 2018; Useni Sikuzani et al. 2018; Zefferman et al. 2018). This poses a dilemma for urban conservation; it remains a complex question as to how to increase diversity and abundance of desirable native species and simultaneously stop the propagation and spread of invasive species.

Genetic tools can aid in understanding and managing invasive species without hindering efforts to promote native species conservation in cities (**Table 1.1**). Investigation into gene flow and genetic connectivity of invasive species in cities may allow researchers to identify variation in how native and invasive species utilize urban green spaces, which can subsequently inform adaptive city planning to reflect these differences. For example, Ishii et al. (2016) found that after complete removal of the invasive warm-temperate palm *Trachycarpus fortunei* in an urban

forest, new colonization of invasive species was concentrated around edges, while the native species thrived on the interior of the urban forest. Similarly, Kong et al. (2010), using a least-cost path method, found that adding narrow green strips along roads only marginally improved green space connectivity for native species, but other studies show that these same green spaces are frequently exploited by invasive species (Jodoin et al. 2008; Christen and Matlack 2009; Gippet et al. 2016).

Anthropogenic corridors for dispersal are highly context dependent. For example, road networks can both enhance and inhibit gene flow in the same invasive species. In instances of human-mediated dispersal, road networks increase gene flow. Adult *Aedes albopictus* can hitchhike in vehicles, traveling much further than their natural flight distance (Eritja et al. 2017). Similarly, invasive snails show increased levels of gene flow between populations located close to roads (Medley et al. 2015; Balbi et al. 2018; Schmidt et al. 2018). However, traveling across roads (rather than along roads) may impede gene flow of these same species (Schmidt et al. 2018), revealing a complex pattern of dispersal driven by human travel. Landscape genetic approaches allow researchers to compare hypotheses about how features influence connectivity and can assess how the same feature may affect gene flow differently depending on context. Paired with the knowledge of invasion history gleaned from population genetics, these tools permit researchers to evaluate invasion scenarios and gain a more accurate picture of the nuances involved in fine-scale invasion spread across a complex urban landscape.

Spread through the urban-rural gradient

Many of the same landscape features and dispersal processes that govern the movement of invasive species within a city apply to their outward spread into rural areas. For example, researchers reported dispersal into more rural areas via human-transportation along roads for several invasive mosquito species, including *Aedes japonicus* and *Aedes albopictus* (Medley et al. 2015; Egizi et al. 2016). Medley et al. (2015) showed that highways are important corridors connecting *Aedes albopictus* populations between the core and edges of their range. Roadside ditches provide both habitat and dispersal corridors for invasive plants, and aquatic plants have invaded nearby wetlands via these ditches (Jodoin et al. 2008; Christen and Matlack 2009).

Natural corridors can also facilitate the spread of invasive species outward from cities. Multiple studies have shown that invasive plants spread via urban river networks into

uncolonized areas (Säumel and Kowarik 2010; Arredondo et al. 2018). Arredondo et al. (2018) implicated streams as dispersal corridors for an invasive grass *Brachypodium sylvaticum* by investigating genomic signatures of unidirectional gene flow. In addition to natural features, fauna can also facilitate invasion along the urban-rural gradient. Both native and invasive species can be vectors of spread for invasive plants and parasites (Gosper et al. 2005; Buckley et al. 2006; Rollinson et al. 2015; Thibault et al. 2018). The native Wahlberg's epaulette fruit bat *Epomophorus wahlbergi* feeds primarily on the invasive syringa fruit *Melia azedarach* in urban areas during the winter when other foraging options are limited and then disperses the seeds to nearby areas (Rollinson et al. 2015). Comparably, the invasion of the red-vented bulbul bird *Pycnonotus cafer* in New Caledonia increases the risk of spreading invasive fruit species from the city to nearby, rural islands in the archipelago due to an apparent feeding preference on invasive rather than native species (Thibault et al. 2018).

Considerations for spread out of urban areas

When discussing invasive species connectivity and dispersal, several considerations that are important for movement into rural areas. Spread out of cities into adjacent rural landscapes exposes invading populations to novel landscapes, habitats, and species that may prevent or enhance their further spread. For instance, food sources may change or decrease, competition from non-urban populations may increase, and there may be a release from anthropogenic causes of mortality. Further, abiotic properties differ. Rural habitats may be cooler due to distance from urban heat islands, and soil and moisture conditions can vary considerably along the urban-rural gradient (Ariori et al. 2017). How an urban invasive species can spread into other habitats depends on its ability to adapt to these different conditions. If an invader is preadapted to be an urban specialist, the likelihood of outward spread may be low (Hufbauer et al. 2012). Instead, we might see a pattern of range expansion via city-hopping and human-mediated dispersal between urban areas. Other invasive populations could be generalists, in which case outward spread into rural landscapes may occur through both natural and assisted dispersal.

Several studies suggest that urban-adapted populations disperse less than their rural conspecifics, implying an anthropogenic adaptation against invasion into rural areas (Cheptou et al. 2008; Ożgo and Bogucki 2011; Vangestel et al. 2011). Cheptou et al. (2008) found that urban environments rapidly selected against the production of dispersing seeds in the weed *Crepis*

*sancta*. However, there is also evidence that evolution in invasive species increases dispersal ability, including in species that are urban invaders (Williams et al. 2016; Wagner et al. 2017; Williams et al. 2019). This rapid evolution may be fueled by multiple introductions and admixture likely to be found among invasive populations in cities and may result in advantageous traits beyond dispersal ability. Wagner et al. (2017) experimentally found that bean beetles *Callosobruchus* genetic diversity and admixture increased the speed of the invasion. For invasive species that do spread outward into rural environments, the initial urban populations could be sources for continual genetic enrichment. The invasive glossy buckthorn *Fragula alnus* was introduced multiple times to American cities, and urban populations show high levels of allelic richness and heterozygosity. Consequently, these urban populations have been identified as a source for continuous migrations and genetic enrichment to rural populations (De Kort et al. 2016). Propagules derived from a genetically diverse source, such as an established urban population, may be more successful when spreading outward to novel environments (Wagner et al. 2017), demonstrating the mechanisms by which multiple introductions can increase invasion success.

The selection pressures on interior, urban populations compared to rural populations may result in signals of differential evolution. Johnson et al. (2018) found that the ability of the invasive white clover *Trifolium repens* to produce an antiherbivore chemical increased with distance from cities along twenty urban-rural gradients, but also found extensive gene flow between populations along the same gradients. This illustrates the disparate selection pressures on rural and urban populations, which can lead to unique evolutionary responses even when there is continuous genetic exchange (Johnson et al. 2018). These results also emphasize the importance of quantifying the effects of landscape and environment on genetic connectivity and traits (**Table 1.1**).

## Conclusion

Population genetic research has incredible potential for aiding adaptive management of invasive species. The most cost-effective strategy for controlling invasive species is preventing propagules from arriving and establishing in urban environments in the first place (McGeoch et al. 2016; Faulkner et al. 2017). Identifying source locations and routes of spread using genetic tools will allow managers to develop prevention strategies and policies (Cristescu 2015; Garnas

et al. 2016). Further, genetic tools can be used to detect cryptic invasions, for example when populations are small, or the invaders are morphologically similar to native species (Morais and Reichard 2018).

For invasive species that are already established, an understanding of their genetic characteristics within and beyond cities will aid management decisions. Richardson et al. (2017) and Combs et al. (2018) used the results of their respective cityscape genetics studies to target eradication units for invasive Norway rats in Salvador, Brazil, and brown rats in New York City, USA, to reduce the risks of reinvasion. Landscape genetics can also identify features that facilitate gene flow of invasive species; these features can be monitored, removed, or altered to limit dispersal (Beninde et al. 2016; Baudouin et al. 2018).

In this review, we have outlined evidence that urban areas facilitate the introduction, establishment, and spread of invasive species and identified ways that genetic tools can enhance our understanding of the urban invasion process. We have highlighted opportunities where the results of landscape and population genetics studies can be translated into actionable items for management and briefly highlighted how urban areas impact aquatic invasions (**Box 1**). Cities are complex landscapes that catalyze adaptation and drive rapid evolution. Studying the population genetics of invasive species can illuminate the inner workings of the invasion process and is critical for targeted, adaptive, and proactive management interventions in multifaceted urban environments.

Urban areas present an inimitable biome for applying population and landscape genetic approaches to understanding invasion. The threat of invasive species to global biodiversity gives immediacy to our need to understand how urban systems affect their ability to spread and adapt.

**Box 1. Aquatic Invasions and urbanization *in brief***

Cities are also hubs for invasions and subsequent spread in marine and freshwater habitats. The extent of anthropogenic disturbance and hard structure in aquatic environments has increased with global urbanization; this applies to both and freshwater habitats (e.g., “ocean sprawl,” Firth et al. 2016) damming, channelization, water transfers, and geochemical shifts from urban and agricultural pollutants; (Beaumont 1978; Anastacio et al. 2018). The arrival of aquatic invaders is often linked to pathways that mirror terrestrial invasions. Foremost are trade routes (e.g., Cook et al. 2007; Drake and Lodge 2007; Ojaveer et al. 2018). For example, the United States invasion

of the zebra mussel *Dreissena ploymorpha* was likely introduced via Eurasion ballast water (Johnson and Padilla 1996). Intentional or accidental introductions from the aquarium trade (Chang et al. 2009) and purposeful introduction of recreationally or economically important species also represent important vectors for introduction and spread (Gozlan et al. 2010).

Like terrestrial invasions, a pattern of disturbance-facilitated spread may hold true in aquatic systems. Anthropogenic structures such as canals can serve to increase connectivity between marine systems (Ruiz et al. 1997; Katsanevakis et al. 2013), and some aquatic invaders associate with anthropogenically-modified habitats (e.g., invasive ascidians, a group associated with benthic fouling; Airoldi et al. 2015). Population genetic tools will be crucial to our understanding of marine invasion dynamics and management (see Darling et al. 2017 for a summary). In brief, advancements in genetic technology, such as environmental DNA and bulk DNA methods, are particularly well-suited for the study and control of aquatic invasions. These tools allow us to overcome many of the obstacles to marine invasion research, including detection, surveillance, and assessment of evolutionary potential.

**Further reading rapid-fire**: Leprieur et al. (2008) found that human activity predicted the number of invasive fishes in river basins across the globe, and that this effect was compounded with increased GDP for the basin. Johnson et al. (2008) found that invasive species were more likely to occur and had higher species richness in artificial lakes created by dams than natural lakes. Kwik et al. (2013) found that artificial stormwater retention ponds in Singapore harbored exclusively invasive fish.

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**Table 1.1** Processes and the expected genetic consequences for the stages of biological invasions and genetic tools researchers use to evaluate and test these hypotheses. Arrows indicate the directionality, when appropriate, of the process or impact.

| Stages of Invasion | Processes Contributing to Success | Expected Genetic Consequences | Genetic Tools |
|---|---|---|---|
| Introduction | ↑ Propagule pressure (# individuals and populations) | ↑ Initial genetic diversity<br>↓ Susceptibility to genetic drift | • mtDNA to identify sources<br>• Calculations of Hardy-Weinberg equilibrium & linkage disequilibrium to identify signatures of multiple introductions<br>• $F_{IS}$, allelic diversity, and effective population size to measure genetic variation and drift |
| | ↑ Sources of introductions | ↑ Initial genetic diversity | |
| Establishment | Preadaptation in native range | ↑ Fitness | • Quantitative genetic studies of traits to identify genes contributing to invasion success<br>• Population genomics to identify signatures of adaptation using outlier loci<br>• F-statistics and ordination methods to measure diversity and structure |
| | Rapid evolution in invasive range | ↑ Fitness | |
| | ↑ Population growth rate | ↑ or maintain Genetic diversity | |
| | ↑ No. of introductions | ↑ Genetic diversity<br>↑ Genetic structure | |
| | ↑ Degree of admixture | ↑ Genetic diversity<br>↓ Genetic structure | |

**Table 1.1** (continued).

| Stages of Invasion | Processes Contributing to Success | Expected Genetic Consequences | Genetic Tools |
|---|---|---|---|
| Spread | ↑ Dispersal ability,<br>↑ Frequency of facilitated dispersal | ↑ Probability of establishing new propagules<br>↑ Probability of detecting signatures of range expansion<br>↑ Gene flow<br>↓ Genetic structure | • Quantitative genetic studies to identify genes contributing to increased dispersal and damaging genotypes<br>• Population genomics to identify signatures of adaptation and leading edge of range expansion<br>• Landscape genetics to identify features important to dispersal<br>• Measures of gene flow frequency and direction and genetic connectivity to identify source populations for new propagules |
| | ↑ Adaptive capacity to new environments | ↑ Fitness<br>↑ Probability of establishing new propagules<br>↑ Probability of detecting signatures of range expansion | |
| | ↑ Habitat connectivity | ↑ Genetic diversity<br>↓ Genetic structure<br>↑ Probability of establishing new propagules<br>↑ Gene flow<br>↓ Genetic structure | |

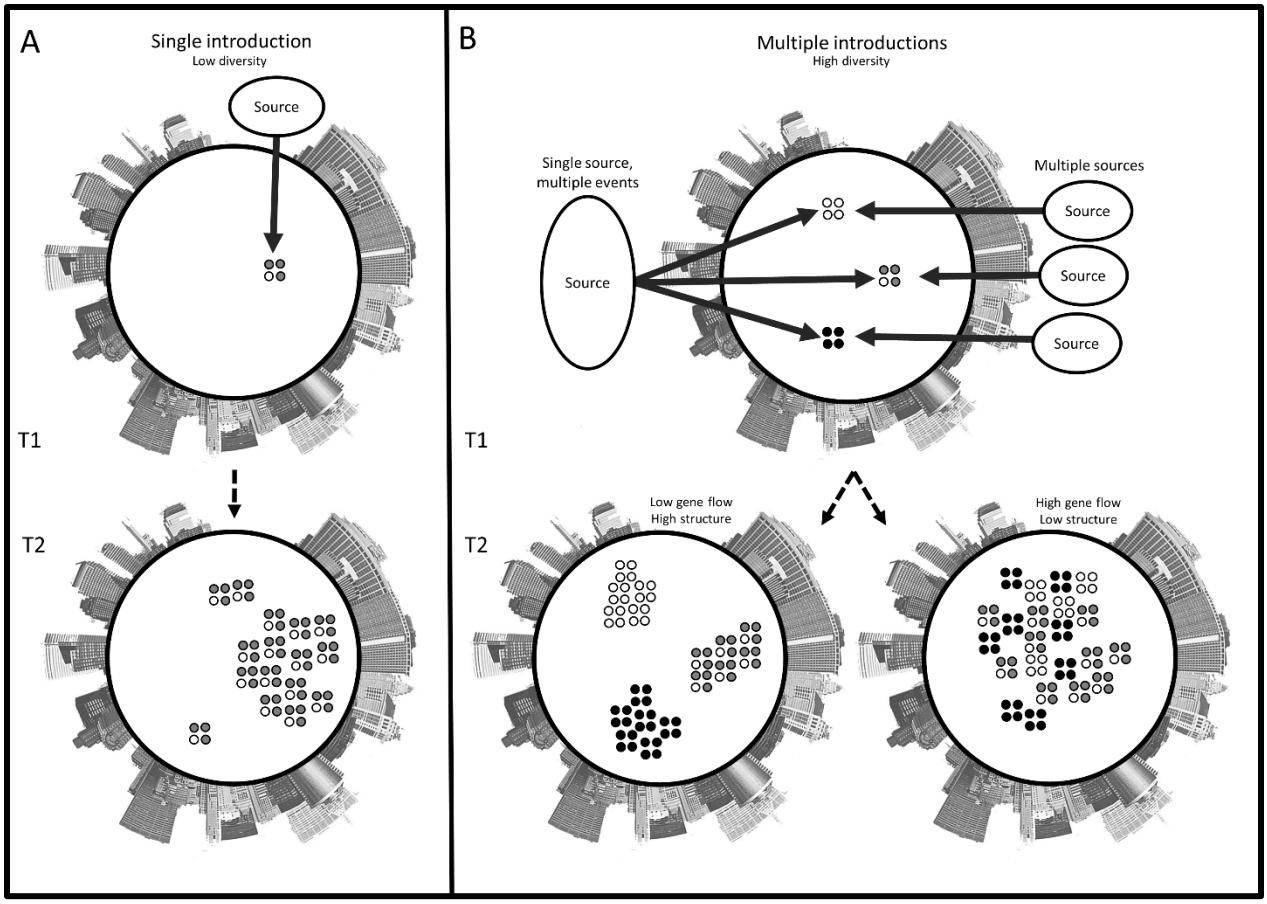


**Figure 1.1** Introduction history and modern gene flow affect genetic characteristics of invasive populations within an urban environment. Studying the level of genetic diversity and degree of genetic structure provides evidence for different introduction and establishment pathways in invasive populations, such as the number of introductions and realized dispersal and connectivity between subpopulations. For example, a) a single introduction during timepoint 1 (T1) results in limited genetic diversity during subsequent establishment and spread within an urban environment (T2). Alternatively, b) if there are multiple introductions, either from a single source over multiple events or from discrete sources, overall genetic diversity will be higher during timepoint 1. If gene flow is limited between individuals from introduction events, then after subsequent establishment (T2) we expect to observe little within-population diversity and high genetic structure (lower left). Conversely, if populations are well connected and gene flow is high, we expect to observe the scenario in the lower right.

# CHAPTER 2

## Heterogeneity in *Aedes albopictus* (Diptera: Culcidae): high variation in abundance and low genetic structure across the urban-rural landscape gradient

### Introduction

Landscape, the spatial pattern of suitable habitat patches and unsuitable matrix, has an undeniable role in determining the distribution, abundance, age structure, and genetic diversity of species. These population dynamics can be altered by land use change, such as urbanization. Urban landscapes are mosaics of habitat patches, such as parks, forests, and the built environment, and are characterized by anthropogenic impacts on abiotic conditions, habitat fragmentation, and increased impervious surface cover (Vitousek 1997; Irwin and Bockstael 2007; Baudouin et al. 2018). Urban areas can be hostile to native flora and fauna, and many species perform poorly in these environments (Czech et al. 2000; McKinney 2008). Yet, some species seem to thrive under urban conditions, including many nonnatives (McKinney 2006; Reed et al. 2020). Nonnative species are often introduced in cities, and those populations may display phenotypic plasticity or have pre-adapted traits that allow them to successfully establish and spread (Hufbauer et al. 2012; McDonnell and Hahs 2015; Williams et al. 2015; Reed et al. 2020). Consequently, their patterns of abundance and population genetic structure may differ from native species along urban—rural landscape networks (McDonnell et al. 1997; Blair and Johnson 2008; Ariori et al. 2017).

The urban—rural gradient was first discussed in an ecological context by McDonnell and Pickett (1990), who used the term "urbanization" to describe the increase in human population density and energy consumption. In turn, the urban—rural gradient describes the gradual reduction in intensity of human influence on the environment (Herrero-Jáuregui et al. 2018). The traditional ecological paradigm along this gradient predicts that population abundance is inversely related to degree of urbanization, and that population genetic differentiation increase with urbanization. There is support for this hypothesis among native taxa (McKinney 2002; Johnson and Munshi-South 2017). For example, Choate et al. (2018) found significantly fewer wild bee species in city centers than in field sites, and Vandergast et al. (2008) observed loss of genetic diversity and higher differentiation in urban populations of a native cricket, which

suggests a population bottleneck and isolation from the rural source. However, species response to urbanization is highly variable and depends both on the ecology of the taxa, the urban area(s) under study, and the surrounding landscape (McIntyre 2000; McDonnell and Hahs 2008). For example, the native European rabbit is more abundant in the urban green spaces of Frankfurt, Germany, likely due to agricultural landscape pressures within surrounding rural areas (Ziege et al. 2020). For a coastal population of butterflies in North Carolina, USA, natural barriers, but not urban features, determined genetic structure (Leidner and Haddad 2010). Response to urbanization is still more variable and context dependent for invasive populations. Numerous studies indicate either no effect of urbanization on genetic diversity or that diversity is higher in more urban areas (Reed et al. 2020).

*Aedes albopictus* is a widespread mosquito that is invasive in most of its range outside of east Asia, and it threatens human and wildlife health as a vector of zoonotic diseases (Hawley et al. 1987; Gratz 2004). As such, this is a good species to examine how urbanization affects a nonnative species ubiquitous in anthropogenic landscapes. *A. albopictus* was first detected in the United States in 1985 in Texas and spread eastward up the Atlantic Coast throughout the 1990s and 2000s (Hawley et al. 1987). It has now become established as far north as New York and as far west as southern California (Bonizzoni et al. 2013). *Aedes albopictus* is currently spreading through Europe, with recently detections in Germany & France and is established parts of Africa and South America (Bonizzoni et al. 2013). Australia has avoided invasion only through vigilant and extensive surveillance and chemical control (Muzari et al. 2017).

Much of *A. albopictus's* invasion success can be attributed to its ecology. *Aedes* mosquitoes in the subgenus *Stegomyia* lay desiccant-resistant eggs in small, ephemeral pools of water, which is why these mosquitoes are commonly referred to as "container-breeding mosquitoes." Containers used for oviposition can be natural (e.g., tree holes, bamboo shoots) or anthropophilic (e.g., planters, clogged gutters, and human refuse). These artificial containers are commonly exploited by *A. albopictus* and have contributed to its spread along with international trade (Benedict et al. 2007).

*Aedes albopictus* larvae also seems to have a competitive advantage over other mosquito species. In parts of the United States and Australasian islands, *A. albopictus* is replacing populations of another invasive *Stegomyia* mosquito, *Aedes aegypti*. In Florida, this replacement is through competitive displacement in both the larval state and through mating competition

(Juliano 2010; Bargielowski and Lounibos 2016; Burford Reiskind et al. 2018; Muzari et al. 2019).

The global distribution of *A. albopictus* is limited primarily by climate (Cunze et al. 2016). At finer spatial scales, *A. albopictus* presence and abundance depends on microclimate and landscape features (Spence Beaulieu et al. 2019; Hopperstad et al. 2020; Reiskind et al. 2020). For example, adults are intolerant of high heat and desiccation, which would limit their prevalence in low-latitude urban centers (Reiskind and Lounibos 2013). *Aedes albopictus* abundances can also vary widely over relatively short time periods, making it difficult to estimate true population sizes in an area and disentangle the relative importance of spatial and temporal variation (Crocker et al. 2017).

To better understand how local *A. albopictus* abundances, stability, and connectedness vary across landscapes, I used both abundance and genetic data to describe population-level patterns in two United States counties: Wake County, NC and Palm Beach County, Florida. Despite their geographic distance, both counties detected *A. albopictus* around the same time. First reports from Palm Beach and Wake Counties were in 1992 and 1993, respectively (O'Meara et al. 1993; Kraemer et al. 2015; Kraemer et al. 2017). However, *A. aegypti* has not been detected in North Carolina since 1995 while persisting and competing with *A. albopictus* in Florida (Reiskind and Lounibos 2013; Reed et al. 2019; Reiskind et al. 2020). *A. albopictus* in Wake County are most active during the summer and enter diapause during the winter. In contrast, they are active year-round in Palm Beach Co., and their landscape distribution may be limited by desiccation intolerance (Juliano et al. 2002; Lounibos et al. 2010; Reiskind and Lounibos 2013). Surveys of *A. albopictus* and other container breeding mosquitoes have been conducted in both counties (Reiskind and Lounibos 2013; Reed et al. 2019; Hopperstad et al. 2020; Reiskind et al. 2020). These studies primarily focused on climate variables, and when land-use variables were investigated, researchers found only marginal evidence they correlated with *A. albopictus* abundances. However, several factors may have influenced these studies, including sampling method and the area of land around a collection site used to quantify landscape predictors.

In this study, I analyze the population genetic characteristics of *A. albopictus* in these counties for the first time and expand on previous ecological research. I used abundance data and preserved *A. albopictus* individuals from a 2013 Palm Beach County survey (Hopperstad and

Reiskind 2016) and a 2016 Wake County survey (Reed et al. 2019), both collected using ovitraps, and I resampled each county in 2018 using both ovi- and adult traps. These samples allowed me to look broadly for patterns within and between zones of sites along the urban to rural gradient. Integrating genomic methods with ongoing surveillance further elucidates the effects of landscape and urbanization on the population dynamics of invasive species at fine spatial scales and provides place-based information on *A. albopictus* population structure in these counties for vector control.

## Methods

### Study Sites

Palm Beach County is 6,172 $Km^2$ with an estimated population of 1.5 million people in 2019 for an average population density of 243 people/$Km^2$. Wake County is smaller at 2,220 $Km^2$, but, with a population of 1.1 million in 2019, has a population density of 495.5 people/ $Km^2$. Since 2014, the population of Palm Beach County has grown by 7.1%, while the population of Wake has grown by 13.4%. Urban areas of Palm Beach County are distributed along its coastline, while the rest of the county remains largely agricultural. In contrast, urban areas of Wake County are patchily distributed around Raleigh, its largest and most central city. Climatically, Wake County had an average summer temperature of 79.4, an average winter temperature of 43.9, and 51.60 inches of annual precipitation from 2006-2020. Over the same time period, Palm Beach County average summer and winter temperatures were 83.3 and 68.9, respectively, with 64.87 inches of rainfall annually.

To compare differences along the urban—rural gradient, I grouped sites in each county into geographically-delineated "zones". While quantitative measures of urbanization may lead to more robust analyses, I found that categorial variables were more appropriate for this descriptive study for several reasons. First, the spatial scale effects on *A. albopictus* population dynamics vary for different landscape features (McClure *et al.*, 2018; Montagner *et al.*, 2018; Kache *et al.*, 2019; Sanders *et al.*, 2020). Additionally, in Wake County, I found low variation in landscape features associated with urbanization at my sites, such as percent impervious surface, at multiple spatial scales. An inadequate range of a predictor variable across sites can lead to incorrect inferences about the relationship between that predictor and the response (Eigenbrod *et al.*,

2011). Both counties have spatial patterns of human development, so to avoid these pitfalls, I used geographic boundaries as proxies for level of urbanization.

In Palm Beach County, I defined zones based on proximity from the Intracoastal Waterway, where urbanization decreased with distance from the coast. The 0, 1, and 3 Km zones were the most urbanized, while the 15 Km zone was largely suburban or rural and the 8 Km zone represented a transition along the gradient (Hopperstad and Reiskind 2016). In Wake County, urbanization is highest at its center, where the city of Raleigh is located, and transitions into suburban and rural areas on the outskirts of the county. Consequently, I decided to group sites from Wake County into three zones separated by the major highways surrounding Raleigh: the inner zone was within the interstate I-440 and Interstate 40 beltway; the outer zone was between I-440, the I-540 beltway, and US route 1; and the outside zone was the remainder of Wake County (**Figure 2.1**).

Sampling

I used *A. albopictus* samples collected from two counties: Wake County, North Carolina, and Palm Beach County, Florida USA (Figures 2.2 and 2.3). In each county, I had two years of sampling and two sampling methods: adult and egg/larval collection.

In Wake County, we collected *A. albopictus* eggs from fifteen sites in 2016 weekly between 15 April – 26 October 2016 as part of a state-wide mosquito survey effort to characterize the distribution and abundance of *Aedes* species in North Carolina (Reed et al. 2019; **Figure 2.2A**). We placed traps strategically around the county in areas with some accumulation of human refuse and along forest/open edges. We selected sites in part based on accessibility and convenience. Consequently, the fifteen locations were five waste and recycling management centers, four gas stations, two residential backyards, and four miscellaneous buildings (school; government building; museum; and retail). We collected eggs using ovitraps, which are plastic cups lined with germination paper and filled with tap water. Female *Aedes* mosquitoes lay their eggs on the paper at the water line. We then collect the papers, hatched, reared, and identified the mosquitoes in the lab (for full methods, see Reed et al. 2019).

In 2018, I collected adult *A. albopictus* mosquitoes at 61 sites (**Figure 2.2B**) using BG sentinels baited with BG Lures, a proprietary chemical attractant targeted at anthropophilic *Aedes* species (Biogents GmbH, Regensburg, Germany). Five of the 61 sites were designated

sampling locations used in Wake County department of health and human services mosquito surveillance. To determine the locations of the remaining sites, I used the r.random.cells function in GRASS GIS (GRASS Development Team, 2018) to generate 100 random points across Wake County, each with a 1000m buffer within which no other points could fall. I chose the 1000m buffer size because previous studies on *A. albopictus* movement found that this species rarely naturally disperse further than this distance (Honório et al. 2003). For each of these 100 points, I generated a 100m buffer within which I could place a trap. I then selected points that were deemed "accessible", which I defined as areas within 1 Km of a public road and that were not entirely water, which eliminated 21 potential sampling locations. Of the 79 remaining, I randomly selected 60 using a random number generator in RStudio (RStudio Team 2018). I then exported these points and their buffers to Google Earth (Google Earth, Google 2008). Using Street View, I ruled out 6 of these sites because the buffers fell completely within private lands. The remaining 56 points had buffers that fell at least partially within public land. I received permission to place traps within Wake County public property from the Wake County Department of Health and Human Services. I collected mosquitoes from 7 June – 25 June 2018, sampling each location once a week, leaving the traps out to collect mosquitoes for 24 hours each. I then conducted an extra day of sampling for locations where a trap had failed, either due to a defective battery or removal of the collection funnel and bag (9 occurrences/181 trap*nights).

In Palm Beach County, I used *A. albopictus* egg and larval samples collected over six weeks from May—June 2013 by K.A. Hopperstad along 6 east-west transects at 5 locations each for a total of 30 sampling locations (Hopperstad and Reiskind 2016; **Figure 2.3**). For each transect, the locations were 0, 1, 3, 8, and 15 Km from the coast. These transects followed major roadways (from North to South): North Lake Boulevard (NLA), Okeechobee Blvd (OKE), Southern Blvd (SOU), Lake Worth Road (LWB), Lantana Rd (LAN), and Boynton Beach Blvd (BBB). I resampled 17/30 sites in 2018 using the same egg and larval sampling methods as 2013 and added BG Sentinels baited with BG Lures to capture adult mosquitoes (**Figure 2.3**). Due to limited time and traps, I was unable to resample all transects and locations, instead prioritizing sites and transects where *A. albopictus* had been found in 2013.

Genomic Library Preparation

I selected a subset of collected mosquitoes to build genomic libraries for sequencing following Burford Reiskind et al. (2016), which uses a double-digest restriction-enzyme associated DNA (ddRAD) approach to genomic sequencing. The sites I included for sequencing were ones with enough intact *A. albopictus* samples to extract at least 8 ng DNA/µL for. I extracted DNA from individual mosquitoes and used two restriction enzymes, *SphI* and *MluCI*, to fragment their genomes. I annealed barcodes to each fragment, size-selected fragments between 350-475 base pairs long using the Blu Pippin Prep at the NCSU Genomic Sciences Laboratory, and built and sequenced genomic libraries, each with 48 individuals. In Wake County, I sequenced DNA from 192 individuals in 2016 (4 libraries; all 15 sampling sites) and 336 individuals in 2018 (7 libraries; 42 sites). In Palm Beach County, I sequenced DNA from 96 individuals, 48 each for 2013 and 2018 (2 libraries; **Table 2.1**). I included individuals from each sampling site across libraries to minimize library and lane effects during sequencing. I sequenced two libraries per lane using two Illumina indices. Sequencing for Wake County 2016 libraries and one 2018 library was conducted at the NCSU Genomic Sciences Laboratory using the Illumina HiSeq 4000. Following the retirement of this machine partway through the project, the remaining libraries were sequenced at the Genomics & Cell Characterization Core Facility (GC3F) at the University of Oregon using the Illumina HiSeq 4000. For all libraries, I conducted single-end sequencing of 100 bp fragments.

Bioinformatic Processing

The Illumina platform de-multiplexed to indices differentiating the two libraries in each lane, producing one FASTQ file per library. For each library, I checked the phred score to ensure high quality of sequence reads using FASTQC (Babraham Bioinformatics; http://www.bioinformatics.babraham.ac.uk/projects/fastqc). I then ran the *process_radtags* script in STACKS version 2.00 (Catchen et al. 2011) to filter low-quality reads (phred score < 33), trim reads to 90 bp, and demultiplex barcodes to produce FASTQ files for each individual following Burford Reiskind et al. (2016). I conducted one STACKS *denovo* pipeline with all individuals ($n$ = 624), which generated a unique catalog of SNPs. I used the following parameters: minimum read depth (-*m*) of six; maximum number of mismatches between loci for an individual (-*M*) of 3; maximum number of mismatches allowed between loci for the catalog (-*n*) of 2. I then ran the

generated STACKS catalog through the STACKS *Populations* pipeline to removed SNPs that did not meet the following parameters: (1) present in a minimum of two populations (-*p* = 2) and (2) were not present in 75% of individuals in a population containing that SNP (-*r* = 0.75). I ran the *Populations* pipeline with five groups: (1) all individuals; (2) Palm Beach County individuals; and (3) Wake County individuals; (4) Wake County 2016 individuals; and (5) Wake County 2018 individuals.

Following the *populations* pipeline, I further filtered SNPs using PLINK, removing variants with a minimum allele frequency (MAF) of less than 0.01 and a genotyping rate (GENO) of less than 0.5. I then removed individuals that had less than 25% of the remaining SNPs genotyped (MIND). Finally, I used the hw.test function in the R package *pegas* v 0.14 (Paradis 2010) to identify SNPs out of Hardy-Weinberg Equilibrium and removed variants with a *P* value below the threshold value after applied a Bonferroni correction ($P < 0.05/\#$ SNPs).

Abundance Analyses

I tested for significant differences in *A. albopictus* abundance between zones and between years using generalized least squares (GLS) with a covariance structure to account for spatial autocorrelation (F. Dormann et al. 2007) with the *gls* function in the R package nlme v.3.1-152 (Pinheiro et al. 2021). If I detected significant differences in the generalized least squares model, I used the *glht* function in the R package multcomp (Hothorn et al. 2008) to conduct post-hoc multiple comparisons of means between groups. The way I designated zones resulted in inherent differences in the distance mean and variability between sites within each category. Consequently, I determined that GLS was the most appropriate model because it could account for distances between sites.

Genomic Analyses

I ran population genomic analyses to investigate patterns in genetic diversity, differentiation, and structure between sampling sites. To measure genetic diversity, I calculated expected heterozygosity ($H_E$) and the inbreeding coefficient ($F_{IS}$) corrected for small sample size for each location using the genetic_diversity function in the R package *gstudio* v1.5.2 (Dyer 2016). I tested for significant differences in mean genetic diversity between zones using GLS, described above. To test for genotypic differentiation, I ran exact tests using GENEPOP v.4.2 (Rousset

2008) between sites with the following parameters: dememorization 10,000, batches 500, iterations per batch 5,000. I also used GENEPOP to estimate pairwise $F_{ST}$ between sampling sites.

I evaluated genetic structure in two ways. First, I used the program STRUCTURE v.2.3.4 (Pritchard et al. 2000; Hubisz et al. 2009), which implements an individual-based Bayesian iterative algorithm to assign individuals to user-defined *k* clusters. I ran STRUCTURE using the admixture ancestry model with 10,000 burn-ins, 10,000 MCMC replications, and a *k* ranging from 1 to 10 with 10 iterations per *k* for the following datasets: all Palm Beach County individuals, Wake County 2016 individuals, and Wake County 2018 individuals. I used STRUCTUREharvester (Earl and vonHoldt 2011) to determine the value of *k* with the highest likelihood using the Evanno method (Evanno *et al.*, 2005). Second, I evaluated structure by conducting a discriminant analysis of principal components (DAPC), implemented in the R package *adegenet* v2.1.3 (Jombart 2008). DAPC can reveal more complex spatial genetic structure than k-mean clustering algorithms such as STRUCTURE and does not make assumptions based on population genetic models (Plue et al. 2018). I chose the optimal number of principal components (PCs) based on the PC value with the lowest root mean squared error and the highest mean success rate after cross-validation with a training set size of 0.95, 1000 replicates, and a maximum number of PCs equal to a third of the individuals included in the dataset to prevent overfitting the data (Jombart et al. 2008; Plue et al. 2018). I used the same data sets as with STRUCTURE and also ran the DAPC with Palm Beach County 2013 and 2018 separately.

I further investigated population genetic differentiation in DAPC within and between zones for each county. In Palm Beach County, I grouped sites at 0-3 Km from the Intracoastal Waterway, into a single zone because I only sequenced individuals at one site within these distances per transect. To investigate genetic structure within zones, I used cross-validation in DAPC to decide the optimal number of PCs to retain. To look at differences in degree of differentiation between zones, I fit zones separately with the same number of PCs and compared correct assignment rates. I interpreted higher rates as indicating greater genetic differentiation between sites within a given zone. To account for different numbers of sites within zones in Wake County 2016 and 2018, I used a rarefaction method to generate a frequency distribution for zones with more sites. To do this, I determined the zone with the fewest sites and randomly

selected an equal number of sites from the remaining zones. I then found the correct assignment rate for that subset of populations. I repeated this process over 1000 iterations to generate the frequency distribution for the zone and from which I calculated a mean and 95% confidence interval.

## Results

Mosquito Abundance

*Wake County 2016*

In Wake County 2016, the sites in the outer zone between beltlines I-440 and I-540 had the highest average abundance with 110.88 ± 31.48 eggs per ovitrap per week, followed by the outside zone (97.61 ± 22.70 eggs per trap per week) with the lowest in the inner zone (65.37 ± 31.62 eggs per trap per week). There were no significant differences in abundance between zones ($P$ = 0.3945; **Figure 2.4**).

*Wake County 2018*

In 2018, I trapped 2553 mosquitos, 2086 of which were *A. albopictus* (81.71%). Of the 61 sites I sampled, I found *A. albopictus* at all but two (**Figure 2.5**). Overall, the trap rate per daylight hour, defined as the hours between sunrise and sunset on the day(s) of collection, of *A. albopictus* across all sites was 0.9757 ± 0.2143 adults/daylight hour. The highest average abundance was in the inner zone, with 1.58 ± 0.79 *A. albopictus* adults per hour of daylight, while the lowest average abundance was in the outside zone with 0.612 ± 0.17 *A. albopictus* adults per daylight hour across 44 sites. The outer zone average was close to the inner zone, with 1.53 ± 0.56 adults trapped per daylight hour. As in 2016, there were no significant differences in adult abundance between zones ($P$ = 0.0880).

*Palm Beach County 2013*

In Palm Beach County 2013, average egg abundance increased with distance from the coast, with an average of 1.14 ± 0.37 *A. albopictus* eggs per trap per week at 0 Km and 34.3 ± 2.22 at 15 Km (**Figure 2.6A**). No *A. albopictus* were found at 7/30 sites (23.33%), all between 0-3 Km (**Figure 2.7A**). Distance had a statistically significant effect on egg abundance ($P$ = 0.0247), and post-hoc

tests showed that mean abundance at 15 Km was significantly greater than abundances at 0, 1, and 3 Km.

*Palm Beach County 2018*

In Palm Beach County 2018, I placed 92 ovitraps and found mosquito eggs on 52. From these, I counted 1,039 mosquito eggs. 383 mosquito larvae hatched, and I identified 204 *A. aegypti* and 179 *A. albopictus*. When I corrected for hatching rates, I calculated an average of 6.79 ± 0.49 *A. albopictus* eggs per trap. I found no eggs at one location and detected no *A. albopictus* eggs at 4/15 sites (26.67%). I did not detect *A. albopictus* eggs at any of the sites where *A. albopictus* eggs were absent in 2013. In addition, there were two sites where *A. albopictus* eggs were detected in 2013 but not 2018 (**Figure 2.6**).

I only sampled one site at the 0 Km distance in 2018, so this site was grouped with 1 Km sites for analysis. The 0-1 Km zone had the highest average abundance of *A. albopictus* eggs (12.71 ± 3.88), followed by the 8 Km zone (8.64 ± 2.25). The 3 Km zone had the lowest abundance on average, with 1.81 ± 0.34 *A. albopictus* eggs per trap per week. I detected *A. albopictus* eggs at only two sites between 0-1 Km, but they were present at high densities (LAN01: 22.86 and OKE01: 15.27 *A. albopictus* eggs per trap; **Figure 2.6B**). There were significant differences in mean abundance between zones ($P = 0.0202$), and post-hoc tests found that mean abundance in the 0-1 Km zone was significantly higher than the 3 and 15 Km zones (**Figure 2.7B**).

I captured 386 adult *A. albopictus* with the BG Sentinel trap in PBC 2018 out of the 2202 mosquitoes I identified (17.53%). Across all sites, I caught an average of 3.99 ± 0.33 *A. albopictus* adults each trap night. I did not detect adult *A. albopictus* at two sites, both of which had *A. albopictus* eggs present in 2013 but not 2018 (**Figure 2.6C**). Consistent with 2018 trends in egg abundance, the 0-1 Km distances had the highest *A. albopictus* adult abundance (7.06 ± 2.00) and the 3 Km distance the lowest (0.95 ± 0.134). I only detected adult *A. albopictus* at 3/5 sites sampled at these distances. However, I found high densities of adults at the same two sites with high *A. albopictus* egg abundance: LAN01 (22.0) and OKE01 (12.8). I did not find any significant differences in mean adult abundance between distances ($P = 0.3139$; **Figure 2.7C**).

There was a significant positive correlation between egg and adult abundance in 2018 ($P$ = 0.0002). However, there was no relationship between 2013 egg abundance and 2018 abundance for eggs or adults ($P$ = 0.6397 and 0.9023, respectively).

Population Genetics

*Wake County 2016*

In Wake County 2016, the STACKS *de novo* pipeline identified 4,003,920 SNPs across 192 individuals from 15 sites, and 166,852 were retained after the Populations pipeline. 15,669, 116,489, and 6,348 SNPs were removed for minimum allele frequency, genotyping rate, and HWE, respectively. Eleven individuals were removed for low genotyping rates, leaving a final dataset of 181 individuals and 28,347 SNPs (**Table 2.2**).

Expected heterozygosity of sites ranged from 0.1203—0.1389 and mean $H_E$ did not differ between the inner, outer, and outside zones ($P$ = 0.4360). The inbreeding coefficient $F_{IS}$ ranged from -0.0250—0.0953. Only one site, WA, had a negative $F_{IS}$ value, which indicates that individuals sampled at this location had higher levels of heterozygosity than expected under Hardy Weinburg Equilibrium (**Table 2.3**). Zone had a significant effect on $F_{IS}$ ($P$ = 0.0463), with the outside zone having a significantly higher mean $F_{IS}$ value (0.0826) than the inner zone (0.0401).

I found evidence of genetic differentiation within Wake County 2016 after accounting for multiple comparisons using a sequential Bonferroni correction (**Table 2.4**). Between sites, 8/105 pairwise comparisons were statistically significant. This pattern was driven by two sites, DPD and WA, which were more genetically differentiated than the remaining locations. Site DPD was a collection center in northeast Wake County, outside the central city of Raleigh, while WA was a restaurant located in a mixed residential/commercial area close to downtown Raleigh. DPD had an average of 48.09 *A. albopictus* eggs trapped per week compared to an overall average of 93.43 eggs, and WA had the lowest abundance of *A. albopictus* from all 15 sites (an average of 10.75 *A. albopictus* eggs per week). Pairwise $F_{ST}$ values ranged from 0.0017 to 0.0776. STRUCTUREharvester identified an optimal $k$ = 3 using the Evanno method (**Figure 2.8A**).

In DAPC, 50 PCs were retained after cross-validation with a correct assignment rate of 0.8066. Sample sites primarily formed one cluster, with two sites, WA and KDB, isolated from the remaining individuals (**Figure 2.9**). There was no signal of isolation by distance (Mantel test:

observation = -0.3934; $P = 0.977$). When I divided zones based on their location, the inner zone had four populations (42 individuals), the outer zone had five populations (56 individuals), and the outside zone had six populations (83 individuals). I found no signal of isolation by distance within the inner and outer zones, but I did find evidence of isolation by distance in the outside zone (Mantel test: observation = 0.3833; $P = 0.01$).

For comparisons within zones, the inner beltway had 13 PCs retained after cross validation, which yielded a correct assignment rate of 0.9048 (**Figure 2.10A**); the outer beltway had eight PCs retained and a correct assignment rate of 0.6964 (**Figure 2.10B**); and the outside beltway retained 26 PCs with a correct assignment rate of 0.7831 (**Figure 2.10C**). To detect if there were any significant differences in correct assignment rates between zones, I used 13 PCs for all zones. The inner zone again had a correct assignment rate of 0.9048, the outer zone a mean of 0.8380 (95% confidence interval: 0.7556 – 0.8889), and the outside zone a mean of 0.6654 (95% confidence interval: 0.5472 – 0.7924).

*Wake County 2018*

In Wake County 2018, the STACKS *de novo* pipeline identified 3,096,027 SNPs. 183,753 SNPs were retained in the Populations pipeline. During PLINK filtering, 14,040 SNPs were removed for MAF < 0.01 and 153,647 for genotyping rates < 0.50. Thirteen individuals were removed for having over 75% missing data. An additional 4,761 SNPs were removed after testing for HWE, leaving a final dataset of 276 individuals and 11,305 SNPs (**Table 2.2**).

There was minimal variation in expected heterozygosity, which ranged from 0.0937 to 0.1058, and I did not find evidence for differences in mean $H_E$ between zones ($P = 0.1019$). $F_{IS}$ was more variable and ranged between 0.0201—0.1562. The inner zone had the highest inbreeding coefficient (0.1120), but the difference between zones was not statistically significant ($P = 0.3040$; **Table 2.3**). Similarly, I found no significant genetic differentiation between sampling sites and no evidence of isolation by distance. Pairwise $F_{ST}$ values ranged from -0.017 to 0.0195. Of the 861 pairwise comparisons, 422 (49%) had zero or negative $F_{ST}$ values. STRUCTUREharvester identified an optimal $k = 4$, though most individuals had the majority of their ancestry assigned to the same cluster, which is a pattern consistent with a true value of $k = 1$ (**Figure 2.8B**).

In DAPC, 40 PCs were retained after cross validation for all individuals, with a correct assignment rate of 0.6200 across 42 populations (**Figure 2.11**). Most populations formed a single cluster, with some differentiation from the major cluster and sites S24, S26, and S41. Site S24 was located in a wooded area between businesses and a residential community; site S26 was located in an herbaceous area adjacent to croplands and residential single-family homes; and site S41 was located between a railroad and industrial zone.

When divided among inner, outer, and outside beltway zones, the inner zone contained four sites and 28 individuals, the outer zone had 14 populations and 87 individuals, and the outside zone 24 populations and 161 individuals. After cross-validation, seven principal components were retained in the inner zone with a correct assignment rate of 0.6786 (**Figure 2.12A**), 26 PCs were retained in the outer zone (correct assignment rate = 0.7011; **Figure 2.12B**); and 50 PCs were retained in the outside zone (correct assignment rate = 0.7826; **Figure 2.12C**).

As before, to compare correct assignment rates, I used rarefaction methods with the outer and outside zones and used seven PCs for all zones. The mean correct assignment rate for the outer zone was 0.6008 (95% CI: 0.4583 – 0.7917) and 0.5169 for the outside zone (95% CI: 0.3571 – 0.6786) compared to the baseline 0.6786 for the inner zone.

*Palm Beach County*

The STACKS *de novo* pipeline identified 1,558,453 SNPs across 96 individuals collected in Palm Beach County from 2013 and 2018. Of these, 132,007 were retained post-*populations* pipeline. 5,882 SNPs were removed for MAF < 0.01, and 110,673 were removed for have a genotyping rate across individuals of less than 50%. Six individuals were then removed for having a genotyping rate of less than 25% across all remaining SNPs. A further 1,451 SNPs were removed for not meeting the expectations of HWE across all individuals, leaving a final genomic dataset of 90 individuals (n = 45 for both 2013 and 2018) and 14,001 SNPs (**Table 2.2**).

I estimated genetic diversity separately for each year. In 2013, expected heterozygosity ranged from 0.0867—0.1269 within sites. There was significant variation in $H_E$ between distances ($P$ = 0.0180). The 8 Km distance had the highest heterozygosity and was significantly different from the 0-1 Km distance, which had the lowest (**Table 2.3**). Estimates of $F_{IS}$ ranged from -0.1238—0.1118. Mean $F_{IS}$ increased with distance from coast, but this trend was not

statistically significant ($P = 0.5710$). In 2018, expected heterozygosity ranged from 0.0938—0.1279 and $F_{IS}$ ranged from -0.1266—0.1022. As in 2013, $H_E$ was highest at the 8 Km distance and lowest in the 0-3 Km zone, but unlike 2013, these differences were not significantly different ($P = 0.1313$). In congruence with 2013, mean $F_{IS}$ was lowest between 0-3 Km, but again differences between distances were not significant ($P = 0.0803$).

Between all sites in 2013 and 2018, there was no significant genetic differentiation (exact G-test). Average $F_{ST}$ estimates between sites sampled in the same year were comparable to between-year pairwise comparisons (**Table 2.5**), and I found no evidence of isolation by distance. Combined across years, STRUCTUREharvester identified an optimal $k = 5$ from STRUCTURE results following the Evanno method (**Figure 2.8C**).

DAPC cross validation selected an optimal value of 25 PCs for combined 2013 and 2018 PBC samples, which yielded a correct assignment rate of 0.9222. The DAPC scatterplot showed most sampled individuals grouped into one cluster, though several sampling locations were isolated, including all SOU 2013 sites, BBB03 and 08 2018, and OKE15 2018. When individuals were separated by sampling year, DAPC was able to correctly identify mosquitoes from 2013 v. 2018 at a rate of 0.9111 ($p = 0.0008$ based on a null distribution) with 45 PCs.

When I ran 2013 sites alone through DAPC, I again saw that all sampled individuals across the SOU transect clustered separately, as did LAN08. Cross validation identified an optimal 20 PCs with a correct assignment rate of 0.9111 (**Figure 2.13A**). If grouped by transect vs. grouped by distance, distance has a higher accuracy rate with 20 PCs (0.8889 v. 0.8444). Each distance zone (0-1 Km, 8 Km, 15 Km) had three populations (SOU, LAN, and OKE) and 15 individuals. To examine genetic structure in DAPC within zones, I used a maximum of five PCs during cross-validation. The 0-1 Km zone retained four PCs with a correct assignment rate of 0.9333; the 8 Km zone retained three PCs with a correct assignment rate of 0.8000; and the 15 Km zone retained two PCs with a correct assignment rate of 0.7333 (**Figure 2.14A-C**). When I used the same number of PCs (two) to compare assignment rates between zones, both the 0-1 Km and 8 Km zones had rates of 0.8000, which equates to three incorrectly assigned individuals, compared to four at the 15 Km distance.

In 2018, cross-validation identified an optimal 12 PCs with a correct assignment rate of 0.8667 (**Figure 2.13B**). Again, there was no evidence of isolation by distance (Mantel test: observation = 0.2369, $P = 0.111$). In contrast with 2013, individuals grouped by transect had a

higher accurate assignment rate than distance (0.7556 v. 0.7333). Most sampled sites grouped into one cluster, with the exception of OKE15 and BBB03. When I investigated genetic differentiation between sites as each distance, each distance zone had three populations (BBB, LAN, OKE) and between 12-17 individuals. The 1-3 Km zone retained three PCs and had a perfect assignment rate (1.000); the 8 Km zone retained four PCs with a correct assignment rate of 0.6250; and the 15 Km zone retained five PCs with a correct assignment rate of 0.9412 (**Figure 2.14D-F**). When I compared assignment rates between all zones with three PCs, the 8 Km zone had a correct rate of 0.6250 (10/16 individuals assigned to the correct site) and the 15 Km zone had a rate of 0.9412 (16/17 individuals assigned to the correct site); these rates where the same as the rates with more PCs, but different individuals were assigned to different sites.

**Discussion**

Patterns within years and counties implicate urbanization as a factor in determining *A. albopictus* abundance, genetic diversity, and genetic structure. Broadly, I found that mean egg abundance was lower in urban centers, with the exception of the 0-1 Km zone in Palm Beach County 2018 (**Figure 2.7**). I did not find a consistent trend with adult *A. albopictus* abundance between Palm Beach County and Wake County 2018. Expected heterozygosity was lowest in sites within urban zones, indicating a loss of genetic diversity, but populations sampled in rural zones were more inbred (**Table 2.3**). I also observed more genetic structure between sites within urban zones than transitioning or rural zones in Wake County, but no in Palm Beach County. This indicates the important role that city history and land use in surrounding rural landscapes play in local population dynamics of *A. albopictus*.

Abundance

Wake County and Palm Beach County varied markedly in abundances of *A. albopictus* when compared to other mosquito species; *A. albopictus* made up over 80% of adults and eggs trapped in Wake County compared to Palm Beach with 55% of eggs in 2013, 46% of eggs or 14% of adults in 2018. This was not unexpected given the presence of *A. aegypti* in Palm Beach County, and the more diverse mosquito fauna in Florida attracted to our adult traps (Wilke *et al.*, 2019).

Trends in abundance in Wake County between 2016 eggs and 2018 adults were somewhat contradictory. Specifically, the inner zone had the lowest egg abundance in 2016 and

the highest adult abundance in 2018, though differences were not statistically significant in either year. While the scope if this study does not permit us to identify whether this switch was due to changes in mosquito population dynamics, land-use changes, or sampling methods between years, the latter seems most likely. In 2016, sites were specifically chosen based on where mosquitoes were likely to be found, while in 2018 sample sites were chosen randomly. Many of the sites in the 2018 outside zone were in landscapes less hospitable to high densities of *A. albopictus*. For example, sites were located by agricultural fields, in the forest interior, and in high grass, all areas not associated with high *A. albopictus* abundance (Barker et al. 2003, Reiskind et al. 2017). These results are also consistent with rural land use bifurcation (Warren et al. 2018). A limitation of urban-rural gradient studies is that they do not account for different land types within the rural regions, which could explain the differences in mean abundances within the outside zone in Wake County 2016 and 2018.

It is possible that the difference within the outside zone reflects differences in trapping approaches. Traps for host-seeking adult mosquitoes may be favored where host density is lower, while ovitraps may be favored in areas where larval habitat is scarce. I did not measure either competing larval habitats or host density, so these potential drivers cannot be discounted in explaining the observed patterns.

In Palm Beach County, I found opposing trends near the coast in 2013 and 2018. In 2013, mean abundance increased with distance from the coast, with a marked increase in abundance at the 8 and 15 Km zones. However, in 2018 mean abundance for both *A. albopictus* eggs and adults was highest at the 0-1Km distance. This was driven by two hotspots at the 1 Km distance with high *A. albopictus* density (**Figure 2.6B-C**). Both of these sites were in wooded areas with low surrounding impervious surfaces. One, LAN-01, was located in a naturally wooded area behind an apartment complex near garbage collection, and the second, OKE-01, was in a wooded but manicured park. If these sites were not included, mean abundance followed the same pattern as 2013, with the highest abundance found at the 15 Km distance. Importantly, I also sampled fewer sites in 2018. Therefore, 2018 *A. albopictus* abundances may not reflect a true change in population densities at these distances and should be interpreted with caution. The 3 Km distance consistently had a low average abundance across years and trapping methods, which is different than the historical distribution of this mosquitoes in Palm Beach County (Braks et al. 2003; Reiskind and Lounibos 2013; Hopperstad and Reiskind 2016). There may also have been higher-

than-average abundances of *A. albopictus* eggs in 2018 due to a lack of alternative oviposition sources at some sites. *Aedes albopictus* eggs require containers that go through wet-dry cycles and are often found at high densities in human refuse, yard ornaments, and used tires, while adults require areas that offer shelter and where blood meals are available (Hawley 1988). In 2013, Palm Beach County experienced a wetter-than-average summer, received over 8 inches more precipitation in July than the 30-year normal, while in 2018 summer precipitation was slightly below average (climatecenter.fsu.edu). This is further evidence that the life stages of *A. albopictus* may respond differently to landscape features. There is also the possibility that I was observing a shift in the niche of *A. albopictus* in South Florida, possible due to adaptation or as a response to the expansion of *A. aegypti* populations in the region (Leisnham & Juliano, 2010; Bargielowski *et al.*, 2013; Hopperstad & Reiskind, 2016).

Population Genetics

In both counties over both years, expected heterozygosity was lowest at sites in the most urbanized zone (inner zone in Wake County, 0-3 Km zone in Palm Beach County) and highest in the intermediate zone (outer zone, 8 Km zone). However, I only found a statistically significant difference between the Palm Beach County 2013 0-3 Km and 8 Km zones (**Table 2.3**). This suggests that genetic diversity is lower for *A. albopictus* in built landscapes and could indicate a recent population bottleneck and genetic isolation. This supports Manica et al's (2016) finding that highly developed areas were not high-quality habitats for *A. albopictus*. In contrast, patterns of mean inbreeding coefficients ($F_{IS}$) were more variable. For example, $F_{IS}$ was lowest for sites in the most urban zones in Palm Beach County and Wake County 2016, but highest in the same zone in Wake County 2018 (**Table 2.3**).

The inbreeding coefficient, despite its name, is not a direct measure of relatedness between individuals. Instead, it indicates the degree to which observed levels of heterozygosity within a population deviate from the expected levels of heterozygosity given the population's allele frequencies at the genetic markers under study. If we consider two populations with the same level of expected heterozygosity, the population with the higher $F_{IS}$ value will have more inter-individual genetic variation, and the population with lower $F_{IS}$ will have more variation within the individual. Therefore, the relatively low levels of expected heterozygosity paired with low $F_{IS}$ in urbanized zones indicate that there were fewer biallelic SNPs in those populations, but

that individuals were more likely to be heterozygous at those loci. This could indicate frequent mating events between unrelated individuals, perhaps from migration events. I observed an extreme example of this relationship in Palm Beach County, where $F_{IS}$ values were negative in the most urban zones. Negative $F_{IS}$ values can be difficult to interpret and represent deviations from HWE from excess heterozygotes (Johnson and Shaw 2015). This may be due to genetic structure within the sites that I did not account for, recently established or ephemeral populations, or bias in the parameter estimates (Guries and Ledig 1981; Ackerman et al. 2017; Zhang et al. 2020). To differentiate between these options would require more sampling for a longer time period and over multiple generations.

Between years and in both counties, there was little consistency in genetic differentiation. In Wake County 2016, I observed significant genetic structure across the county. However, this was not reflected in the STRUCTURE results, which did not show strong differentiation between the two sites implicated in the pairwise G-test, WA and DPD (**Figure 8**). Based on the DAPC analysis, the inner zone was the most differentiated, followed by the outer and outside zones. Sites within the inner zone were more genetically different from one another than within the outer and outside zones, indicating little gene flow between the urban-most locations. For example, in Wake County 2016, WA formed a separate genetic cluster in both DAPC scatterplots (**Figure 9**), despite being located less than 1000m from another sampled site, NRC. Overall, sites in the inner zone were genetically dissimilar, while sites in the outer and outside zones showed little genetic differentiation. This suggests that there is minimal gene flow between more urban locations but does not preclude gene flow in or out of urban areas to more rural populations.

Despite covering a much smaller area than Wake County, our sampling in Palm Beach County showed more genetic differentiation between sites. Because *A. albopictus* was first observed in both counties during a similar time period, this pattern is unlikely due differences in the length of time the species has been established. This observed genetic structure may be due to the presence of *A. aegypti*, a conspecific competitor of *A. albopictus*. Previous research has observed spatial partitioning of the two species in Palm Beach County, *A. aegypti* more abundant in the more urban coast and *A. albopictus* dominating the more rural interior (Reiskind and Lounibos 2013; Hopperstad and Reiskind 2016). However, if *A. aegypti* presence were the main force driving genetic structure of *A. albopictus* in Palm Beach County, I would expect strong

differentiation between *A. aegypti*-dominated sites near the coast (0-3 Km) and *A. albopictus*-dominated sites (15 Km). However, I observed genetic differentiation between sites within the 0-3 Km and 15 Km zones, with the least genetic differentiation at the 8 Km distance in both 2013 and 2018, which is the distance *A. albopictus* and *A. aegypti* co-occur most often (Hopperstad and Reiskind 2016).

When all individuals were combined, I saw more differentiation between sites in 2013 than in 2018. Examining historical data from Palm Beach County and our abundance data from 2018, there may be a process by which the competitive interaction between these species is becoming less important in determining the landscape distribution of *A. albopictus* (Braks et al. 2003; Reiskind and Lounibos 2013; Hopperstad and Reiskind 2016). This, in turn, may affect the potential for gene flow across the landscape. An alternative explanation could be sampling method, which would be consistent with observations of genetic differentiation between 2016 eggs and 2018 adults in Wake County. While I controlled for sampling siblings by limiting the number of larvae sequenced per ovitrap, female *A. albopictus* display skip-oviposition, where one female will lay eggs in multiple containers (Rey and O'Connell 2014; Davis et al. 2015). In addition, for the adult mosquitoes present at a site, only a selection of females will be gravid at a given time. This may limit the gene pool of larvae sampled at a site and lead to an underestimation of genetic diversity within and an overestimation of genetic differentiation between sites.

The patterns I observed across these two counties point to several areas for future research. First, I saw more genetic differentiation and variation in abundance when I sampled eggs rather than adults. While beyond the scope of this study to confirm that trap type yields different population genetic results, it is still worth noting, because many population and landscape genetic studies on *A. albopictus* use egg or larval samples (e.g., Ayres et al. 2002; Schmidt et al. 2017; Adilah-Amrannudin et al. 2018). If these sampling methods are used in research designed to inform mosquito control, especially those targeted for adult mosquitoes, overestimating genetic differentiation may lead to the misidentification of population management units. To devise location-based adaptive management practices, especially in light of the increase in genetic control methods of mosquito control, additional studies should measure gene flow and directional migration between populations, especially between and within urban

and rural locations, and investigate the role of the intervening landscape matrix in determining genetic diversity and connectivity.

Finally, this study emphasizes the challenging nuances of urban ecology, especially for nonnative species. I found evidence both consistent with and in opposition to the traditional urban—rural ecological paradigm (McDonnell and Pickett 1990; McDonnell et al. 1997). Genetic differentiation was generally highest in the most urbanized areas, but genetic diversity was overall similar across zones and patterns of abundance varied between years in both Wake and Palm Beach counties. Careful consideration of species ecology, introduction history, and location-specific features of the urban—rural network are necessary to predict population responses to human development.

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**Table 2.1** Summary of sequenced *A. albopictus* individuals for each County/Year combination, with the total number of sequenced individuals (*N*) and the range of individuals sequenced per site (*n*).

| **County/Year** | ***N*** | **No. sites** | **Range *n* per site** |
|---|---|---|---|
| Palm Beach County 2013 | 96 | 9 | 5 |
| Palm Beach County 2018 | 96 | 9 | 3-6 |
| Wake County 2016 | 192 | 15 | 7-18 |
| Wake County 2018 | 336 | 42 | 5-10 |

**Table 2.2** Summary of bioinformatic processing and single nucleotide polymorphism (SNP) filtering for three groups of samples: Palm Beach County (2013 and 2018), Wake County 2016, and Wake County 2018.

| County/Year | | Denovo Pipeline (STACKS) | Populations Pipeline (STACKS) | MAF (Plink) | GENO (Plink) | MIND (Plink) | HWE - Final Dataset |
|---|---|---|---|---|---|---|---|
| **Palm Beach County** | **Individuals** | 96 | 96 | 96 | 96 | 90 | 90 |
| | **Variants** | 1558453 | 132007 | 126125 | 15452 | 15452 | 14001 |
| **Wake County 2016** | **Individuals** | 192 | 192 | 192 | 192 | 181 | 181 |
| | **Variants** | 4003920 | 166853 | 151184 | 34695 | 34695 | 28347 |
| **Wake County 2018** | **Individuals** | 336 | 289 | 289 | 289 | 276 | 276 |
| | **Variants** | 3096027 | 183753 | 169713 | 16066 | 16066 | 11305 |

**Table 2.3** Mean expected heterozygosity ($H_E$) and inbreeding coefficient ($F_{IS}$) within groups for each county/year pair. Asterix (*) indicate groups whose differences in genetic diversity were statistically significant.

| County/Year | Zone | $H_E$ | $F_{IS}$ |
|---|---|---|---|
| Wake 2016 | Inner | 0.1300 | 0.0401* |
| | Outer | 0.1350 | 0.0717 |
| | Outside | 0.1320 | 0.0826* |
| Wake 2018 | Inner | 0.0984 | 0.1120 |
| | Outer | 0.1020 | 0.0790 |
| | Outside | 0.1000 | 0.0850 |
| Palm Beach 2013 | 0-1 Km | 0.1050* | -0.0024 |
| | 8 Km | 0.1230* | 0.0033 |
| | 15 Km | 0.1210 | 0.0017 |
| Palm Beach 2018 | 1-3 Km | 0.1060 | -0.0439 |
| | 8 Km | 0.1210 | 0.0547 |
| | 15 Km | 0.1150 | 0.0307 |

**Table 2.4** Pairwise $F_{ST}$ values between Wake County 2016 sites. Background color of site names shows the zone that site belongs to: red = inner, gray = outer, and blue = outside. Comparisons that were identified as significantly differentiated following an exact G-test with a sequential Bonferroni correction are bolded with a grey background.

| | CDM | NRC | VDR | WA | BPG | GWA | KDB | OOR | WNS | DPD | KER | MMR | OSR | SRC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NRC | 0.018 | | | | | | | | | | | | | |
| VDR | 0.017 | 0.028 | | | | | | | | | | | | |
| WA | 0.052 | 0.063 | 0.063 | | | | | | | | | | | |
| BPG | 0.014 | 0.023 | 0.031 | 0.067 | | | | | | | | | | |
| GWA | 0.016 | 0.013 | 0.022 | **0.054** | 0.014 | | | | | | | | | |
| KDB | 0.027 | 0.02 | 0.026 | 0.061 | 0.028 | 0.019 | | | | | | | | |
| OOR | 0.026 | 0.026 | 0.034 | **0.078** | 0.023 | 0.022 | 0.028 | | | | | | | |
| WNS | 0.011 | 0.013 | 0.019 | 0.057 | 0.023 | 0.009 | 0.024 | 0.023 | | | | | | |
| DPD | 0.014 | 0.020 | **0.028** | 0.050 | 0.021 | 0.011 | 0.028 | 0.030 | **0.019** | | | | | |
| KER | 0.012 | 0.010 | 0.022 | **0.050** | 0.015 | 0.007 | 0.016 | 0.017 | 0.013 | **0.011** | | | | |
| MMR | 0.007 | 0.014 | 0.023 | 0.045 | 0.020 | 0.012 | 0.019 | 0.030 | 0.013 | 0.012 | 0.006 | | | |
| OSR | 0.013 | 0.015 | **0.026** | 0.055 | 0.019 | 0.011 | 0.025 | 0.030 | 0.016 | 0.012 | 0.012 | 0.011 | | |
| SRC | 0.022 | 0.024 | 0.036 | 0.058 | 0.036 | 0.025 | 0.032 | 0.037 | 0.024 | 0.023 | 0.024 | 0.021 | 0.017 | |
| WDB | 0.010 | 0.014 | 0.022 | **0.050** | 0.017 | 0.007 | 0.016 | 0.020 | 0.012 | 0.013 | 0.002 | 0.007 | 0.010 | 0.021 |

**Table 2.5** Pairwise $F_{ST}$ values for Palm Beach County. Site names highlighted in blue are from 2013, while names highlighted in yellow are from 2018. $F_{ST}$ estimates between sites in the same year are shown in blue and yellow for 2013 and 2018, respectively, while comparisons between years are green. There were no significantly differentiated populations identified by the exact G test.

| | 2013 | | | | | | | | | 2018 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LAN01 | LAN08 | LAN15 | OKE00 | OKE08 | OKE15 | SOU01 | SOU08 | SOU15 | BBB03 | BBB08 | BBB15 | LAN01 | LAN08 | LAN15 | OKE01 | OKE08 |
| LAN08 | 0.144 | | | | | | | | | | | | | | | | |
| LAN15 | 0.091 | 0.051 | | | | | | | | | | | | | | | |
| OKE00 | 0.124 | 0.080 | 0.033 | | | | | | | | | | | | | | |
| OKE08 | 0.082 | 0.061 | 0.000 | 0.043 | | | | | | | | | | | | | |
| OKE15 | 0.115 | 0.066 | 0.011 | 0.048 | 0.027 | | | | | | | | | | | | |
| SOU01 | 0.127 | 0.093 | 0.042 | 0.086 | 0.050 | 0.061 | | | | | | | | | | | |
| SOU08 | 0.096 | 0.052 | 0.001 | 0.026 | 0.013 | 0.015 | 0.058 | | | | | | | | | | |
| SOU15 | 0.129 | 0.100 | 0.043 | 0.073 | 0.056 | 0.049 | 0.090 | 0.047 | | | | | | | | | |
| BBB03 | 0.205 | 0.178 | 0.105 | 0.153 | 0.116 | 0.131 | 0.158 | 0.107 | 0.137 | | | | | | | | |
| BBB08 | 0.136 | 0.093 | 0.057 | 0.092 | 0.050 | 0.064 | 0.086 | 0.057 | 0.089 | 0.146 | | | | | | | |
| BBB15 | 0.087 | 0.060 | 0.024 | 0.046 | 0.028 | 0.034 | 0.049 | 0.032 | 0.056 | 0.124 | 0.042 | | | | | | |
| LAN01 | 0.088 | 0.065 | 0.011 | 0.049 | 0.029 | 0.015 | 0.048 | 0.014 | 0.043 | 0.083 | 0.037 | 0.032 | | | | | |
| LAN08 | 0.091 | 0.058 | 0.000 | 0.042 | 0.000 | 0.017 | 0.051 | 0.008 | 0.043 | 0.105 | 0.054 | 0.023 | 0.017 | | | | |
| LAN15 | 0.094 | 0.064 | 0.020 | 0.059 | 0.031 | 0.033 | 0.066 | 0.032 | 0.062 | 0.114 | 0.061 | 0.043 | 0.029 | 0.023 | | | |
| OKE01 | 0.149 | 0.098 | 0.043 | 0.081 | 0.041 | 0.061 | 0.086 | 0.042 | 0.078 | 0.173 | 0.079 | 0.062 | 0.061 | 0.034 | 0.069 | | |
| OKE08 | 0.097 | 0.058 | 0.017 | 0.039 | 0.017 | 0.039 | 0.030 | 0.019 | 0.056 | 0.134 | 0.064 | 0.034 | 0.020 | 0.020 | 0.027 | 0.065 | |
| OKE15 | 0.133 | 0.096 | 0.042 | 0.082 | 0.050 | 0.063 | 0.080 | 0.048 | 0.092 | 0.162 | 0.103 | 0.072 | 0.053 | 0.046 | 0.072 | 0.094 | 0.045 |

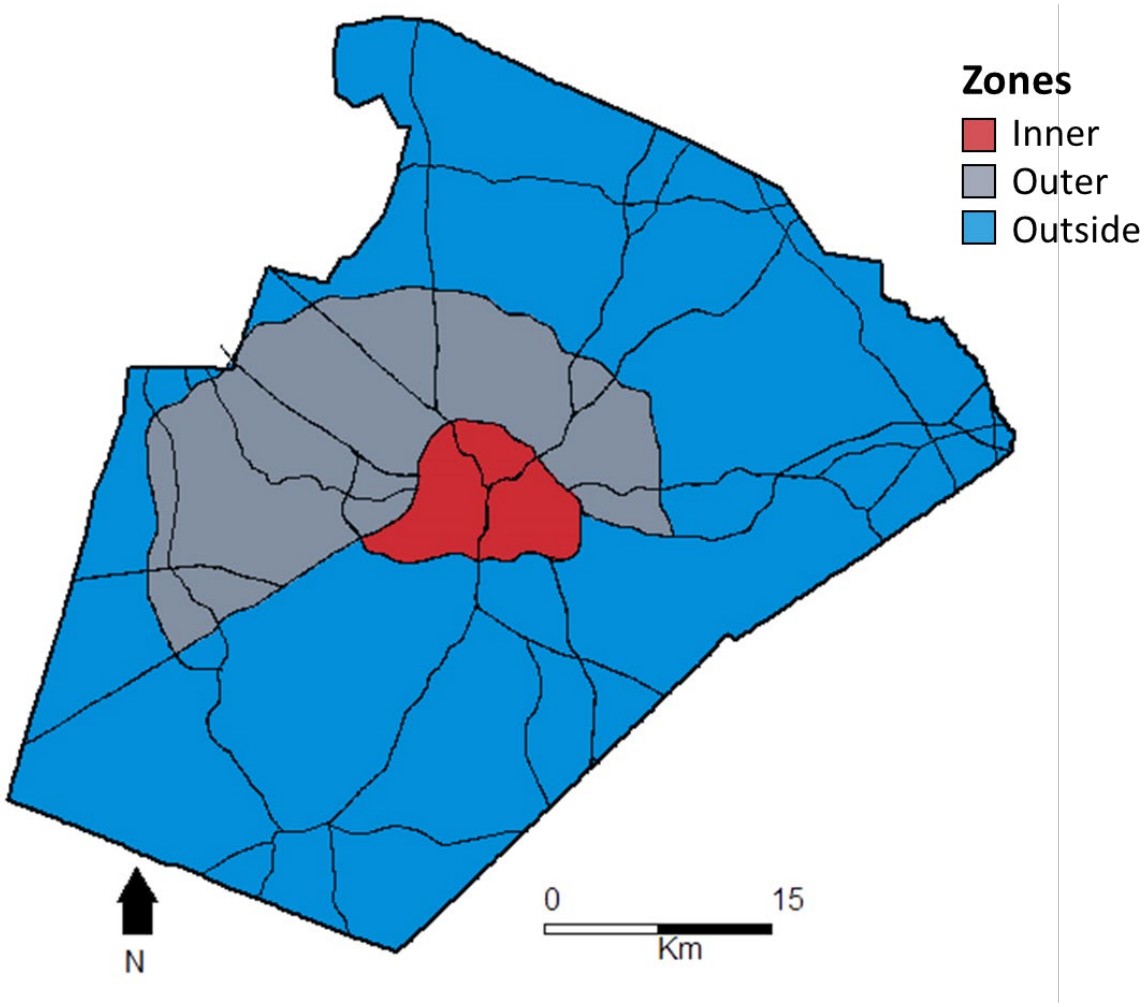


**Figure 2.1** Delineation of zones for Wake County which act as proxies for overall pattern of urbanization. In order from most to least urbanized: the inner zone is defined as inside the interstate 440 beltline; the outer zone is within the between the Interstate 540 beltline and I-440; and the outside zone the remainder of the county.

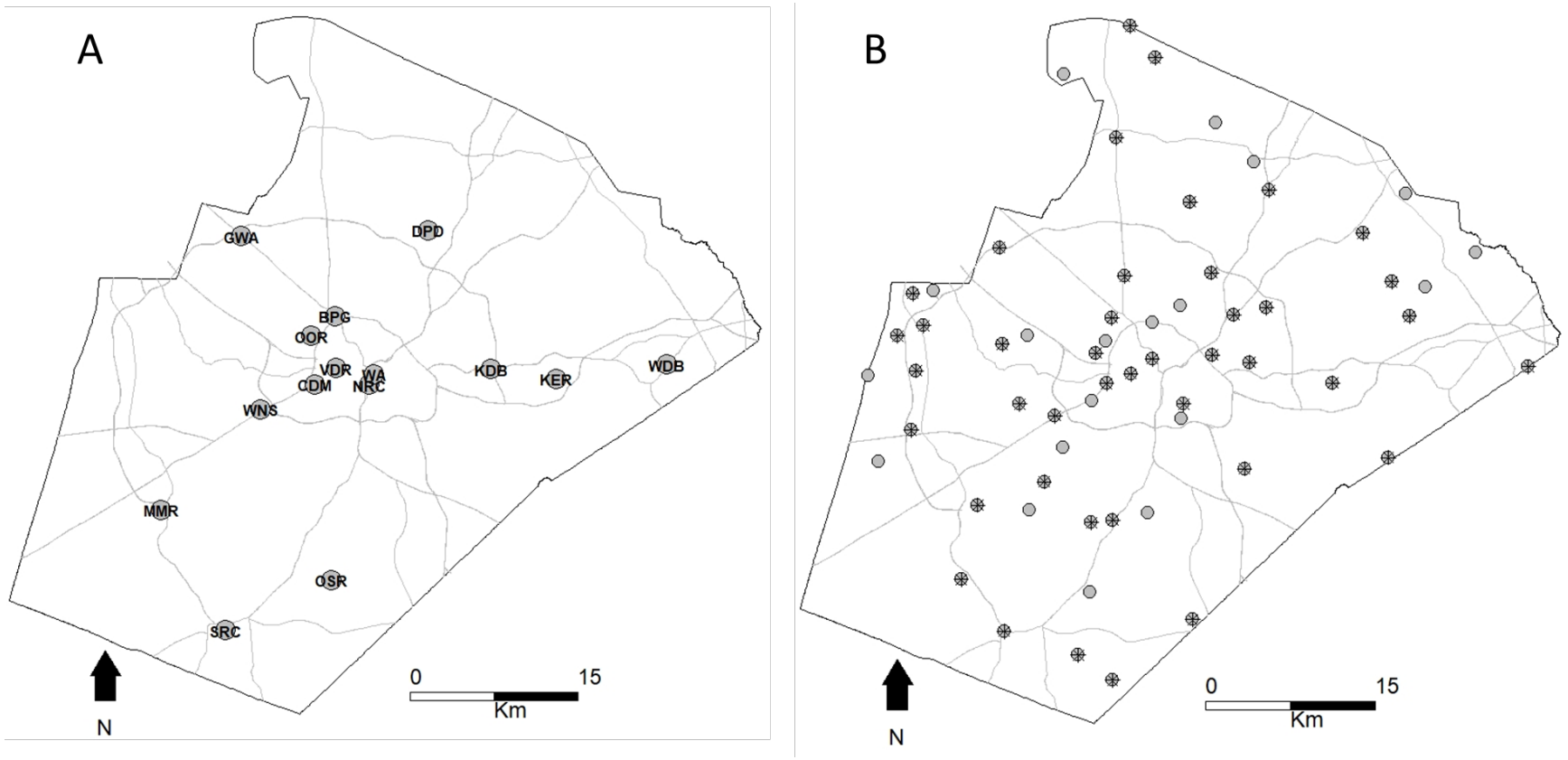


**Figure 2.2** Wake County sampling locations. (A) sites and site names from 2016; all have genetic data for a portion of collected individuals. There are four sites in the inner zone, five populations in the outer zone, and six populations in the outside zone. (B) Sampled sites from 2018; points overlaid with asterisks are sites that also have genetic data for mosquitoes. The number of sites in each zone (and number of sites used in population genetic analyses in parentheses) are as follows. Inner zone: six (four); outer zone: 18 (14); outside: 37 (24).

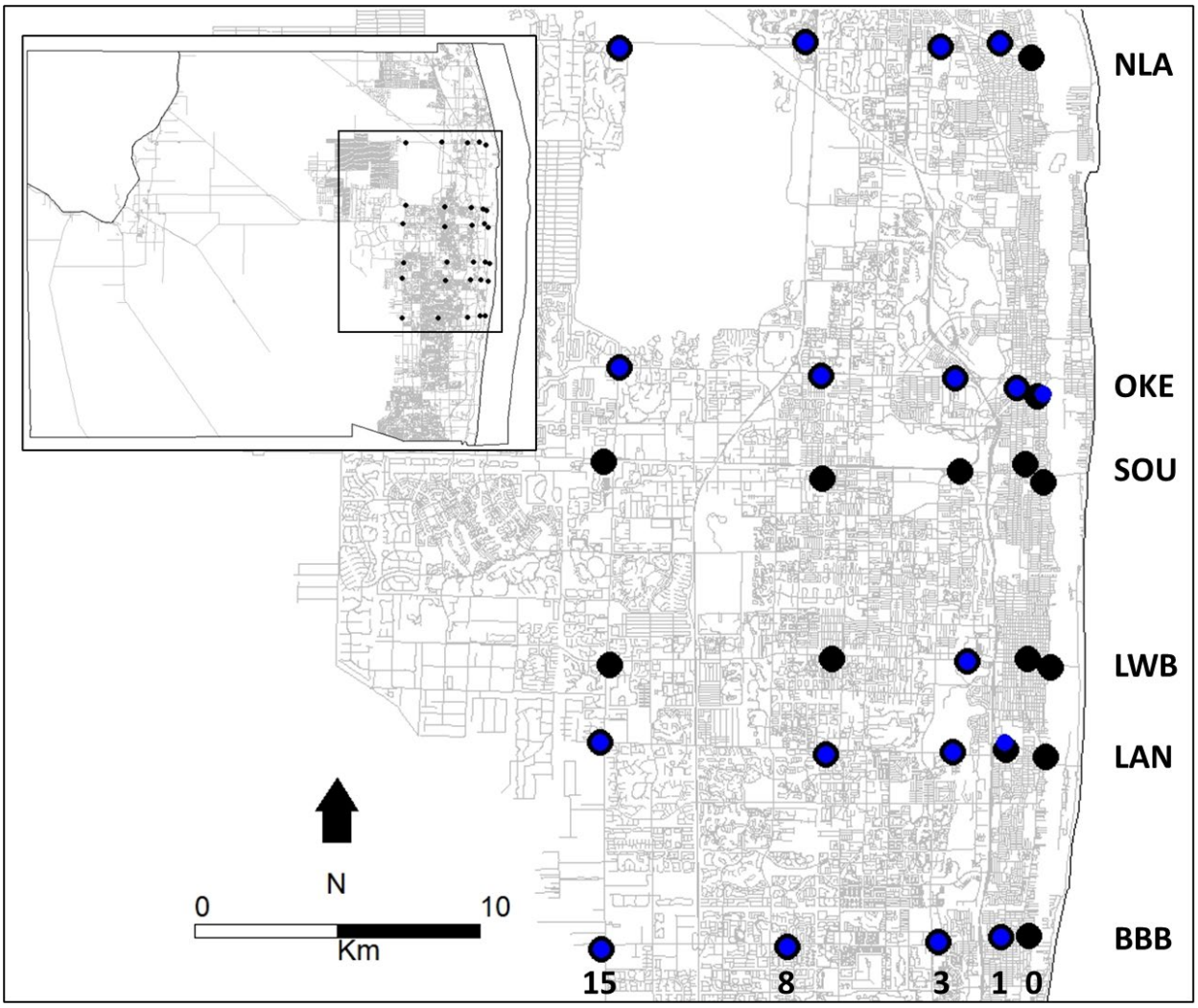


**Figure 2.3** Palm Beach County Sampling Sites. Black and blue circles are sites sampled in 2013 and 2018, respectively. Black and blue asterisks are sites with sequenced DNA data in 2013 and 2018, respectively (some sites were not selected because of too few *A. albopictus* specimens). Letters along the Y axis represent sampled transects, while numbers along the X axis represent approximate distance from coast (Km). Inset: full map of Palm Beach County and sampling points.

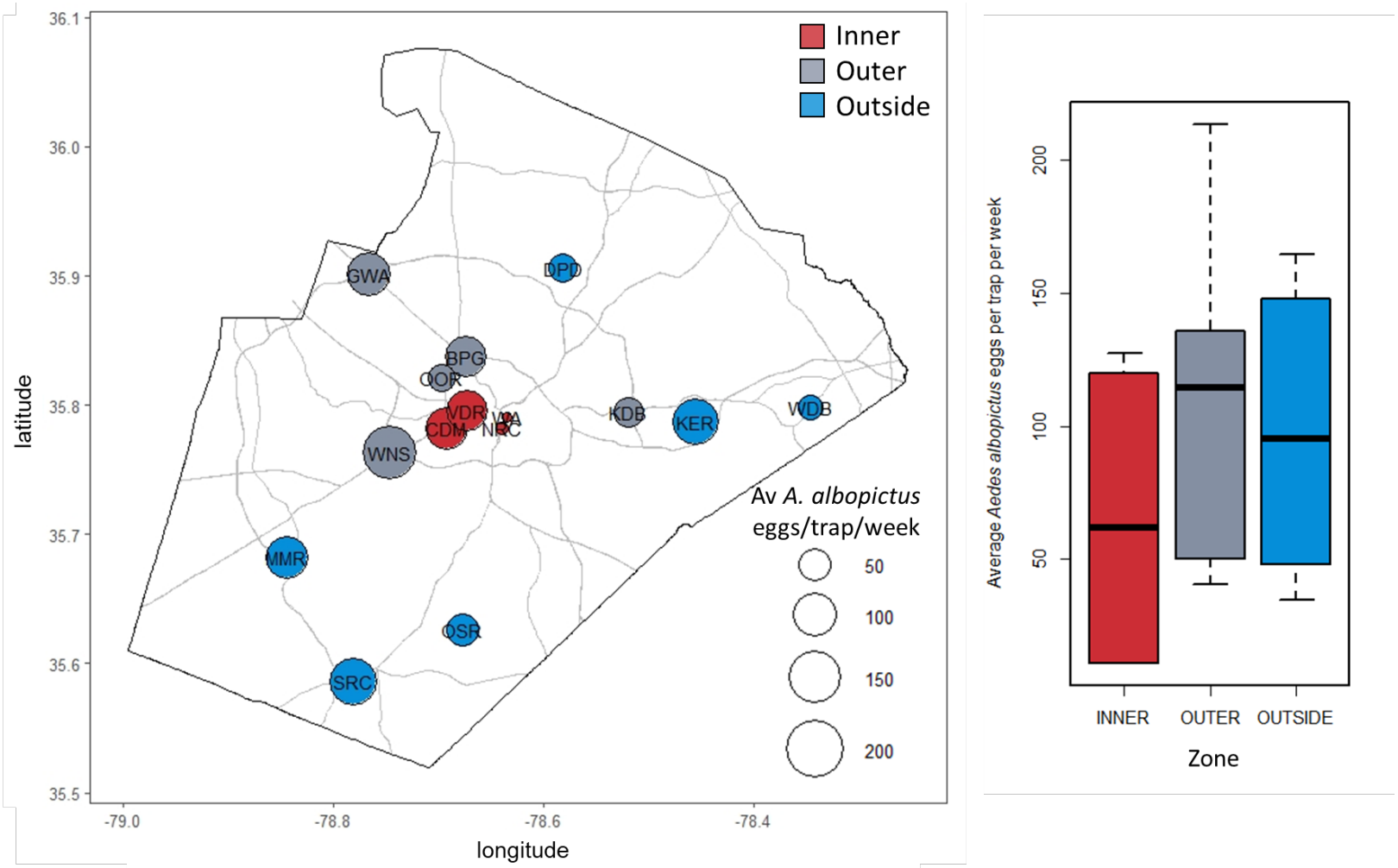


**Figure 2.4** Average *A. albopictus* egg abundance per week in Wake County 2016. Larger points indicate higher average egg abundance. *Aedes albopictus* eggs were found at all sampling locations, and average count ranged from 10.75-213.59 per ovitrap per week. There were no significant differences in mean abundance between zones. Sites in the inner zone are shown in red, those in the outer zone in gray, and in the outside zone, blue.

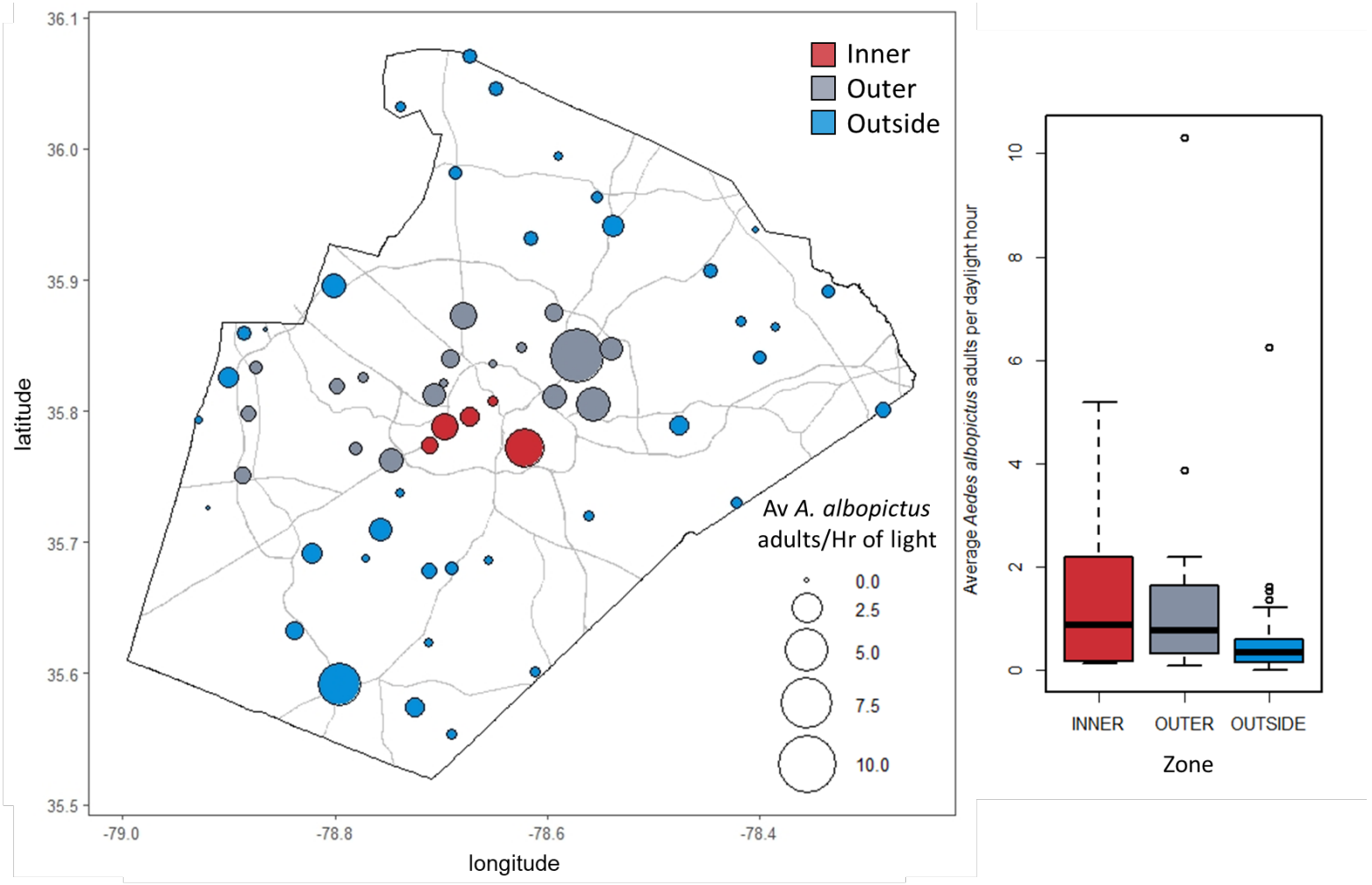


**Figure 2.5** Average *A. albopictus* adult abundance per light-hour in Wake County 2018 at 61 locations. Larger points indicate higher average adult abundance. *Aedes albopictus* adults were found at 59/61 sites, and average abundance ranged from 0.02—10.31 adults per light-hour. There were no significant differences in mean abundance between zones. Sites in the inner zone are shown in red, those in the outer zone in gray, and in the outside zone, blue.


**Figure 2.6** Relative abundances of *A. albopictus* in (A) 2013 ovitraps *(n* = 30), (B) 2018 ovitraps *(n* = 15), and (C) 2018 adult traps (*n* = 18). Black dots are sites where no *A. albopictus* were detected. Dot size is proportionate to the maximum number of *A. albopictus* trapped at a site for that year. Point color indicates the site's zone, based on distance from the intracoastal waterway. The 0 Km -3 Km zones are shown in the same color because these distances were grouped into the same zone for population genetic analyses.

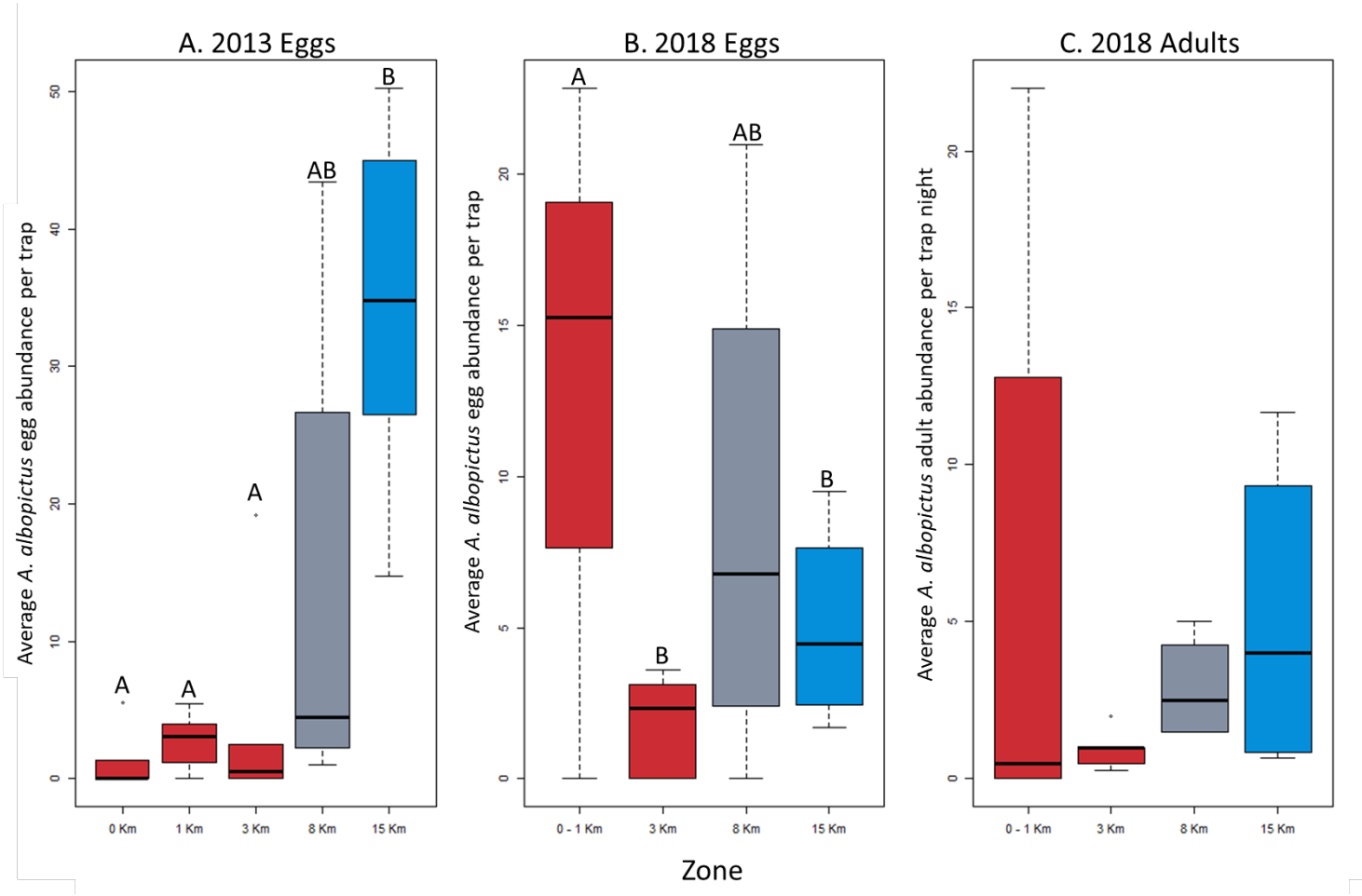


**Figure 2.7** Box plots of average *A. albopictus* abundance for (A) Palm Beach County 2013 eggs, (B) Palm Beach County 2018 eggs, and (C) Palm Beach County 2018 adults, divided into groups based on distance from the coast (0-3 Km, 8 Km, and 15 Km). I found significant differences in mean *A. albopictus* egg abundance between the 15 Km zone and the 0-3Km zones in Palm Beach County 2013 and between the 0-1 Km zone and the 3 and 15 Km zones in 2018. I found no differences in mean adult abundance between zones.

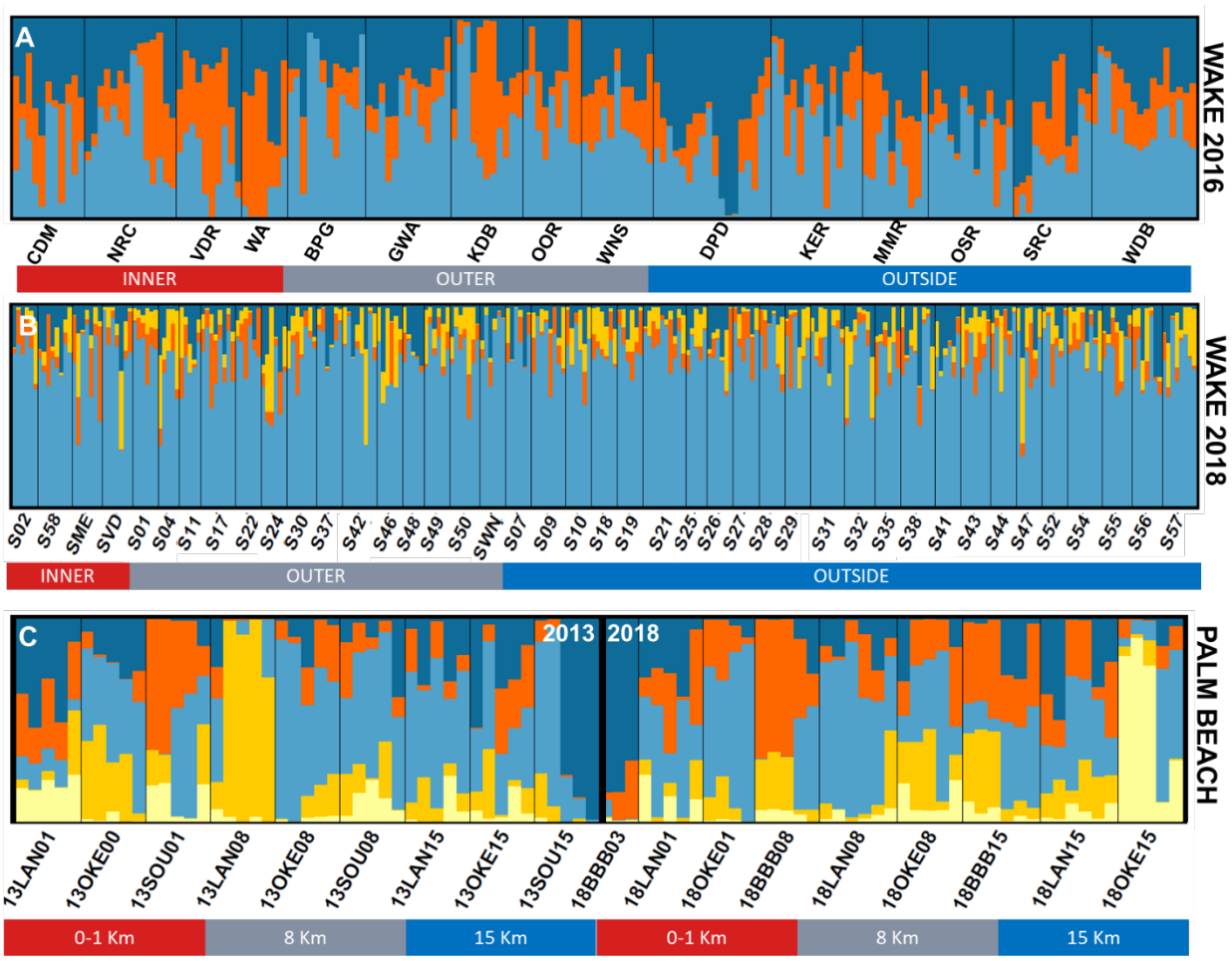


**Figure 2.8** STRUCTURE bar plots with the optimal value of *k* determined by the Evanno method in STRUCTUREharvester for (A) Wake County 2016 (*k* = 3); (B) Wake County 2018 (*k* = 4); and (C) Palm Beach County (*k* = 5). Sampling sites are organized by zone (Wake County: inner, outer, and outside; Palm Beach County: 0-3 Km, 8 Km, 15 Km) and alphabetically within groups. Bar plots were generated using DISTRUCT v.1.1 (Rosenberg 2003).

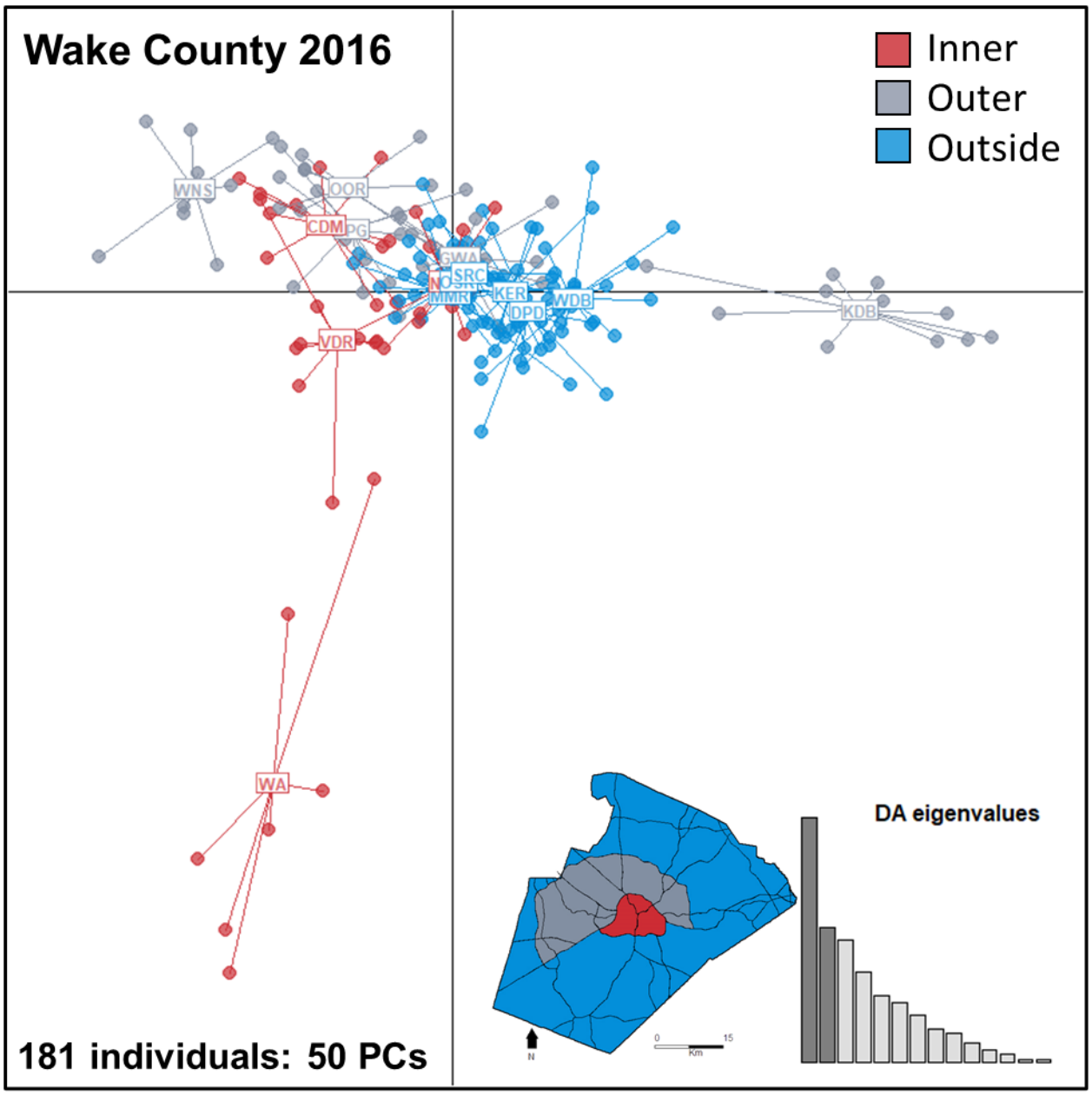


**Figure 2.9** DAPC scatterplots for the 15 sites sampled in Wake County 2016, with the first two discriminant functions (DAs) on the *x* and *y* axes. Cross-validation retained 50 principal components, which produced a correct assignment rate of 0.8066.

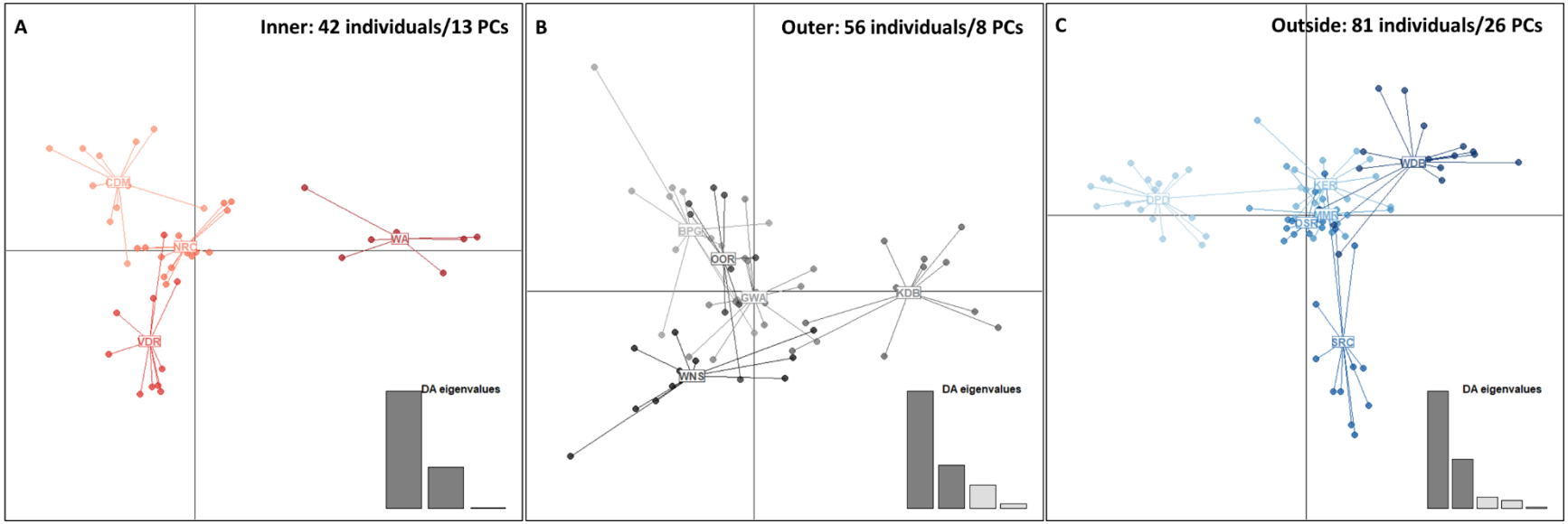


**Figure 2.10** DAPC scatterplots for each zone of Wake County 2016. The inner zone had four populations with 42 individuals, the outer zone had five populations with 56 individuals, and the outside zone had six populations with 81 individuals. The *x* and *y* axes show the first two discriminant functions. After cross validation, the inner zone (A) retained 13 principal components (PCs) with a correct assignment rate (CAR) of 0.9048, the outer zone (B) retained eight PCs (CAR = 0.6964), and the outside zone (C) retained 26 PCs (CAR = 0.7831).

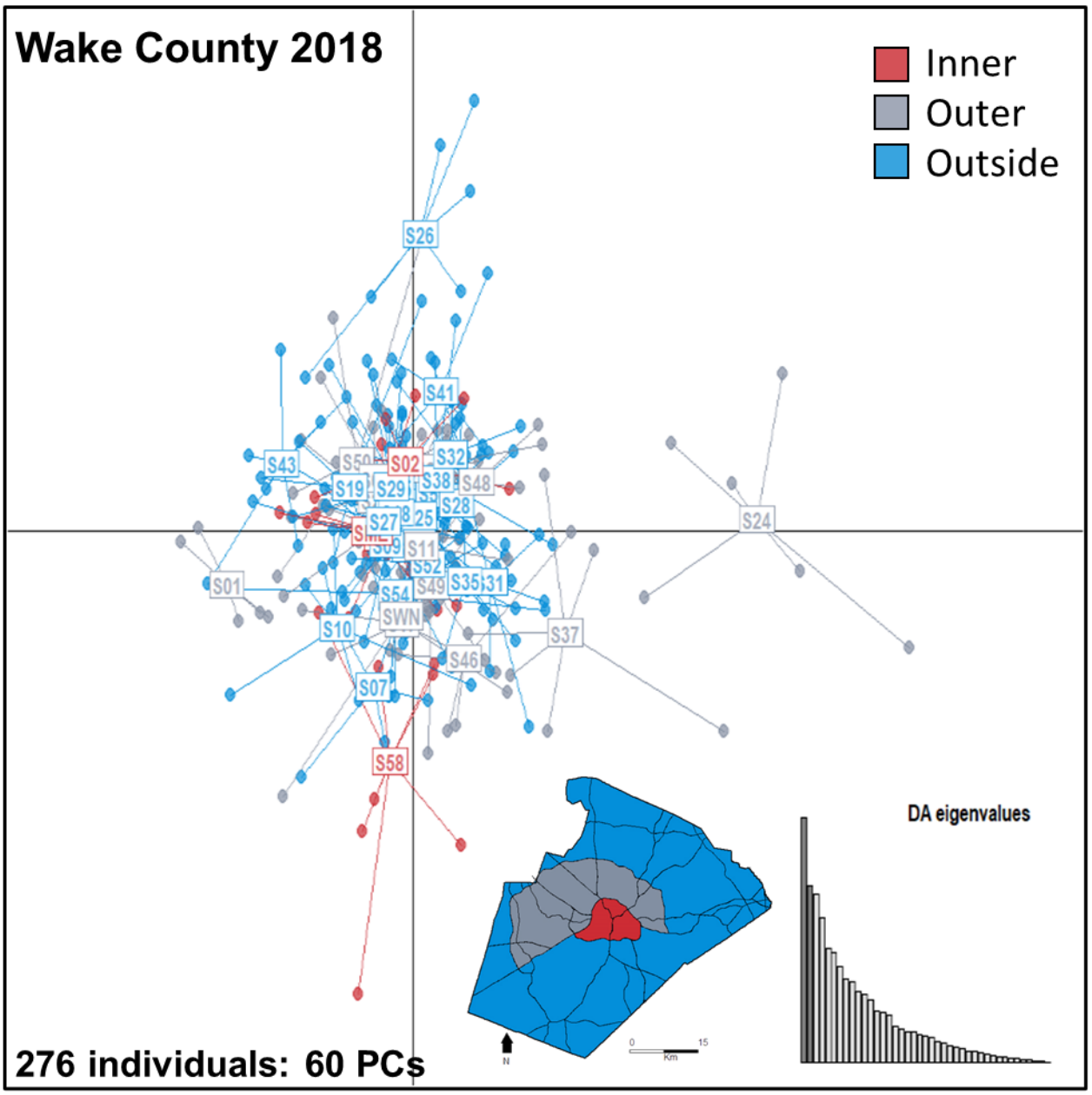


**Figure 2.11** DAPC scatterplot for the 42 sites sampled in Wake County 2018, with the first two discriminant functions (DAs) on the *x* and *y* axes. Cross-validation retained 60 principal components, which produced a correct assignment rate of 0.6200.

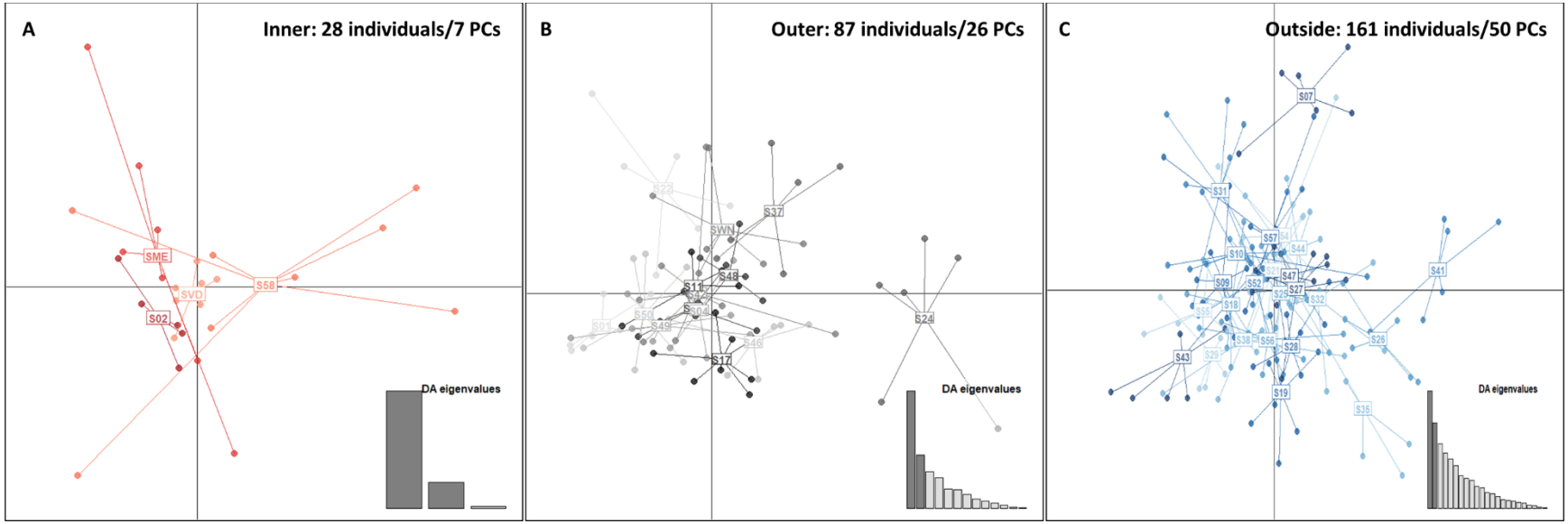


**Figure 2.12** DAPC scatterplots for each zone in Wake County 2018. The inner zone had four populations with 28 individuals, the outer zone had 14 populations with 87 individuals, and the outside zone had 24 populations with 161 individuals. The *x* and *y* axes show the first two discriminant functions. After cross validation, the inner zone (A) retained seven principal components (PCs) with a correct assignment rate (CAR) of 0.6787, the outer zone (B) retained 26 PCs (CAR = 0.7011), and the outside zone (C) retained 50 PCs (CAR = 0.7826).

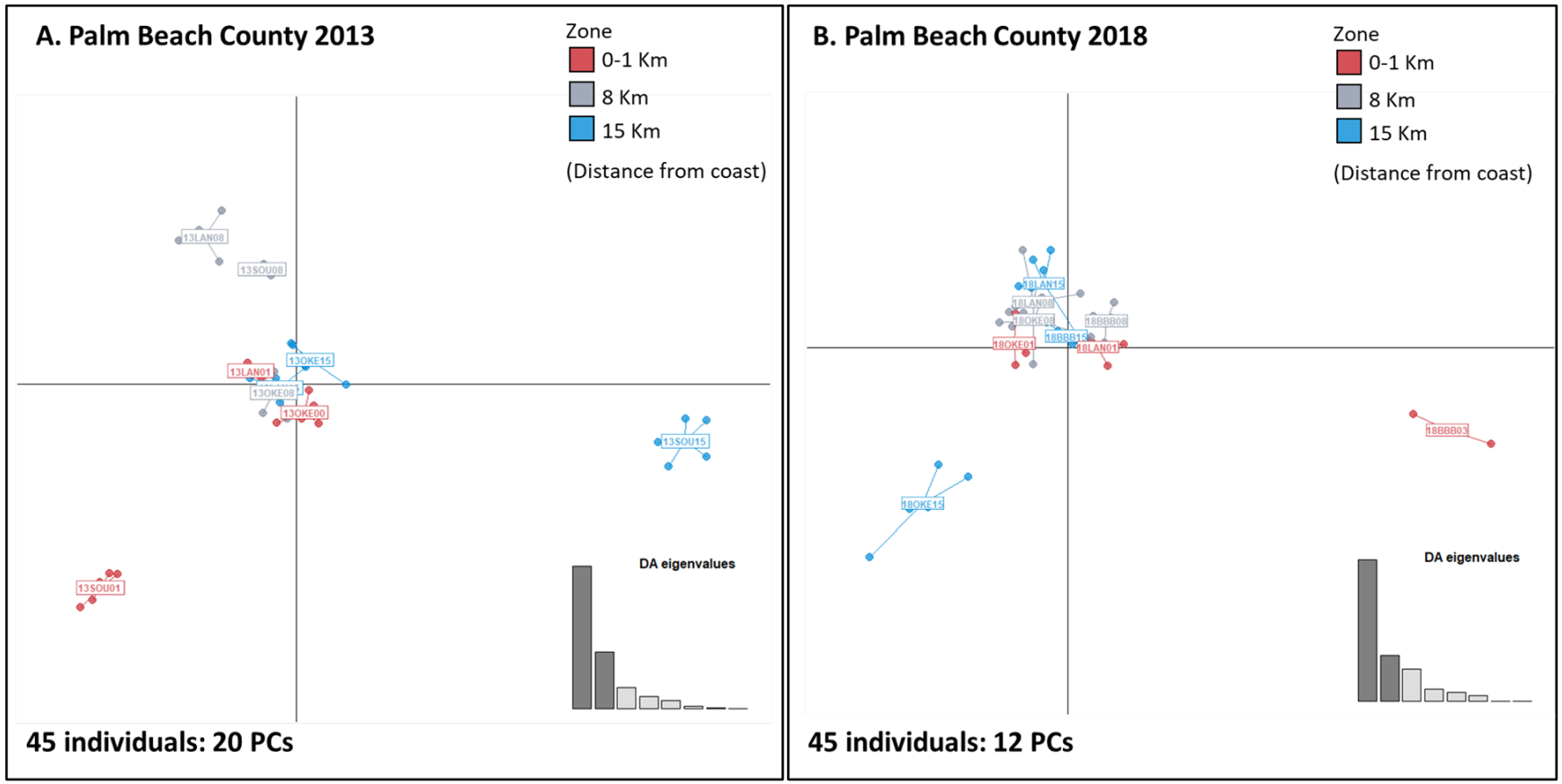


**Figure 2.13** DAPC scatterplots for the nine sites sampled in Palm Beach County (A) 2013 and (B) 2018, with the first two discriminant functions (DAs) on the *x* and *y* axes. (A) Cross-validation retained 20 principal components for 2013 individuals, which produced a correct assignment rate of 0.9111. (B) For 2018 individuals, 12 principal components were retained with a correct assignment rate of 0.8667.

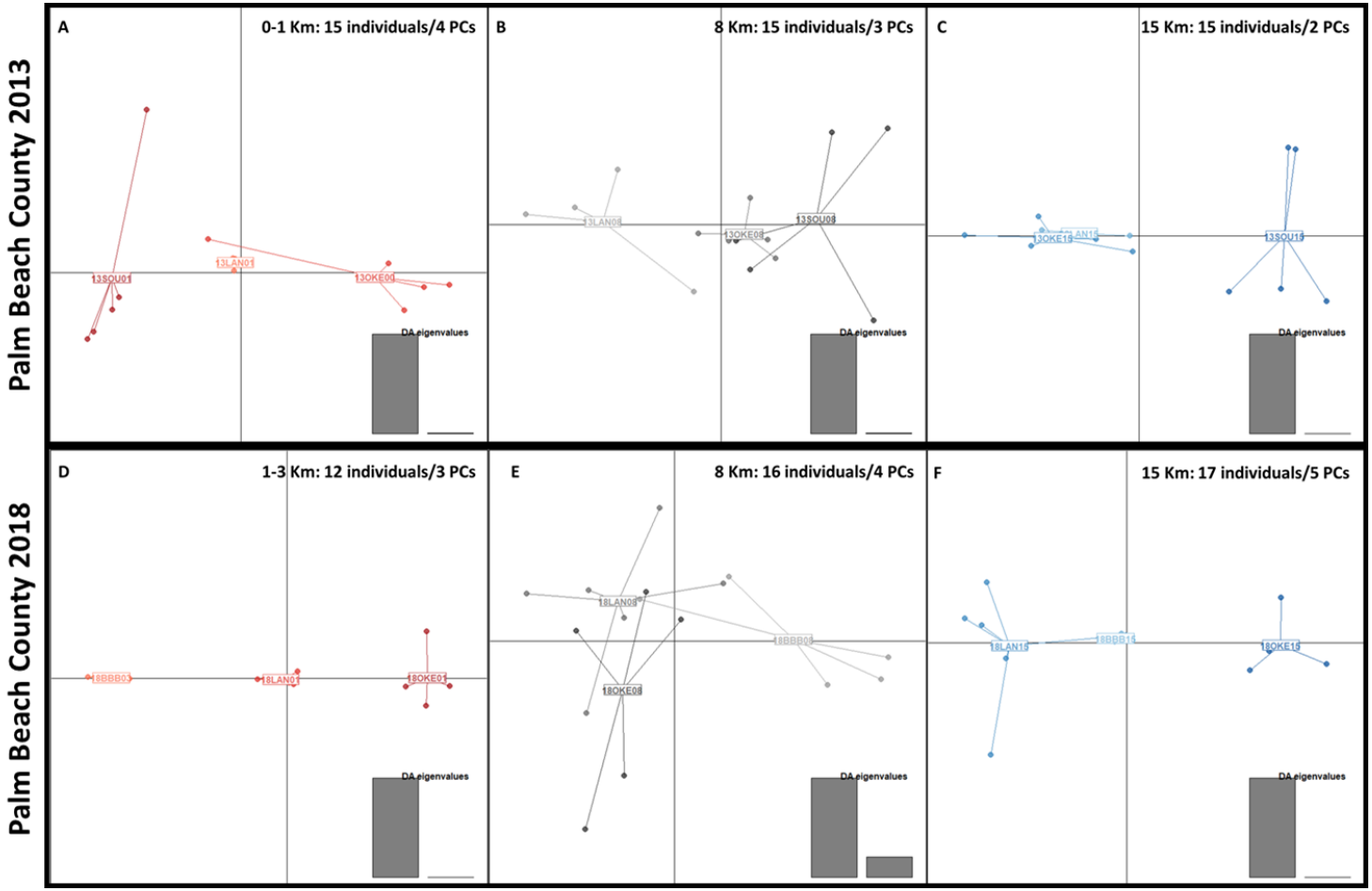


**Figure 2.14** DAPC scatterplots for Palm Beach County 2013 (A-C) and 2018 (D-F) within zones, defined as distance from coast. In both years, each zone had three sites and 12-17 individuals. Cross validation retained (A) four PCs in the 2013 0-1 Km zone with a correct assignment rate (CAR) of 0.9333; (B) three PCs (CAR: 0.8000) in the 2013 8 Km zone; (C) two PCs (CAR: 0.7333) in the 2013 15 Km zone; (D) three PCs (CAR : 1.0000) in the 2018 1-3 Km zone; (E) four PCs (CAR: 0.6250) in the 2018 8 Km zone; and (F) five PCs (CAR 0.9412) in the 2018 15 Km zone.

# CHAPTER 3

## Fine-scale landscape genetics show resistance to human development and asymmetric patterns of gene flow within a local geopolitical boundary for the invasive tiger mosquito *Aedes albopictus* (Diptera: Culicidae)

### Introduction

*Aedes albopictus* is an invasive, cosmopolitan mosquito adapted to a range of climatic and anthropogenic environments (Manica *et al.*, 2016). Native to northern Asia, *A. albopictus* is now globally distributed and currently spreading through much of Europe and North America (Bonizzoni et al. 2013). This spread has raised international concern and increased monitoring and control of the species, as *A. albopictus* is both a nuisance pest and a vector for zoonotic disease (Benedict *et al.*, 2007). Much of its introduction success can be attributed to its oviposition ecology and adaptation to anthropogenic landscapes. As a container-breeding species, *A. albopictus* females lay their eggs in small, ephemeral bodies of water. The eggs go through a drying and re-wetting cycle before hatching and can survive in both natural and artificial containers (Hawley 1988). This ability has allowed them to exploit international trade, especially of used automobile tires, to move around the globe (Hawley et al. 1987). However, there is less certainty about the major corridors and barriers for *A. albopictus* movement at smaller spatial scales.

Landscape genetics is a popular approach to identify and model fine-scale dispersal across heterogenous environments. Landscape genetics applies population genetics in a spatially explicit context, which permits researchers to study the effects of land use and climate change on functional dispersal and gene flow at multiple scales (Balkenhol et al. 2015; Fenderson et al. 2019; Klinga et al. 2019; Aylward et al. 2020). Consequently, landscape genetics is increasingly used to inform species and resource management. For example, previous research has used these approaches to investigate the effects of new development on metapopulations (Yu et al. 2017; Cheeseman et al. 2019), evaluate the efficacy of landscape corridors and habitat restoration (Sawaya et al. 2014; Marquardt and Marcus 2018; Soanes et al. 2018), and manage the spread of zoonotic diseases (Biek and Real 2010; Richardson et al. 2017; Hemming-Schroeder et al. 2018). This is crucial for nonnative species whose trajectories are linked to human-induced global climate and land use change.

For landscape genetics to be relevant for invasive species management, it is important to consider the extent and scale of the system. Scale can have significant impacts on conclusions leading to management recommendations (Cushman and Landguth 2010). Thus, to design an informative landscape genetics study, it is necessary to *a priori* define scales that are appropriate for the species under study, the genetic data available, the landscape variables of interest, the computational intensity for geospatial data, and the level that management operates. These different factors can be at odds with one another. For example, management interventions, unlike species or landscapes, are beholden to geopolitical boundaries. A mismatch between the scale of control actions and the scale of a landscape genetics study scale may render the research conclusions irrelevant or unreliable to practitioners.

Mosquito control in the United States is often guided by local governments, but many of the landscape genetics on *A. albopictus* and congeneric species are conducted at regional, national, and continental scales. These studies have found that human transportation networks can drive gene flow of container-breeding *Aedes* species regionally (Medley et al. 2015; Schmidt et al. 2017; Hopperstad et al. 2019). However, at a finer scale, Schmidt et al. (2018) found that highways interrupted genetic connectivity in *Aedes aegypti*. Hopperstad et al. (2019) detected signals of directional migration between two *Aedes aegypti* populations along an interstate highway in Florida, with gene flow from out of the more densely populated urban center. This is one of only a few population genetic studies of *Aedes* mosquitoes that measured directional gene flow in heterogeneous landscapes. Yet, this is a valuable approach to evaluate and predict genetic and demographic population sources and sinks (Sundqvist et al. 2016; Gustafson et al. 2018).

Understanding how landscape drives population connectivity and directs migration will inform adaptive and effective mosquito management, especially for genetically modified mosquito releases aimed at controlling disease spread and suppressing mosquito populations (Unlu et al. 2016; Dimopoulos 2019). If the ecological and landscape processes governing *A. albopictus* dispersal are scale-dependent, findings from regional landscape genetics studies may not be appropriate for local control.

Here, I investigate genetic connectivity, gene flow, and landscape genetic patterns of *A. albopictus* within one geopolitical boundary: Wake County, North Carolina. Wake County conducts mosquito surveillance, but mosquito control is privatized and generally targeted to households or structures. Wake County is a rapidly urbanizing area and its largest city, Raleigh,

was the second fastest growing city in 2019 (2019 Population Estimates, U.S. Census Bureau). Mosquitoes, including *Aedes albopictus*, have been monitored annually in the county since 2015, but the population genetics of the population have not been characterized (Reed et al. 2019; Spence Beaulieu et al. 2019; Hollingsworth et al. 2020; Reiskind et al. 2020). In this study, I describe patterns of genetic connectivity of *A. albopictus* adults across Wake County, measure *A. albopictus* migration rates between urban, suburban, and rural areas of the county, and identify local barriers and corridors to gene flow using a landscape genetics approach.

## Methods

### Study system

I conducted my research in Wake County, North Carolina. Wake County is home to Raleigh, the state's capital and second largest city with an estimated population of 457,159 in 2018 (United States Census Bureau). Surrounding Raleigh is a mosaic of land use types, including suburbs, agriculture, and preserved open and forested green spaces. Between 2010 and 2018, Wake County's population has grown by 20.3% from 906,882 to 1.091 million residents. To accommodate this growth, Wake County is converting rural areas into low-density housing developments. This rural/suburban transition has spread outward from the Raleigh epicenter to towards the outskirts of the county in a pattern consistent with descriptions of urban sprawl in the southeast (Terando *et al.*, 2014). The areas west of Raleigh, which includes the Apex, Cary, and Morrisville municipalities, is the most developed region of Wake County, and the eastern and southern portions of the county are slated for residential and mixed-use development in the near future (PLANWake, Wake County Government).

### Sampling

I sampled 61 sites for *A. albopictus* within Wake County from June 7 to June 25, 2018. (**Figure 3.1**). I used Biogents BG sentinels baited with BG Lures ™, a chemical attractant designed for *A. albopictus* and *A. aegypti* (Biogents GmbH, Regensburg, Germany). Of the 61 sites, Wake County had designated five as surveillance locations used to monitor mosquito abundance for the county. To identify the remaining sites, I used a random point generator using the *r.random.cells* function in GRASS GIS (GRASS Development Team, 2018). I used this function to select 100 points across Wake County with a minimum 1000m distance between points, as previous studies

indicated that *A. albopictus* rarely disperse further than 1 Km (Honório et al. 2003). For each point, I determined whether or not the site was accessible by examining the landscape within a 100m buffer of the point using Google Earth (Google Earth, Google 2008). A site was deemed inaccessible if its buffer was entirely water or on private land. Additionally, sites needed to be within 1 Km of a public road to minimize the amount of on-foot travel required to reach the sampling location. These requirements eliminated 27 locations, and I randomly selected 60 of the 73 remaining sites using the *sample.int* command in RStudio (RStudio Team 2018). Four of the 60 sites were located on private roads not marked on Google Earth and were not used, leaving 56 randomly sampled sites.

I sampled each location once a week for three weeks, leaving traps out to collect mosquitoes for approximately 24 hours. I placed twelve traps per day and randomly selected which traps were set on a given day for each week. This ensured that sites were visited at different times and days of the week over the sampling period. I included a fourth week of sampling for locations where a trap had failed ($n$ = 9/181). A trap failed if its battery died or if the collection funnel and bag were removed.

Once I retrieved a trap from a site, I placed the mesh collection bag in a plastic bag to prevent mosquitoes from escaping and I put both in a cooler for storage until all traps were retrieved for the day. I then froze the plastic bags for 24 hours to kill the mosquitoes. The following day, I sorted and identified any *A. albopictus*, and placed them in a centrifuge tube of 95% ethanol to preserve mosquitoes before DNA extraction.

Genomic Library Preparation

I sequenced mosquito genomic data from sampled sites using double-digest restriction-enzyme associated DNA sequencing (*ddRADseq*) following Burford Reiskind et al. (2016) with *SphI* and *MluCI* restriction enzymes. I determined which sites to sequence based on quantity and quality of mosquito DNA extractions. I detected *A. albopictus* at 59/61 sampled sites and collected six or more individuals at 47 sites (Reed 2021, Chapter 2). After extraction, I had high-concentration DNA samples for at least six individuals at 42 sites, and I obtained sequence data from 289 adult *A. albopictus*.

I extracted DNA from *A. albopictus* individuals using the Qiagen DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA, USA) and quantified DNA concentration using a Qubit

2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). I built seven ddRAD genomic libraries each with 48 individuals from sites that had six or more *A. albopictus* individuals with DNA concentrations of at least 8 ng/μL. To build the genomic libraries, I fragmented genomes using restriction enzymes and annealed variable-length DNA barcodes to each fragment. I then size-selected fragments between 350-475 base pairs (bp) in length using the Blu Pippin Prep at the North Carolina State University Genomic Sciences Laboratory. I amplified the size-selected DNA and annealed an Illumina index to each library. This allowed me to sequence two genomic libraries per lane and de-multiplex post-sequencing. I submitted sequence-ready genomic libraries to the Genomics & Cell Characterization Core Facility (GC3F) at the University of Oregon, who used the Illumina HiSeq 4000 to create single-end sequencing reads of 100 bp.

Bioinformatic Processing

Post-sequencing, the Illumina platform de-multiplexed the Illumina indices to create two FASTQ files per lane, one for each library. I checked the library quality using FASTQC (Babraham Bioinformatics; http://www.bioinformatics.babraham.ac.uk/projects/fastqc) and filtered reads with a phred score lower than 33 using *process_radtags* in STACKS version 1.09 (Catchen et al. 2011). This step also trimmed sequences to 90 bp and demultiplexed individual barcodes to create FASTQ files for each individual mosquito. I created a catalog of single nucleotide polymorphisms (SNPs) with the STACKS *denovo* pipeline. I required a minimum read depth of six using the *-m* flag; a maximum of three mismatches between loci within an individual with the *-M* flag; and a maximum of two mismatches between loci in the catalog with the *-n* flag.

I filtered SNPs from the STACKS catalog using the *Populations* pipeline. I only included SNPs that were present in at least 75% of individuals in a population and that occurred in at least two populations. I further filtered SNPs by removing individuals with over 75% missing data, and variants with a minimum allele frequency (MAF) less than 0.01 and a genotyping rate of less than 0.75 in PLINK v1.19 (Purcell et al. 2007). I used a more stringent genotyping rate to accommodate for the sensitivity to missing data in landscape genetic and connectivity analyses (Arnold et al. 2013; Gautier et al. 2013). I converted PLINK files to Genetix files using PGDSpider v2.1.1.0 (Lischer and Excoffier 2012) and imported these files to RStudio. With the *hw.test* function in the R package *pegas* v.0.14 (Paradis 2010), I removed SNPs that were not in Hardy-Weinberg Equilibrium after a Bonferroni correction ($P < 0.05/\#$ SNPs). Finally, I

converted the filtered file to a *locus* object as implemented in the R package *gstudio* (Dyer 2016). Locus objects behave as data frames in RStudio, allowing users to easily summarize, manipulate, and transform marker-based genetic data and integrate genetic and spatial data for downstream analyses. I selected SNPs in *gstudio* that were present in 90% of individuals to use as our final dataset for connectivity and landscape genetic analyses.

Genetic Connectivity and Gene Flow

I evaluated genetic connectivity with a graph theoretic approach following Dyer and Nason (2004). I built population graphs using the R packages *iGraph* and *popGraph*, which use a multivariate network model to define relationships among groups of populations simultaneously. In contrast to traditional approaches such as *F*-statistics, this method addresses multiple population genetic questions at once, such as genetic diversity, differentiation, and connectivity. Briefly, the population graph is composed of a set number of nodes (in this case, the 42 sampled sites) and edges that connect nodes. Started with a fully saturated graph where all nodes are connected, the population graph algorithm attempts to prune the maximum number of edges possible and accurately describe the among-population genetic variation. The longer the edge length, the more genetic variation between the two connected nodes (Dyer and Nason 2004).

Once I defined the population graph, I extracted node-specific parameters for each site: node size, closeness, betweenness, degree, and eigenvector centrality. Size is a measure of the within-population contribution to genetic variation observed across all sites, akin to a genetic diversity measure. Closeness is defined as the number of steps required for a node to access every other node in the graph, weighted by the edge value. Therefore, higher closeness values indicate higher genetic differentiation. Betweenness describes the number of shortest paths along edges between any two nodes that includes the given node. A high betweenness value may indicate the site is a hub for flow along the graph's topology. Degree is a count of the number of edges connected to a given node, where sites with low degree values are more genetically isolated. Finally, eigenvector centrality, referred to as centrality from hereon, is the value of the first eigenvector in the graph's adjacency matrix. It is proportional to the sum of centralities of connected nodes, and higher centrality scores indicate a node is directed connected to other well-connected nodes; in a social network, high centrality may be described as a "super influencer". When considered together, parameters identify hubs of gene flow and putative locations of

genetic sources and sinks. These population graphs are undirected and assume equivalent emigration and immigration between nodes.

To assess source and sink dynamics, I measured gene flow between sites using the program *BA3-SNPs* (Mussmann et al. 2019). I conducted five runs in *BA3-SNPs* with 5,000,000 iterations each, a burn-in period of 1,000,000, and an interval of 1000 iterations between samples. I tested for model convergence by comparing the parameterized migration rates between runs. Only migration rates with a 95% confidence interval greater than zero were included in the results. I evaluated Source/sink dynamics by subtracting the sum of immigration rates from emigration rates following Gustafson et al. (2018). Higher emigration indicates the region is a source, while higher immigration indicates a sink.

Due to the computationally intensive process of estimating unidirectional migration rates and the rapid increase in pairwise calculations necessary with additional sites, I chose to group sites geographically into ten regions, each with three to five sites (**Figure 3.2**). I assigned sites to regions based on their proximity to Wake County municipalities and major roadways. Because of their size, I divided the Raleigh jurisdiction into three regions (RAL: central Raleigh; NER: northeast Raleigh; NWR: northwest Raleigh) and Cary into two regions (MW: midwestern Wake; W: western Wake). Central Raleigh sites fell within the boundary of interstate 440, and NWR and NER were divided by Lead Mine Road. Sites closest to Fuquay-Varina formed the southern region (S); sites south of Raleigh and north of Fuquay Varina formed the mid-southern region (MS); sites east of Raleigh formed the eastern region (E); sites in Wake Forest and north of interstate 87 formed the northeast region (NE), and sites west of Wake Forest and north of Raleigh formed the northwest region (NW). These regions are at different stages of urbanization. The most developed areas are in Raleigh and Cary, while the least developed are in the northwest and eastern parts of the county. In addition to assessing migration rates, I also averaged node characteristics by region and tested for differences between groups using a generalized linear model.

Landscape Resistance Analysis

To understand how landscape variables influenced observed genetic variation and connectivity, I modeled correlations in genetic distance and landscape distance pairwise matrices. I selected three measures of genetic distance commonly used in landscape genetic studies. The first,

conditional genetic distance (cGD), uses the population graph described above and is the shortest distance between nodes following the retained edges (Dyer et al. 2010). I also calculated Cavalli-Sforza and Edwards chord distance (CSE) and linearized $F_{ST}$ ($F_{ST}$ / 1- $F_{ST}$) using the *hierfstat* package (Goudet 2005). Linearized $F_{ST}$ adjusts the commonly used pairwise $F_{ST}$ to support an isolation-by distance-model (Rousset 1997). CSE is less sensitive to missing data and is calculated based on geometric distance, thus avoiding the population-level assumptions used to calculate $F_{ST}$ (Pless et al. 2021).

I built resistance surfaces for 35 landscape variables, 31 categorical variables and 4 continuous variables (**Table 3.1**). I based 25 variables on 2016 USGS National Land Cover Database (NLCD) land-use classifications. Each land cover type was converted to a binary surface, creating 15 variables. I then combined land classifications into broader categories for an additional 10 variables. I generated five binary resistance raster layers from vector-based variables for Wake County: railroads, floodplains, highways, major roads, and streets. With the streets vector, I also created a multi-categorical resistance surface weighted by speed limit. The continuous variables included percent impervious surface and three topographical features: slope, aspect, and roughness, derived from a digital elevation model for Wake County (North Carolina Spatial Data Download: https://sdd.nc.gov/). Slope measures change in elevation, while aspect indicates the slope orientation. Roughness incorporates both slope and aspect and is a measure of terrain complexity (Wilson et al. 2007). I included these variables because heterogenous terrain may hinder natural dispersal in *A. albopictus* by inhibiting flight distance and may affect the micro-distribution of oviposition sites by directing water flow (Bonnet and Worcester 1946; Shabani et al. 2018).

For all variables, I calculated resistance surfaces for four values: 1.5:1, 2:1, 5:1, and 500:1. The first three values were treated as either degree of conductance (easier to move through) or resistance (harder to move through), and the 500:1 surface represented strong barriers for gene flow. All surfaces had a resolution of 30 x 30m. Finally, I also included an isolation by distance surface to measure Euclidean distance as a null model.

I calculated pairwise distances between sites using least cost paths and all paths in the *gdistance* package in R (van Etten 2017). I used the *commuteDistance* function for the all paths method , a functional equivalent to the connectivity software CIRCUITSCAPE (McRae 2006; Kivimäki et al. 2014). To compare the resulting pairwise resistance distances to genetic distance,

I used multiple regression on distance matrices (MRDM; Legendre et al. 1994) with 100,000 permutations in the R package *ecodist* (Goslee and Urban 2007). For least cost paths and all paths, I performed forward model selection as described in (Legendre et al. 1994). I identified the landscape variable and associated cost value whose distance matrix yielded the smallest *P* value, removed all other cost matrices for that variable, and continued iteratively until the model *P* value was greater than 0.05/the number of variables. I then checked the retained landscape variables for collinearity. If $r > 0.70$ for any predictor pairs, I removed the predictor that contributed least to the full model (lower $R^2$). This resulted in six final models: a least cost path-based model and an all-path model for each genetic distance.

**Results**

Sampling & Bioinformatic processing

The STACKS *de novo* pipeline identified 3,096,027 SNPs, 183,753 of which were retained post-*populations* pipeline. PLINK filtering removed 180,009 SNPs and 13 individuals that did not meet thresholds for minimum allele frequency and genotyping rates. I removed an additional 2,553 SNPs using *gStudio* to minimize missingness for a final dataset of 276 individuals and 1,193 loci.

Genetic connectivity and migration

The population graph retained 61 edges between nodes, and all nodes were connected to at least one other site, although this is not a requirement of the algorithm (**Figure 3.3**). Node degree ranged from one to five (four sites and three sites, respectively). I found node closeness and betweenness were positively correlated (linear regression, $P < 0.001$; $R^2 = 0.3826$) and centrality was negatively correlated with both closeness and betweenness (closeness: $P < 0.001$ and $R^2 = 0.6633$; betweenness: $P = 0.0266$; $R^2 = 0.0950$).

When I compared node characteristics between regions defined *a priori* (**Figure 3.2**), I found the strongest patterns of genetic connectivity and variation in the regions north and east of Raleigh. The eastern region (E) had the highest average node degree and centrality, which indicates that the sites in this region have high genetic connectivity with other areas and strongly influence the graph topology. In contrast, the northeast region (NE) had the lowest average betweenness, degree, and centrality, suggesting that these sites are genetically isolated relative to

the rest of the county. The northwest region (NW) had the highest average node size, which indicates that this area has high within-site genetic diversity. While these were compelling trends, I did not find significant differences among regions for any of the nodes.

Of the 90 estimated migration rates between sites, I found 27 that had 95% confidence intervals above zero (**Figure 3.4**). Northeast Raleigh (NER) had an immigration rate eight times greater than its emigration rate and was estimated to receive 52% of total emigrants from other regions, which indicates a genetic sink. The only regions that did not have statistically significant immigration rates into NER were the northwest (NW) and southern (S) regions. The latter, along with NE were also identified as potential sinks. In contrast, rates of gene flow into and out of northwest Wake (NW) were approximately equal. The largest source population was from the eastern region, which contributed 20% of immigrants to the county. This reflects the high degree of connectedness seen in the population graph node parameters for this area. Central Raleigh (RAL) and eastern Cary (MW) both contributed seven times more immigrants and were also identified as potential source populations, as were the northwest Raleigh (NWR), midsouth (MS) and western (W) regions, albeit to a lesser extent.

Landscape Resistance

Of the three genetic distances I used to test for landscape resistance to gene flow, linearized $F_{ST}$ and CSE were correlated ($R^2$ = 0.7867), but not with cGD (Linearized $F_{ST}$: $R^2$ = 0.0810; CSE: $R^2$ = 0.0885). I found no evidence of isolation by distance, and none of the final landscape models retained Euclidean distance as a significant variable (**Table 3.2**).

The all-paths analysis explained more genetic variation than the least-cost path approach for all three genetic distances and retained more landscape variables that were statistically significant. Of the 36 landscape variables used in model selection, 12 were retained in at least one of the six final models: 10 NLCD land-use variables, floodplains, and percent impervious surface (**Table 3.2**). Medium development and mixed forest were the most common variables, each retained in 3/6 models. Medium development was included in the three all-path models with a resistance cost of 5:1, while mixed forest facilitated gene flow for linearized $F_{ST}$ and CSE with a conductance value of 1/5 but inhibited gene flow in the cGD all-paths model with a resistance cost of 500:1.

## Discussion

Overall, I detected asymmetric gene flow across Wake County from urban to rural regions and found evidence that urban features acted as barriers to dispersal between sampled sites. The highest levels of within-site genetic diversity were in undeveloped areas despite having relatively low *A. albopictus* abundance, consistent with previous results (Reed 2021, Chapter 2). The described patterns of genetic connectivity, directional migration, and landscape resistance all indicate that urban sprawl strongly influences population dynamics of *A. albopictus* in Wake County.

The geographic locations of sites did not correspond to genetic distances or to the topology of genetic variance in Wake County (**Figure 3.3**), and I found no evidence for isolation by distance (IBD). This is consistent with previous research that found a lack of genetic structure by geography in *A. albopictus* populations (Vazeille et al. 2001; Maia et al. 2009; Medley et al. 2015; Goubert et al. 2016; Schmidt et al. 2017; Latreille et al. 2019), although IBD has been detected in the species at finer spatial scales (Schmidt et al. 2017). A lack of geographic and genetic distance correlations in *A. albopictus* is often attributed to recent introductions or continuous passive dispersal (Latreille et al. 2019; Schmidt et al. 2020). However, *A. albopictus* has been established in Wake County since at least the 1990s (Kraemer et al. 2015) and transportation networks were not retained in any landscape genetic models. This suggests that the configuration of landscape features and networks of urban sprawl, not physical distance, strongly influence *A. albopictus* population genetic dynamics.

Both the population graph and migration estimates showed that levels of gene flow and isolation varied between regions of Wake County. Genetic material tended to flow from urban to rural or between rural regions. For example, western and central Wake County have high levels of urbanization, and 46% of total immigration between regions originated from this area. In contrast, northern and southern Wake are less developed, and these regions were identified as potential genetic sinks (S, NE) or at a gene flow equilibrium (NW). My results contrast with Sherpa et al. (2020), who observed less dispersal in diffuse urban areas of southeast France. However, they conducted their study at the range edge of a recent *A. albopictus* invasion and in a region with a different topology and urban distribution than Wake County. This reinforces both the need for replication in landscape genetics studies (Holderegger & Wagner, 2008; Richardson *et al.*, 2016) and the importance of place-based research for management. Still, my findings were

consistent with patterns of asymmetric migration observed in *A. aegypti* by Hopperstad et al. (2019) in Florida, who detected dispersal from a densely urbanized metropolis to a smaller city. These patterns suggest that urban and high-density residential areas can maintain *A. albopictus* population, conserve genetic diversity, and facilitate the spread of potentially adaptive genotypes across the landscape.

There were two regions that challenged this trend. The largest genetic sink was in northeast Raleigh (NER), which contributed only six percent of immigrants but received 52% of the total migration across the county despite being high urbanized. Similarly, the eastern region was the greatest single source of gene flow but is sparsely developed. However, almost half of the emigration from this region flowed into NER, which could explain its deviation from the overall trend. Furthermore, I did not detect significant migration between most pairs of regions. When estimating migration rates, population-based Bayesian and maximum likelihood programs such as *BA3-SNPs* and MIGRATE yield more accurate and precise results when genetic variation between subpopulations is high (Beerli 2006; Faubet et al. 2007). If there is not strong differentiation between sample sites, error terms for estimates can be reduced with larger sample sizes (Sundqvist et al. 2016; Li et al. 2020). While I attempted to constrict confidence intervals for migration rates by grouping sample sites into regions, this may have decreased between-population variation.

Finally, I limited my sampling to Wake County, and thus did not measure the genetic contribution of *A. albopictus* populations outside of Wake County. As noted by Meirmans (2015), biological boundaries do not follow geopolitical jurisdictions, and migration rates likely underestimate the number of immigrants from surrounding areas in the regions along the border of Wake County. However, this does not account for the lack of migration into the urban interior of Wake County. Despite the limited sampling, this study was designed to be context-dependent within a geopolitical boundary and provides evidence for complex landscape-driven dispersal that warrants further investigation. A more targeted study could further clarify patterns of asymmetrical migration in *A. albopictus*. For example, I recommend more comprehensive sampling of urban and suburban areas identified *a priori*.

The observed regional patterns corroborated landscape resistance models. Urban features hindered gene flow, which may explain the lack of gene flow into the most urbanized areas of the county. These features included percent impervious surface (CSE and linearize $F_{ST}$ LCP

models), medium development (CSE and linearize $F_{ST}$ all paths models), and open/low development (cGD models). The all paths cGD model explained the most variation of the six final models, and also included two forest types and pastures as significant barriers. Medley et al. (2015) also found that forests and agriculture impede gene flow along the northern US range edge of *A. albopictus*. However, unlike most regional landscape genetics studies, I found no evidence that transportation networks hindered or facilitated genetic connectivity within Wake County. This supports previous research that that passive dispersal of *A. albopictus* via human-mediated transportation drives genetic connectivity at larger spatial scales, but not locally (Medley et al. 2015; Sherpa et al. 2020). While there is evidence of short-distance transport of *A. albopictus* along road networks (Eritja et al. 2017), it may occur too infrequently or within such short distances as to be inconsequential to local dispersal dynamics.

My results also suggest that *A. albopictus* do not tend to disperse into developed areas, even those that are predominately residential. Low and medium density residences are a preferred habitat for *A. albopictus* (Armstrong et al. 2017; Montagner et al. 2018; Spence Beaulieu et al. 2019), and large, established populations in suburbs may lead to the high relative emigration rates I observed. Alternatively, people living in suburban areas may be more likely to employ mosquito control methods that render potential backyard environments unsuitable. Hollingsworth et al. (2020) found that barrier sprays and larval habitat removal reduced *A. albopictus* populations in Wake County neighborhoods with evidence of a spillover effect into adjoining yards. Areas with regular mosquito management are therefore likely to act as barriers to dispersal. Collectively, this indicates that urban *A. albopictus* populations may be slow to re-establish following extensive control or eradication efforts in the absence of dispersal corridors.

While I found strong evidence that urbanization hinders gene flow, none of the landscape features I included facilitated *A. albopictus* movement. This is due in part to the high degree of collinearity between distances calculated from conductance surfaces. In particular, many of the landscape distance matrices in the all paths model were correlated ($r > 0.70$) and consequently eliminated during model selection. The degree of collinearity is likely a product of the degree of land-use fragmentation in Wake County; the costs accumulated moving between areas that facilitate connectivity outweigh the benefits of travelling within the conductive patches. Consequently, in highly fragmented landscapes such as Wake, I would suggest conductance and resistance surfaces are first combined before calculating pairwise commute distances.

*Aedes albopictus* is a ubiquitous nuisance species and vector of zoonotic disease, and itis important to adopt adaptive, science-based strategies to control its spread. Landscape genetic approaches are fast becoming a primary tool for integrating conservation and invasion biology and management but translating research findings to action comes with significant challenges, particularly for *A. albopictus*. Based on bidirectional migration estimates and landscape resistance models, I recommend that mosquito control focus surveillance and management efforts on areas actively growing and urbanizing. While more research is needed to generalize these recommendations to other counties, fine-scale, place-based studies such as this provide support and context to larger-scale research and are directly applicable to local control agents, connecting science to practice.

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**Table 3.1** Landscape variables included in landscape genetic analysis. For all variables, I generated eight resistance surfaces, four that facilitated gene flow (ratios 2:3, 1:2, and 1:5) and four that impeded gene flow (3:2; 2:1; 5:1; 500:1). The ratio 500:1 represents the extreme cases where the variable was an effective barrier to dispersal. *Class value is applicable only to USGS NLCD land use classifications. ^Elevation was not included in model selection.

| Landscape Variable | Class Value* | Source |
|---|---|---|
| Water | 11 | USGS NLCD 2016 |
| Developed, Open Space | 21 | USGS NLCD 2016 |
| Developed, Low Intensity | 22 | USGS NLCD 2016 |
| Developed, Medium Intensity | 23 | USGS NLCD 2016 |
| Developed, High Intensity | 24 | USGS NLCD 2016 |
| Barren Land | 31 | USGS NLCD 2016 |
| Deciduous Forest | 41 | USGS NLCD 2016 |
| Evergreen Forest | 42 | USGS NLCD 2016 |
| Mixed Forest | 43 | USGS NLCD 2016 |
| Shrub/Scrub | 52 | USGS NLCD 2016 |
| Grassland/Herbaceous | 71 | USGS NLCD 2016 |
| Pasture/Hay | 81 | USGS NLCD 2016 |
| Cultivated Crops | 82 | USGS NLCD 2016 |
| Woody Wetlands | 90 | USGS NLCD 2016 |
| Emergent Herbaceous Wetlands | 95 | USGS NLCD 2016 |
| All Developed | 21-24 | USGS NLCD 2016 |
| Low-Highly Developed | 22-24 | USGS NLCD 2016 |
| Less Developed | 21-22 | USGS NLCD 2016 |
| More Developed | 23-24 | USGS NLCD 2016 |
| Forest | 41-43 | USGS NLCD 2016 |
| Agriculture | 81-82 | USGS NLCD 2016 |
| Wetland | 90, 95 | USGS NLCD 2016 |
| Open Land | 21, 71, 81 | USGS NLCD 2016 |
| Grassy, low vegetation | 21, 52, 71 | USGS NLCD 2016 |
| undeveloped low vegetation | 52, 71 | USGS NLCD 2016 |
| % Impervious Surface | | USGS Imperviousness 2016 |
| Elevation^ | | North Carolina Spatial Data Download |
| Slope | | derived from elevation |
| Aspect | | derived from elevation |
| Roughness | | derived from elevation |
| Railroads | | Wake County Government GIS |
| Floodplain | | Wake County Government GIS |
| Highways | | Wake County Government GIS |
| Major Roads | | Wake County Government GIS |
| Streets | | Wake County Government GIS |
| Streets - speed limit | | Wake County Government GIS |

**Table 3.2** Results of landscape genetic models for least cost paths and all paths analysis of resistance layers for three measures of genetic distance: CSE, linearized $F_{ST}$, and cGD. I performed multiple regression on distance matrices and conducted forward-stepping model selection following Legendre *et al.* (1994). Statistically significant terms ($P < 0.05$) are in italics.

| **Least Cost Paths** | | | | |
|---|---|---|---|---|
| Genetic Distance | Landscape Variable | Cost | *P* | $R^2$ |
| CSE | *Impervious Surface* | *500* | *0.0159* | |
| | Mixed Forest | 1/5 | 0.1339 | |
| | *overall* | | *0.0207* | *0.0761* |
| FST | *Impervious Surface* | *500* | *0.0037* | |
| | *Mixed Forest* | *1/5* | 0.0240 | |
| | *overall* | | *0.0043* | 0.1345 |
| cGD | *Open + Low Development* | *500* | *0.0153* | |
| | Agriculture | 1/5 | 0.2289 | |
| | *overall* | | *0.0163* | *0.0853* |
| **All Paths** | | | | |
| Genetic Distance | Landscape Variable | Cost | *P* | $R^2$ |
| CSE | *Medium Development* | *5* | *0.0028* | |
| | *Open Development* | *2* | *0.0069* | |
| | *Floodplain* | *500* | *0.0248* | |
| | Evergreen Forest | 500 | 0.0858 | |
| | Agriculture | 500 | 0.2585 | |
| | *Overall* | | *0.0016* | *0.1626* |
| FST | *Medium Development* | *5* | *0.0002* | |
| | *Floodplain* | *500* | *0.0053* | |
| | *Grassland* | *500* | *0.0398* | |
| | *Evergreen Forest* | *500* | *0.0316* | |
| | *Wetland* | *2* | *0.0001* | |
| | Pasture | 500 | 0.1251 | |
| | *Overall* | | *0.0001* | *0.2551* |
| cGD | *Deciduous Forest* | *500* | *<0.0001* | |
| | *Open Development* | *500* | *<0.0001* | |
| | *Pasture* | *5* | *<0.0001* | |
| | *Mixed Forest* | *500* | *0.0271* | |
| | Medium Development | 5 | 0.1876 | |
| | *Overall* | | *<0.0001* | *0.3826* |

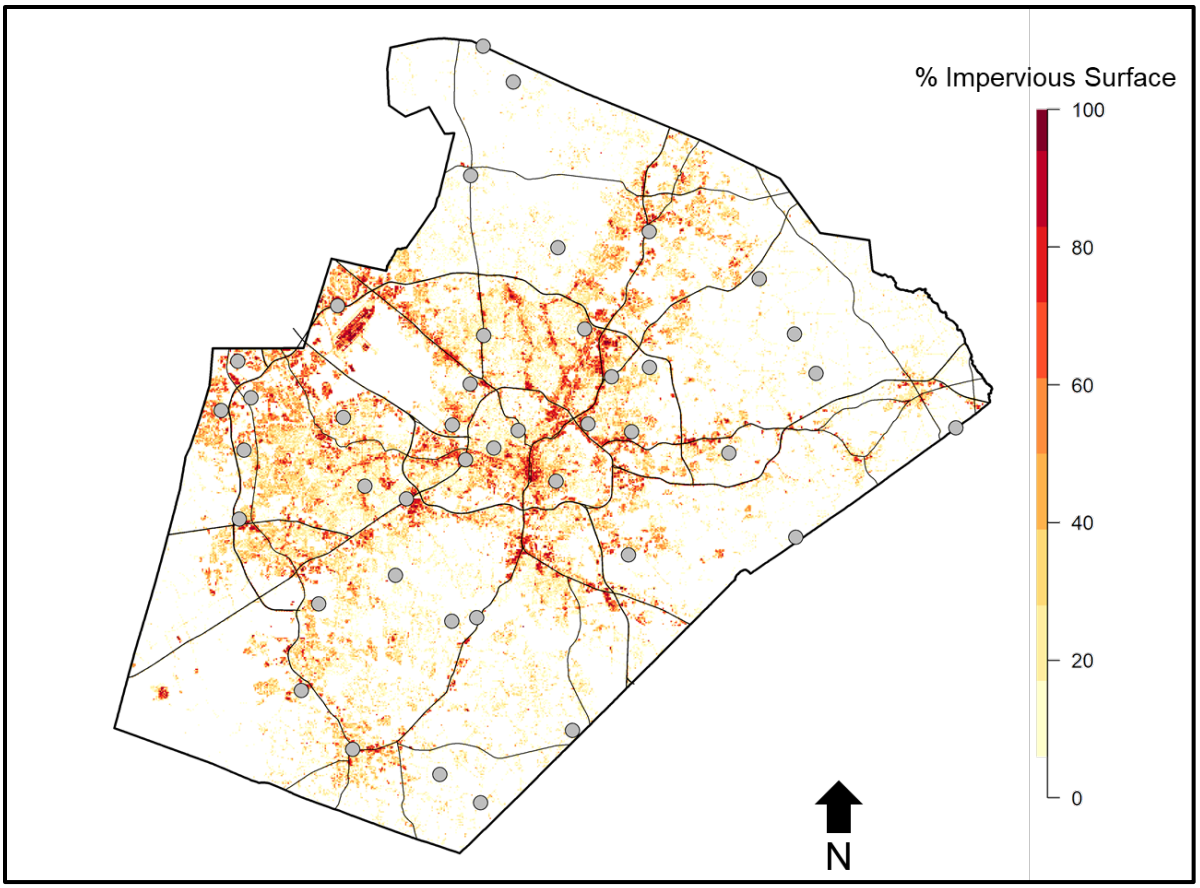


**Figure 3.1** Sample sites for genomic sequencing in Wake County, North Carolina. Background shows percent impervious surface from the USGS 2016 impervious surface dataset and highways in Wake County. Raleigh is located in the center of the county.

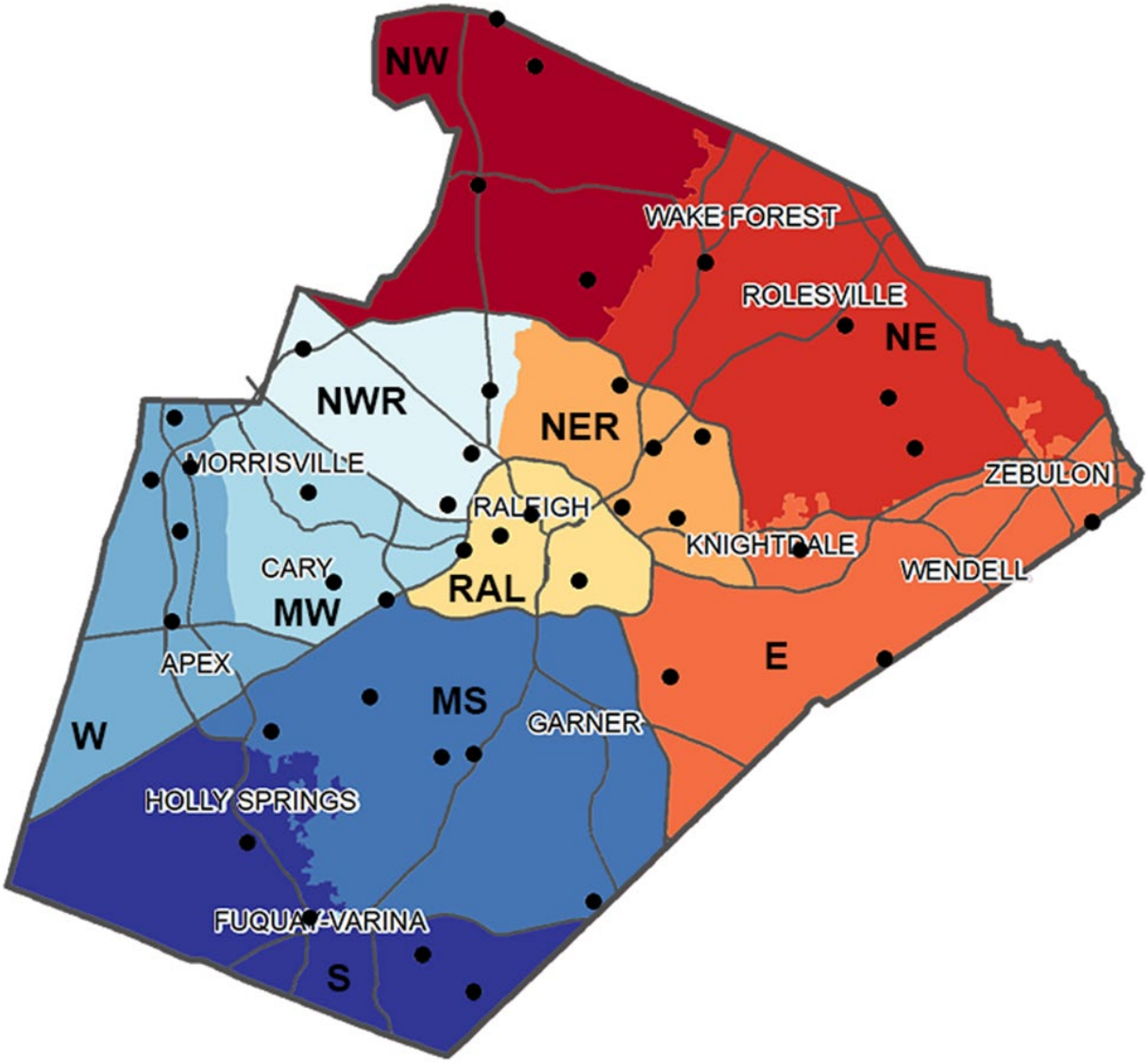


**Figure 3.2** To compare node parameters and quantify directional gene flow, I grouped sample sites in ten regions of 3-5 sites. I determined sites based on proximity to Wake County jurisdictions (black labels with white background) and major road networks. Abbreviations for regions are shown in black: RAL = central Raleigh; NWR = northwest Raleigh; NER = northeast Raleigh, E = east; MS = mid-south; S = south; W = west; MW = mid-west; NW = northwest, and NE = northeast.

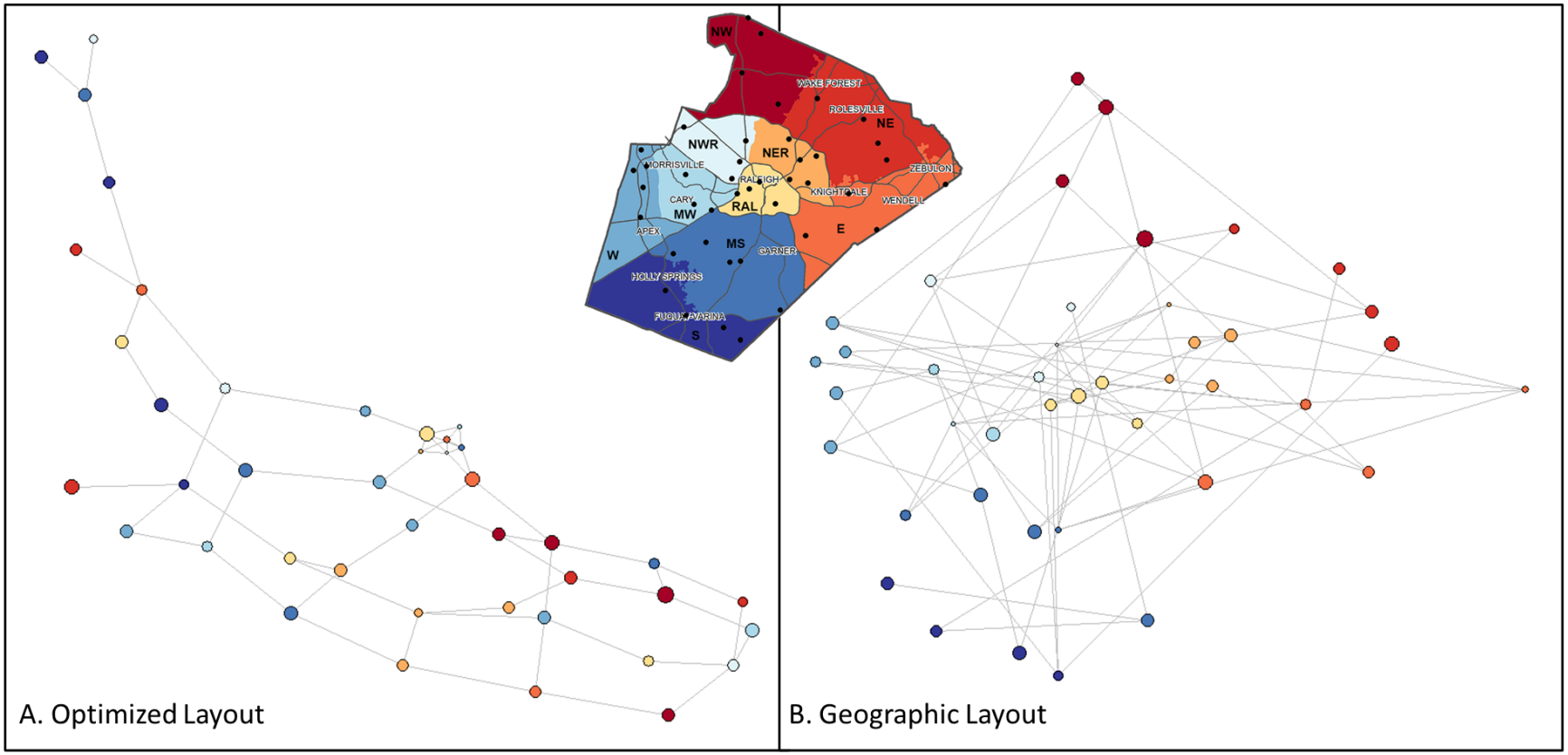


**Figure 3.3** Population graph of the 42 sample sites in Wake County generated and visualized in the R package *popgraph.* Nodes represent sampled sites. Node size reflects the within-site contribution to observed genetic variation across all locations and node color signifies the region in which the site was located. Sites are connected by edges (grey) that were pruned to minimize number of connections while still explaining the between-node contributions to genetic variance. In the optimized layout (A), edge length is proportional to genetic distance, with longer edges showing greater genetic differentiation between connected nodes. (B) shows the same connections with nodes positioned based on their geographic coordinates.

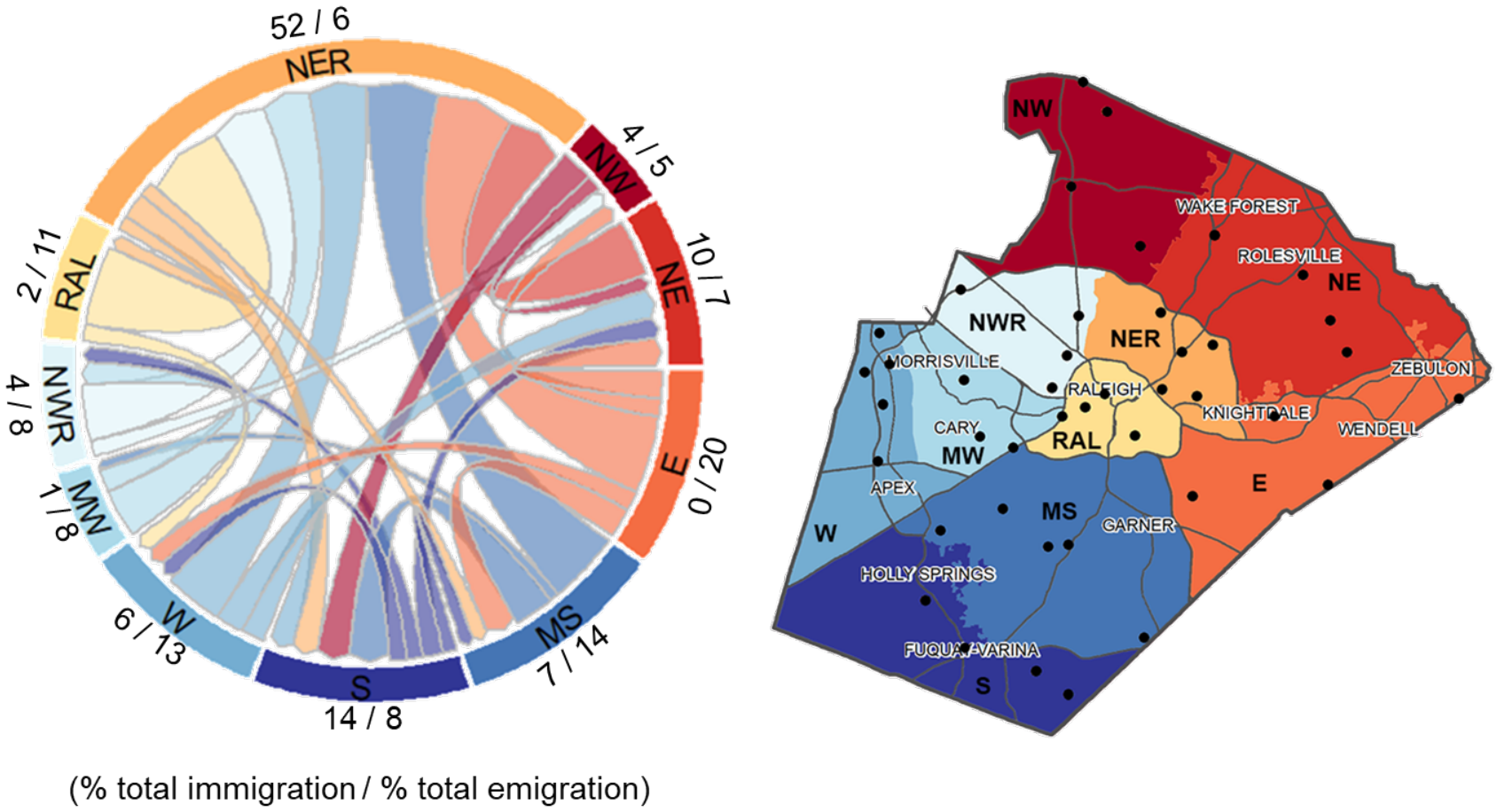


**Figure 3.4** Directional gene flow between regions of Wake County. Only estimates of migration that had a 95% confidence interval that did not include zero are included. Width of arrows indicates migration rate, and fractions along outside of regions indicate the percent of relative immigration and the percent of relative emigration moving into and out of the region, respectively.

# CHAPTER 4

## The *Aedes albopictus* roadmap: using landscape genetics to model past and future gene flow in Wake County

### Introduction

Landscape genetics is a largely exploratory field in which researchers seek to identify landscape features that affect gene connectivity, either by hindering or facilitating gene flow (Storfer et al. 2010). However, there is the potential to apply landscape genetic studies to species management in the face of rapid climate and land use change. Such an approach expands on species distribution models, which are used to predict the effects global change on habitat availability and arrangement (Rodríguez et al. 2007). Predictive landscape genetics can enhance distribution models by considering the effects of land use change between patches.

Landscape genetics can be used to forecast how future land use changes may impact species connectivity (Storfer et al. 2007). By modelling future connectivity, we can evaluate how potential changes to landscape composition, configuration, and quality from the combined effects of global land-use and climate change shape patterns of gene flow within and between populations (Holderegger and Wagner 2006). By predicting future land-use change and its effects on genetic connectivity, we can better manage species through adaptive and strategic interventions (van Strien et al. 2014). In an urban context, such interventions could be extended to city planning. For example, new development and infrastructure could be designed to mitigate or even enhance gene flow for at-risk species (Kong et al. 2010; Cadavid-Florez et al. 2020). With predictive landscape genetic models, we can consider the landscape configuration and composition in urban growth scenarios to identify regions of high and low impact on connectivity (Bolliger and Silbernagel 2020).

Several studies have used landscape genetic models to predict the effect of future land use on population gene flow (e.g., van Rees et al. 2017; Cox et al. 2020). Van Strien et al. (2014) first demonstrated the potential to apply landscape genetics to management in their study of the large marsh grasshopper. Their models forecast a reduction in gene flow between grasshopper population pairs in land use scenarios with new residential construction, but no significant change in gene flow under a forest restoration scenario. They also found that gene flow would increase with building demolition to allow the establishment of a new populations. Using a

similar simulation approach, Wan et al. (2018) evaluated the effects on connectivity under different scenarios of fragmentation, patch configuration, and isolation by resistance for the Mexican spotted owl. They found that landscape resistance increased genetic differentiation more than isolation by distance models, and that this difference was amplified when maximum dispersal distance was increased. In both examples, the study species were of conservation concern and had populations that were patchily distributed with low levels of genetic diversity and high genetic structure.

It is unclear how well approaches used to simulate connectivity in at-risk species would perform for a continuously distributed invasive species with low genetic differentiation across the metapopulation. Invasive species are often generalists and better adapted to a range of habitat types, disturbances, and environmental changes (Moffitt and Osteen 2006). Therefore, it is more difficult to differentiate habitat patches from the landscape matrix and to identify landscape features that govern connectivity and gene flow of the invasive population. This complicates efforts to predict how future land use change will change migration and population genetic dynamics.

Here, I provide a simple framework to simulate future genetic connectivity in the abundant and continuously distributed invasive mosquito *Aedes albopictus* within Wake County, North Carolina. *A. albopictus* was established in Wake County in 1992 and is not currently experiencing the effects of localized population bottlenecks or spread, unlike the populations of at-risk species in the studies described above (Van Strien et al. 2014, Wan et al. 2018). Rapid changes in population size and genetic variation can make it more difficult to detect more subtle landscape effects on gene flow (Balkenhol et al. 2015). In addition, *A. albopictus* habitat associations are complex. In its nonnative range, including Wake County, *A. albopictus* is adapted to anthropophilic environments. However, females will consume blood meals from a variety of sources and oviposit eggs in a diversity of environments (Li et al. 2014; Goodman et al. 2018). Consequently, *A. albopictus* habitat preferences may shift between landscapes with different land use composition and configuration, all of which affect fine-scale patterns distribution, dispersal, and gene flow. Given both its genetic stability and strong association with human-dominated landscapes, the Wake County *A. albopictus* population is a good system to study the effects of future development on genetic connectivity in invasive species.

The population in Wake County is increasing, necessitating urban growth. Since 1990, the population of Wake County has more than doubled, from 430,000 residents to 1.1 million in 2018. Urbanization in the area is characterized by suburban sprawl, which is the conversion of natural land use to expansive multi-use and low-density residential developments (Terando et al. 2014). As the population continues to grow, the county government and the twelve municipalities contained within have developed near-future (2030-2050) land use plans that map priority regions for development. Therefore, I can predict *A. albopictus* genetic connectivity by simulating future land use scenarios rooted in explicit, highly local urban planning data.

**Methods**

Genetic Data

I used genetic data from adult *A. albopictus* sampled across 42 Wake County sites in 2018 following previously described bioinformatic pipelines and genetic markers (Reed 2021, Chapter 3). These data comprised 1,193 single nucleotide polymorphisms (SNPs) with genotyping rates greater than 90% across 276 adult *A. albopictus*, with 5-10 individuals per sampling location. Following Pless et al. (2021), I used linearized $F_{ST}$ ($F_{ST}$ / 1- $F_{ST}$) to measure pairwise genetic distance in the hierfstat R package (Goudet 2005). I selected linearized $F_{ST}$ because it was best explained by previous landscape genetic models over Cavalli-Sforza and Edwards chord distance (Reed 2021, Chapter 3). I also tested conditional genetic distance (Dyer et al. 2010), but resistance surfaces failed to converge during optimization (see below).

Land Use Data & Resistance Surface Optimization

I created resistance surfaces based on modern Wake County land use data. Resistance surfaces are spatially explicit layers that assign cost values to landscape features. Those cost values represent the degree to which the associated feature facilitates or enhances genetic connectivity of the study organism and can be defined by empirical data when available, ecological hypotheses, or expert opinion (Peterman 2018). The cost is often a unitless number that represents the effort needed to traverse the landscape feature relative to other features. Resistance cost can apply to the energy an individual requires to pass through a cell or to multi-generational movement depending on the species and the spatial extent (McRae 2006). Because I did not have *a priori* data or hypotheses about the specific cost values of different land use classifications for

*A. albopictus*, I generated resistance surfaces that are neutral to the mechanisms governing gene flow, only the relative resistance of flow compared to other classifications.

To create resistance surfaces, I used the 2016 USGS National Land Cover Database (NLCD; Dewitz 2019). The 2016 NLCD is a 30-m resolution image classification based on Landsat satellite imagery (Yang et al. 2018). I determined which land use categories to define in resistance layers based on the results in Reed (2021; Chapter 3), which identified development and forest as the most important landscape features for *A. albopictus* genetic connectivity. Based on my previous models, I optimized resistance costs for the four development categories (open, low intensity, medium intensity, and high intensity; values 21-24 respectively) and the three forest categories (deciduous forests, evergreen forests, mixed forests; values 41-43 respectively). These seven categories make up 77% of the land cover in Wake County (**Table 4.1**). Developed open space are areas with minimal constructed material and manicured vegetation. These areas are generally dispersed residences or recreational areas. Low-intensity development is a mix of construction and vegetation with 20-49% impervious surface cover. In Wake County, this tends to be suburban areas dominated by single-family housing units. Medium-intensity development is between 50-79% impervious surface characterized by more dense residential housing, and high-intensity development is between 80-100% impervious surface dominated by apartment complexes and commercial or industrial facilities (Yang et al. 2018; **Figure 4.1**).

Before analysis, I aggregated NLCD raster pixels by a factor of 10 from 30-m resolution to 300-m resolution, where most frequent land-use type determined the value of the aggregate pixel. A 300-m resolution was adequate to study *A. albopictus* gene flow because it is within the maximum dispersal distance of *A. albopictus* (Honório et al. 2003), and because migration between sampled locations likely occurs over several generations (Reed 2021, Chapter 3). In addition, changes in resolution do not have large effects on landscape genetics results if the cell size is fine enough to retain important landscape features (McRae et al. 2008; Peterman and Jarman 2018; Winiarski et al. 2020). However, statistical power to detect the effects of landscape on gene flow increase with finer grain analysis (Cushman and Landguth 2010). I found that a 300-m cell size was an appropriate compromise between reducing computation time and selecting a grain-size relevant to the species.

With the R package ResistanceGA (Peterman and Jarman 2018), I estimated resistance costs for land use classifications. ResistanceGA uses an iterative optimization algorithm to fit

resistance distance against observed genetic distance (Peterman and Jarman 2018). ResistanceGA estimates a resistance matrix for landscape features and tests the predictive power of that matrix against the genetic distances between sites. ResistanceGA tested and optimized surfaces by using the *commuteDistance* function in the R package gDistance (van Etten 2017). The *commuteDistance* algorithm is identical to Circuitscape (McRae et al. 2008) and processes data more quickly (Marrotte and Bowman 2017; Arredondo et al. 2018). Processing time is an important consideration when running ResistanceGA, as its optimization method iteratively varies resistance costs for input features. During each iteration, ResistanceGA runs *commuteDistance* and evaluates performance by regressing resistance distance against genetic distance. I ran ResistanceGA for five combinations of the seven land use categories (four development and three forest): all development categories, all forest categories, and all development plus each forest category (**Table 4.2**). For each model, I treated cells that were not classified as one of the named land use types as a single "other" category. I evaluated models with optimized resistance costs for different sets of land use classifications using log likelihood, Akaike's information criteria (AIC), and the amount of variation explained compared to a null model (marginal $R^2$, **Table 4.2**).

Future land use and connectivity

To model future land use, I compiled government-produced 'future land use' maps and comprehensive plans for Wake County and its twelve municipalities (**Figure 4.2**). Within each municipality, I used available plans and maps to select the areas of priority development. These were areas that were either outlined on the map or highlighted in the plans as future activity centers. One municipality, Wake Forest, did not have future land use maps or plans available, so only areas that were currently under development or were approved for development were included. Geocoded future land use maps were available for seven of the remaining 11 municipalities. For the final four (Rolesville, Wendell, Knightdale, Holly Springs), I used current zoning maps and overlaid them with future land use maps in Adobe Photoshop (Adobe Photoshop CC: 2017 release). I then selected the areas that corresponded with future regions of development in ArcMap v.10.4.1 (ESRI 2016, Redlands CA). Only the NLCD pixels within future development regions could be modified for future land use predictions (**Figure 4.3**).

Pixels within future development regions that were already classified as developed in the 2016 NLCD dataset retained their values, as did pixels denoted as water (value 11) or wetlands (values 90, 95). The remaining pixels were randomly assigned a development value. To determine the probability that a pixel was assigned to each value, I designed four scenarios representing different levels of development intensity (**Table 4.3**). In the first, the probability for each development value corresponded to the frequency of that value within developed areas across Wake County in 2016. This is the "business as usual" scenario, where future development intensity reflects the current level. The second through fourth scenarios increased the frequency of the medium and high intensity developed areas by 50, 100, and 200%, respectively, and subtracted the number of pixels assigned to the open and low development classes accordingly. I performed ten replicates of each scenario and calculated the commute distances between 2018 sampling points for each to assess the degree to which random cell assignments affected change in gene flow and connectivity. I then compared commute distances in each scenario to those calculated from current NLCD land use in Wake County.

I performed ten replicates of each scenario and calculated the resistance distances between 2018 genetic sample points using the *commuteDistance* function in gDistance. For each scenario, I used multiple regression on distance matrices (MRDM) models to assess the degree to which random cell assignments affected change in gene flow and connectivity. I then compared commute distances in each scenario to those calculated from current NLCD land use in Wake County.

## Results

### Resistance Surface Optimization

The top performing model included all four development classes and no forest features (marginal $R^2 = 0.2418$; **Table 4.2**). This was also the only model with a smaller AIC than the null model (null hypothesis: all genetic distances are uniform). Of all models, the isolation by distance model performed the worst, with no improvement over the null model (marginal $R^2 = 0$). For all resistance models, developed and forested land cover hindered gene flow relative to the baseline (e.g., all other land use classes, **Table 4.2**).

In the all-development model, low-intensity development had the smallest cost resistance to gene flow (4.16:1.00, where 1.00 is the cost for all non-development land uses; **Figure 4.4**).

Open development had the next-lowest resistance cost (75.16:1.00). Medium-intensity development had the highest resistance (449.33:1.00), followed by high-intensity development (resistance cost 242.40:1.00).

The model that included only forest land classifications did not converge, and the addition of forest classes to the all-development model did not improve model performance (**Table 4.2**). Of the development + forest models, the model that included evergreen forests performed best (marginal $R^2 = 0.2394$). This model found similar cost values for low, medium, and high-intensity developed after optimization against genetic distance (5.38, 432.97, and 205.31:1.00, respectively). However, unlike the all-development model, this model assigned open development land classes a lower resistance cost than low-intensity development. Evergreen forest had a slightly higher resistance to *A. albopictus* gene flow than open and low-intensity development.

Future land use scenarios and genetic connectivity

The areas prioritized for development covered 30.7% of Wake County, and the undeveloped portions of the priority development area covered 31.78% of undeveloped land (**Figure 4.5**). Within future land use scenarios, all replicates were highly correlation ($R^2 > 0.98$). I used the mean resistance distances between sites across replicates to compare against baseline pairwise distances that used 2016 land-use data. Between scenarios three and four, the dominant development class switched from open to medium-intensity development (**Table 4.3**). Nevertheless, overall predicted levels of genetic and landscape connectivity did not vary strongly between future land use scenarios (**Figure 4.6**). All scenarios predicted an overall decrease genetic connectivity than in Wake County 2016, although this effect was minimal (between 1.04—6.02% increase in mean pairwise commute distances). Of the 861 pairwise distances estimated, pairwise commute distance increased in 59.3, 61.7, 61.2, and 63.0% for scenarios 1-4, respectively. However, when I compared pairwise differences in a spatially explicit context, I observed that genetic connectivity was predicted to increase in certain areas of the county (**Figure 4.7**). For example, in scenarios 1-3, pairwise connectivity increased at eastern and southern sites, while in scenario 4, sites that increased in connectivity were in central Wake County (**Figure 4.7**).

## Discussion

### Resistance Optimization

I found stark variation in optimized resistance costs between the four development classes. These classifications are based largely on percent impervious surface, with set thresholds between classes (open development: < 20%; low intensity: 20—49%; medium intensity: 50-79%; high intensity: 80-100%). Open developments were diffuse residential areas, manicured lawns, and golf courses with minimal construction. Low and medium intensity developed spaces were largely suburban and residential, though at different housing densities, while high intensity development was characterized by multifamily residences such as apartments, office spaces, commercial areas, and industrial facilities (Homer et al. 2020; **Figure 4.1**). I would predict that high-density development, with a large portion of impervious surface area and fewer households, would be the strongest barrier to gene flow. However, the optimized costs for the best-fit resistance surface identified medium development as the costliest land use category hindering *A. albopictus* gene flow. This pattern was consistent between optimization models regardless of which additional land-use classifications were included. Medium-intensity developed was also identified as a barrier to *A. albopictus* gene flow in a previous exploratory landscape genetic study (Reed 2021, Chapter 3).

The ability to optimize costs on resistance surfaces is a powerful step forward for the field of landscape genetics. It reduces reliance on expert opinion to determine the direction and magnitude of effect that a landscape feature will have on a population (Balkenhol et al. 2015; Peterman and Jarman 2018). It also permits a more rigorous investigation of the landscape genetics for species with sparse spatial and movement ecology data (Winiarski et al. 2020), as is the case for *A. albopictus*.

That the all-development model best predicted changes in *A. albopictus* genetic connectivity over other land use categories can in part be attributed to the topography and arrangement of urban centers in Wake County. Development is geographically central in Wake County, and therefore most straight-line distances between sampled sites will pass through developed land use types. The all-forests model failed to converge in resistanceGA because paths between sampled sites did not pass through the forested regions concentrated along the periphery

of county. This result demonstrates that these observations cannot yet be generalized to other areas, but instead serve as a case study to inform local management and urban planning.

Predicted near-future genetic connectivity

Future predictions of gene flow show an overall reduction of gene flow in Wake County with development. I created four future land use scenarios, which represent increasing intensities of urbanization in undeveloped areas of Wake County. I modeled an increase in urban intensity with greater proportions of medium and high-intensity land use compared to land use in 2016. While all four scenarios predicted a decrease in *A. albopictus* genetic connectivity, I also observed areas of the county where gene flow was predicted to increase between sites. In scenarios 3 and 4, I observed an increase in genetic connectivity between sites that were already located in developed areas (**Figure 4.7**). These results suggest that diffuse urban growth may act facilitate *A. albopictus* dispersal relative to denser developments. In other words, with continued suburban sprawl in Wake County, the amount of undeveloped land will decrease, and low-intensity developments will act as de facto corridors to gene flow between *A. albopictus* populations relative to other development types.

Predicted genetic connectivity would likely increase if I used models that included forest classes. In the deciduous forest model and the evergreen forest model, forests were more costly to *A. albopictus* gene flow than open and low-intensity development (**Table 4.2**). Therefore, if forested areas were developed, genetic connectivity would increase as long as development was primarily diffuse residential neighborhoods and suburbs. This would likely be the case in Wake County based on both current land use patterns and the future development plans (**Table 4.1**). Based on the priority areas I identified for future development (**Figure 4.3**), southwest Wake County would see the largest increase in genetic connectivity from deforestation, as this is the region with the largest proportion of deciduous and evergreen forests that is also slated for development (**Figure 4.5**).

The conclusions from the future land use scenarios presented here also come with caveats. Perhaps most important, these scenarios only measured the effects of horizontal anthropogenic expansion. This follows an urban sprawl model consistent with the southeast United States and North Carolina (Terando et al. 2014; Carruthers and Ulfarsson 2016). However, many Wake County municipality plans include further development of their urban

centers which may act to hinder observed increases in connectivity. Additionally, I did not distinguish between types of urban expansion. Instead, I probabilistically assigned NLCD raster pixels to development classes. I chose this approach to reduce potential bias while interpreting the future growth plans of different municipalities. Between municipalities, there are not consistent codes or terminology for zoning or future land use plans. Therefore, it was difficult to compile and group types of development between governances. For example, Apex residential planning includes categories such as medium, medium/high, and high density residential, while the Cary future land use plan defines residential areas with terms such as heritage or traditional neighborhoods.

In the future, governances could coordinate cohesive development plans and use consistent language for definitions of land use types. This would allow me to assign land classification values more accurately for prediction scenarios. Alternatively, simulated future land use models could generate clumps of pixels to represent new developments with similar levels of urban intensity. Such an approach would have an advantage over the random assignment of development classes used here because it would better reflect realistic development. In particular, new higher-density areas may disrupt gene flow in *A. albopictus* that would be more readily detectable with landscape genetic-based predictive models. However, this would require more user-defined parameters that determine the number, shape, size, and growth rates of clusters for each development classification and increase computational load (Batty et al. 1999; Wu 2002).

Recommendations for Wake County Planning

While previous landscape genetic research of this nature aims at maintaining or improving gene flow in imperiled species, here my aim was to understand how landscape and urbanization affect connectivity in a nonnative, nuisance arthropod vector. However, my recommendations are in line with sustainable growth models. To interrupt local *A. albopictus* dispersal, Wake County should focus on building up low-density urban areas and conserve undeveloped lands. While I did find evidence of gene flow within rural areas, we have previously found that adult *A. albopictus* densities are lower outside of human centers in Wake County (Reed et al. 2019; Reiskind et al. 2020; Reed 2021, Chapter 2). Adult mosquitoes are also less likely to interact with humans in these environments, which minimizes the risk of zoonotic disease transmission

and reduces the frequency of nuisance encounters. Therefore, mosquito control should target new developments, especially low-density suburbs, as areas for increased gene flow and *A. albopictus* population growth. Specifically, my results suggest that new residential areas in the eastern and southeastern regions of the county are likely to become hotspots for *A. albopictus* in the near future.

**Conclusion**

I have presented a framework for using landscape genetics to make baseline predictions about population gene flow under future land use scenarios. This case study demonstrates the potential applications of landscape genetic approach for urban planning at a local, management-relevant scale. While the future growth scenarios are relatively simple, I used real-world resources to determine areas of priority development within Wake County. The future land use scenarios can be readily modified as plans change and with practitioner and policy input. Such place-centered research has high potential for direct impact on decision-making, a necessity in a time of rapid global change.

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**Table 4.1** NLCD land use classifications and their percent land cover at a 300m resolution in Wake County in 2016 and under four future land use scenarios.

| | | Percent Cover | | | | |
|---|---|---|---|---|---|---|
| **Land Cover Classification** | **NLCD Value** | **2016** | **S1** | **S2** | **S3** | **S4** |
| Open water | 11 | 2.82 | 2.82 | 2.82 | 2.82 | 2.82 |
| Developed – open space | 21 | 24.17 | 36.15 | 34.80 | 34.02 | 31.59 |
| Developed – low intensity | 22 | 7.53 | 11.06 | 11.04 | 10.58 | 9.78 |
| Developed – medium intensity | 23 | 5.24 | 7.74 | 8.99 | 10.09 | 12.99 |
| Developed – high intensity | 24 | 0.63 | 0.94 | 1.06 | 1.21 | 1.53 |
| Barren | 31 | 0.28 | 0.13 | 0.13 | 0.13 | 0.13 |
| Deciduous forest | 41 | 8.64 | 5.77 | 5.77 | 5.77 | 5.77 |
| Evergreen forest | 42 | 14.72 | 10.43 | 10.43 | 10.43 | 10.43 |
| Mixed forest | 43 | 13.62 | 9.13 | 9.13 | 9.13 | 9.13 |
| Shrub/scrub | 52 | 0.85 | 0.58 | 0.58 | 0.58 | 0.58 |
| Grassland/herbaceous | 71 | 1.70 | 1.08 | 1.08 | 1.08 | 1.08 |
| Pasture/hay | 81 | 8.53 | 5.34 | 5.34 | 5.34 | 5.34 |
| Cultivate crops | 82 | 6.52 | 4.08 | 4.08 | 4.08 | 4.08 |
| Woody wetlands | 90 | 4.74 | 4.74 | 4.74 | 4.74 | 4.74 |
| Emergent herbaceous wetlands | 95 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |

**Table 4.2** Evaluated land use classification models compared to a null model (genetic distances are uniform) and an isolation by distance model (genetic distance increases with Euclidean distance). The five models were combinations of seven NLCD classifications: open development (21), low (22), medium (23), and high-intensity (24) development, deciduous forest (41), evergreen forest (42), and mixed forest (43) at a 300m resolution. Model parameters, or cost values for land classifications, were optimized using ResistanceGA using linearized $F_{ST}$ as a response variable. I compared model performance based on Akaike's Information Criteria (AIC), log likelihood (LogLik), and marginal $R^2$ ($mR^2$: variation explained by land use cost values). The Development model was selected at the best model based on all criteria (AIC, LogLik, $mR^2$). The Forest model failed to converge during optimization.

| | | | | | **Optimized Cost Values** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Model** | **Model Description** | **AIC** | **LogLik** | **$mR^2$** | **Other** | **21** | **22** | **23** | **24** | **41-43** |
| Null | Null | -5975.08 | 2988.54 | 0 | | | | | | |
| Null-IBD | Isolation by distance | -5973.08 | 2988.54 | 0 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 41-43 | Forest | NA | NA | NA | | | | | | |
| 21-24 | Development | -5975.36 | 2993.68 | 0.2418 | 1.00 | 75.16 | 4.16 | 449.33 | 242.40 | |
| 21-24, 41 | Development + deciduous forest | -5972.87 | 2993.43 | 0.2307 | 1.00 | 21.71 | 1.59 | 461.24 | 69.85 | 189.84 |
| 21-24, 42 | Development + evergreen forest | -5973.24 | 2993.62 | 0.2394 | 1.00 | 4.54 | 5.38 | 432.97 | 205.31 | 6.37 |
| 21-24, 43 | Development + mixed forest | -5972.78 | 2993.39 | 0.2282 | 1.00 | 72.95 | 5.69 | 413.16 | 305.80 | 3.90 |

**Table 4.3** Future land use scenarios of urban intensity. I modeled four scenarios of urban growth in regions designated as high priority for development (Figure 2). For each scenario, I varied the probability that a pixel was assigned to a specific development class (21-24; open, low-intensity, medium-intensity, and high-intensity development, respectively). The probabilities in the first scenario reflected the proportions of each development class in the 2016 NLCD dataset. In scenarios 2-4, Medium and high-intensity development increase by 50, 100, and 200% each in newly developed areas compared to 2016, respectively. Commensurate decrease is split proportionately between open and low intensity developments.

| Scenario | Description | Probabilities |
|---|---|---|
| Scenario 1 | Proportions of each development class are consistent with 2016 | 21: 0.6373<br>22: 0.1985<br>23: 0.1381<br>24: 0.0166 |
| Scenario 2 | 50% increase in medium and high-intensity development | 21: 0.5857<br>22: 0.1824<br>23: 0.2071<br>24: 0.0248 |
| Scenario 3 | 100% increase in medium and high-intensity development | 21: 0.5267<br>22: 0.1640<br>23: 0.2762<br>24: 0.0331 |
| Scenario 4 | 200% increase in medium and high-intensity development | 21: 0.4088<br>22: 0.1273<br>23: 0.4142<br>24: 0.0497 |

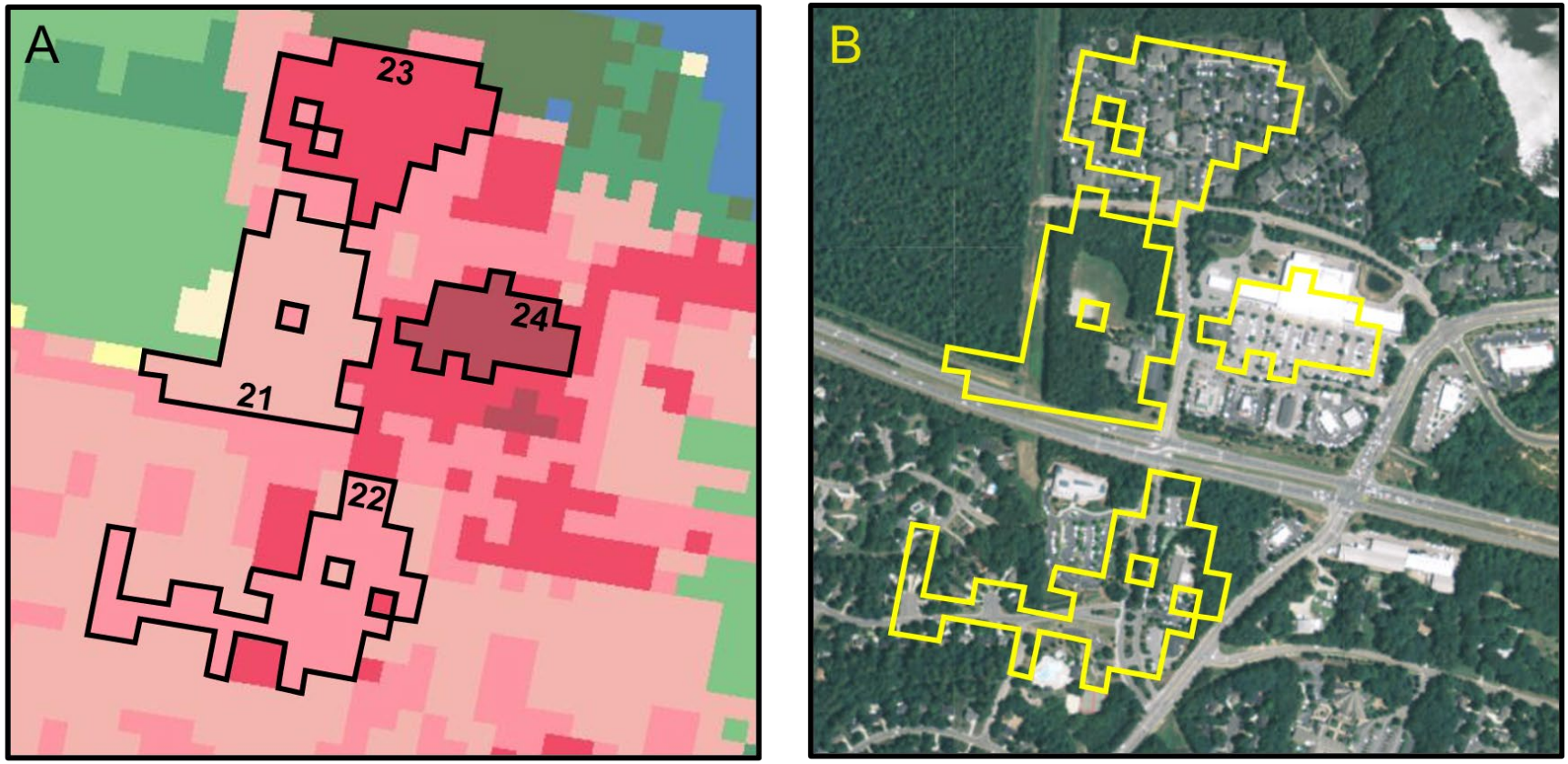


**Figure 4.1** (A) National land classification database (30m pixels) and (B) 2016 satellite imagery of a portion of Wake County, demonstrating classifications for developed land. Open development is assigned value 21; low-intensity, 22; medium intensity, 23; and high-intensity development, 24. Black (A) and yellow (B) shapes show outlines of different patches of each development classification on the NLCD and satellite maps.

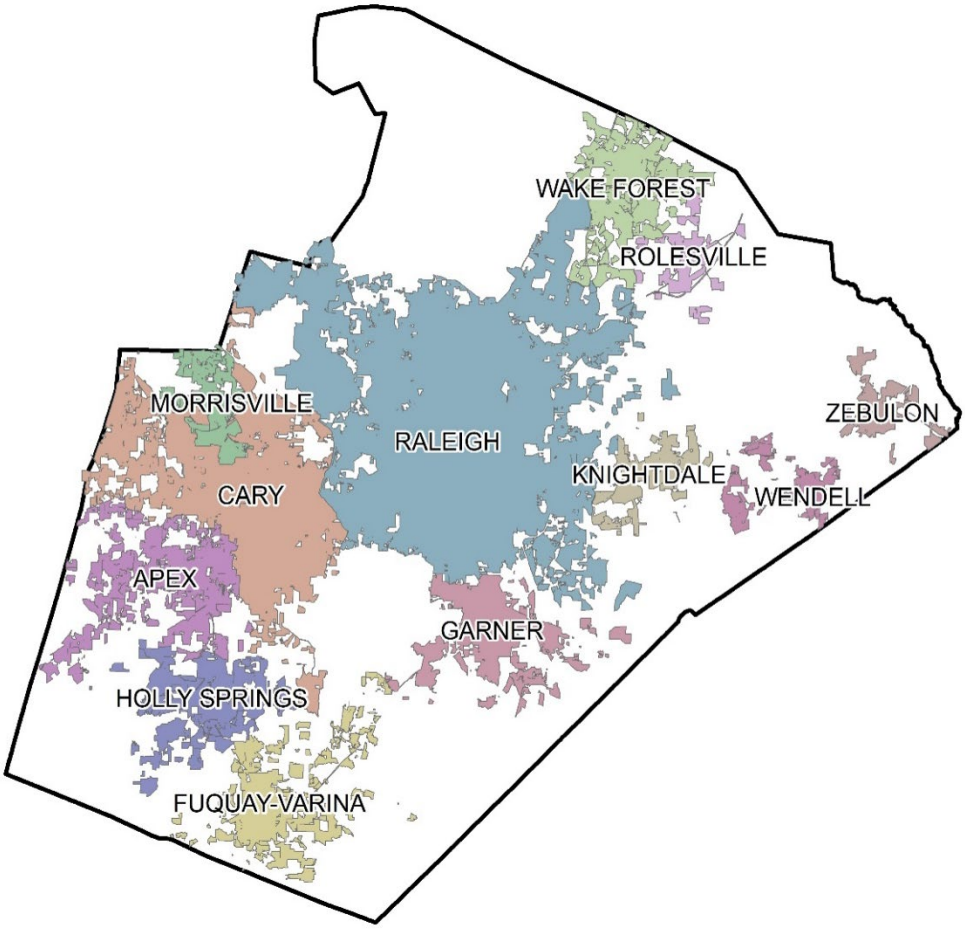


**Figure 4.2** Map of the twelve municipalities within Wake County and neighboring cities (Angier, Durham, and Clayton). I compiled future land use plans and maps for each municipality and Wake County to determine areas of urban growth.

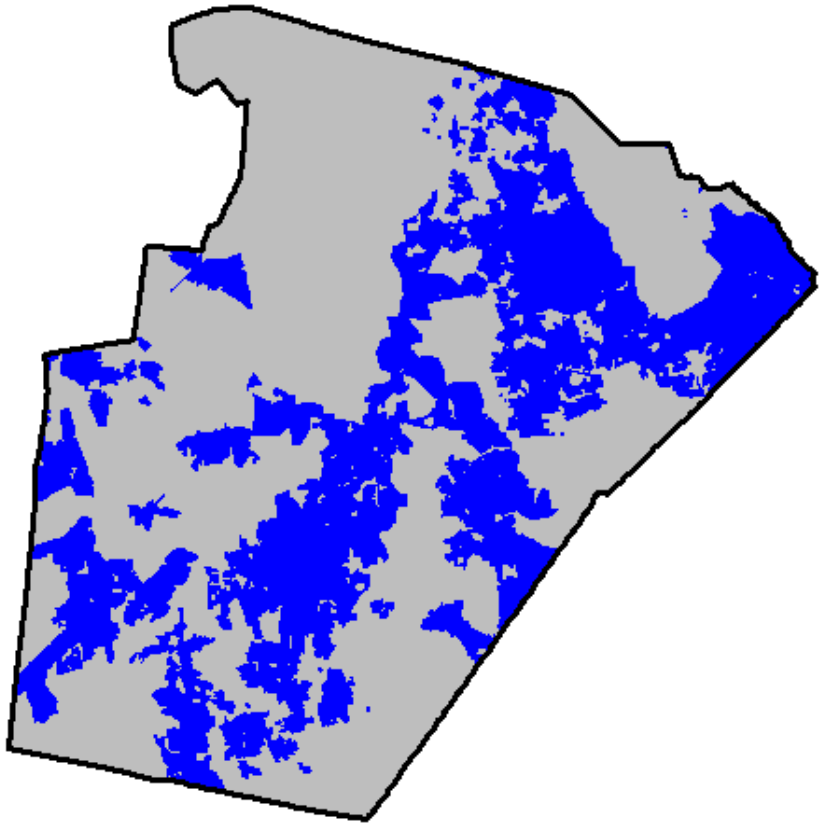

**Figure 4.3** Areas identified as high priority for development across Wake County and its twelve municipalities are shown in blue. Only NLCD values in these areas were modified for future land use scenarios.

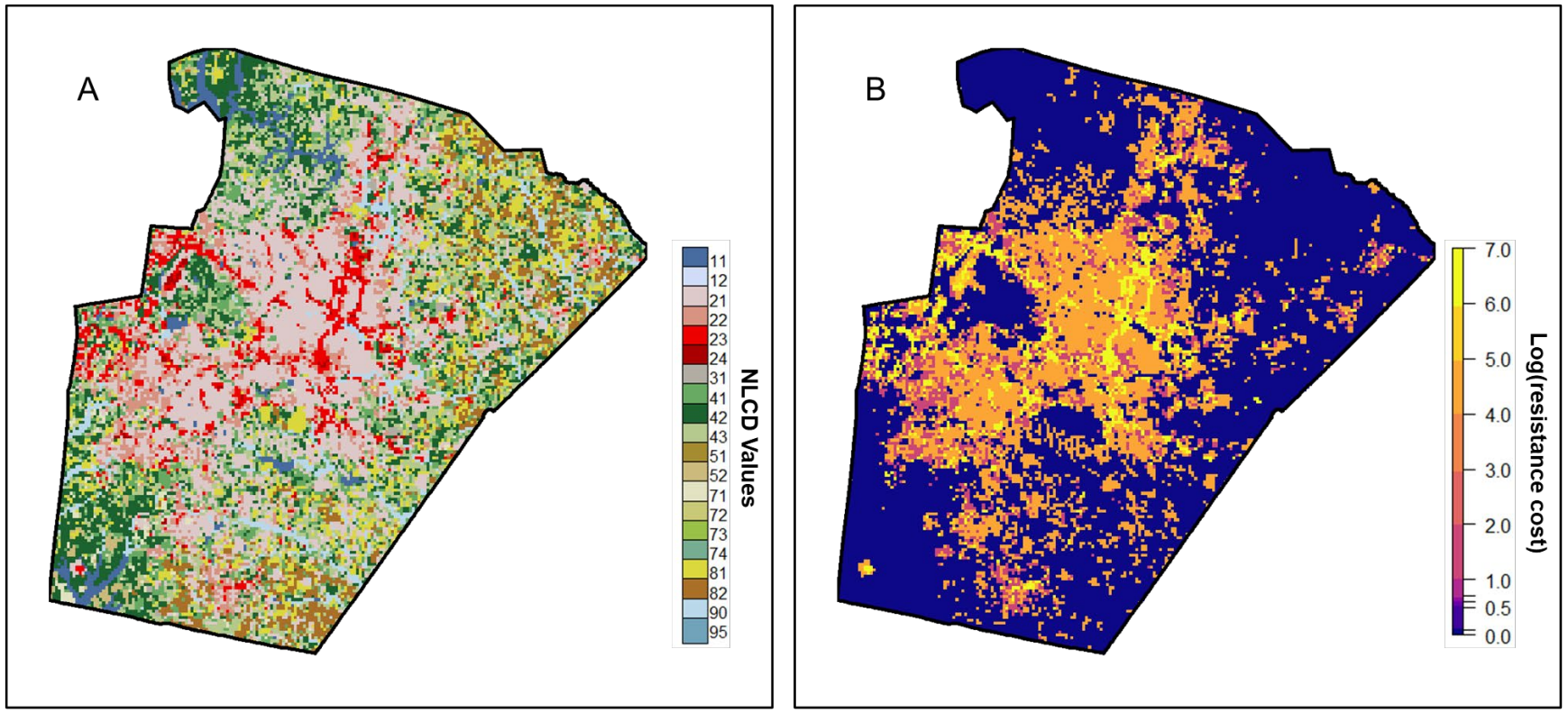


**Figure 4.4** (A) USGS land cover classifications for Wake County (see **Table 4.1** for descriptions of NLCD values) and (B) the Wake County cost surface for the all-development model after resistance optimization. Cost values are log transformed for visualization. Medium-intensity development had the highest cost, followed by high-intensity, open, and low-intensity development (cost values 449.33, 242.40, 75.16, and 4.16 respectively). All other land use types had a cost of 1.00.

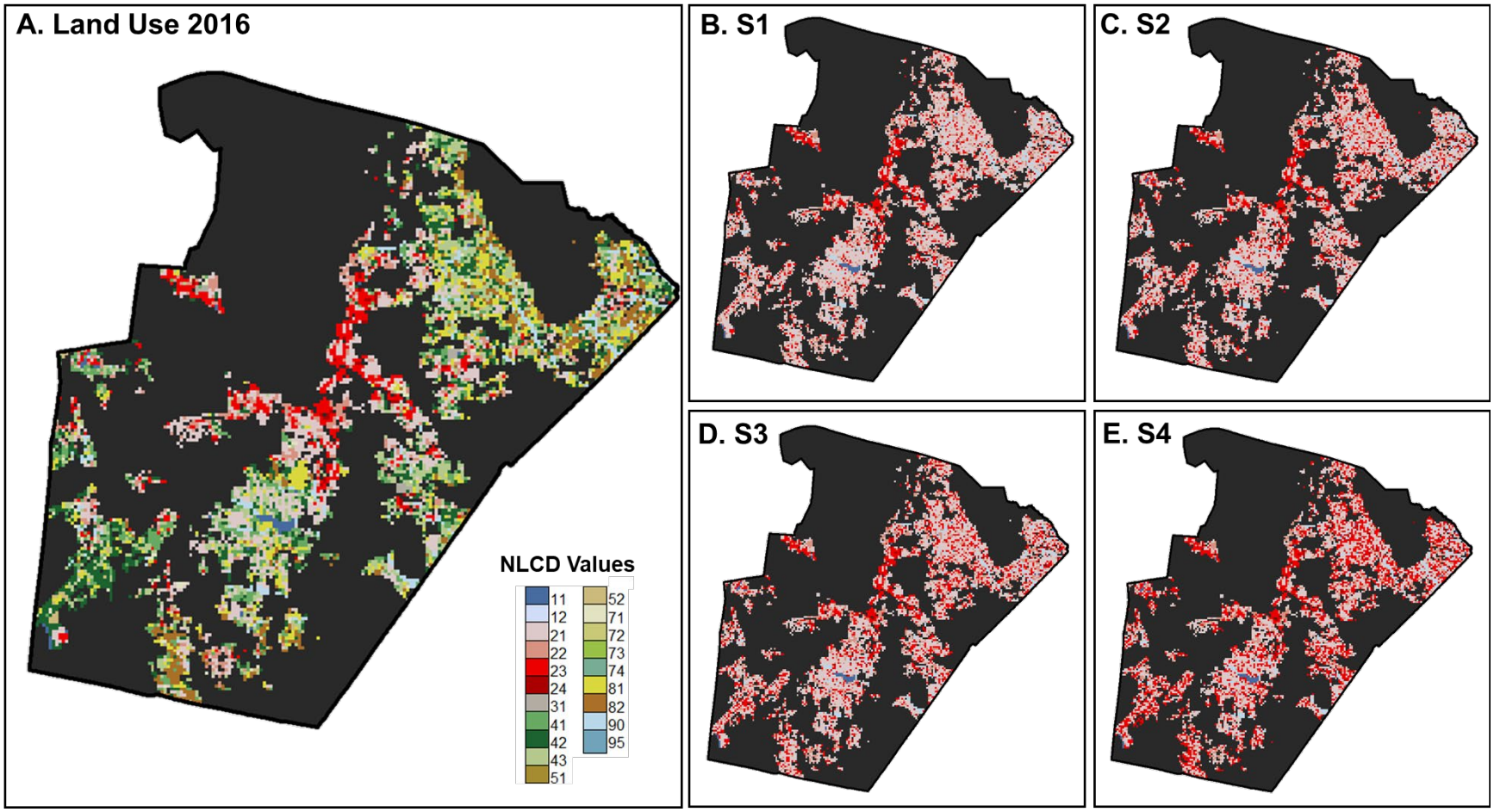


**Figure 4.5** Land use classifications in (A) 2016 and (B-E) future land use scenarios for high-priority development areas of Wake County, defined as locations identified by the county or municipalities in their future land use plans. (B) Scenario 1 (S1) assigned cells to a development class in equal proportions to those classes across Wake County in 2016. In scenarios 2-4, medium- and high-intensity development classes increased by 50% (C), 100% (D), and 200% (E), respectively.

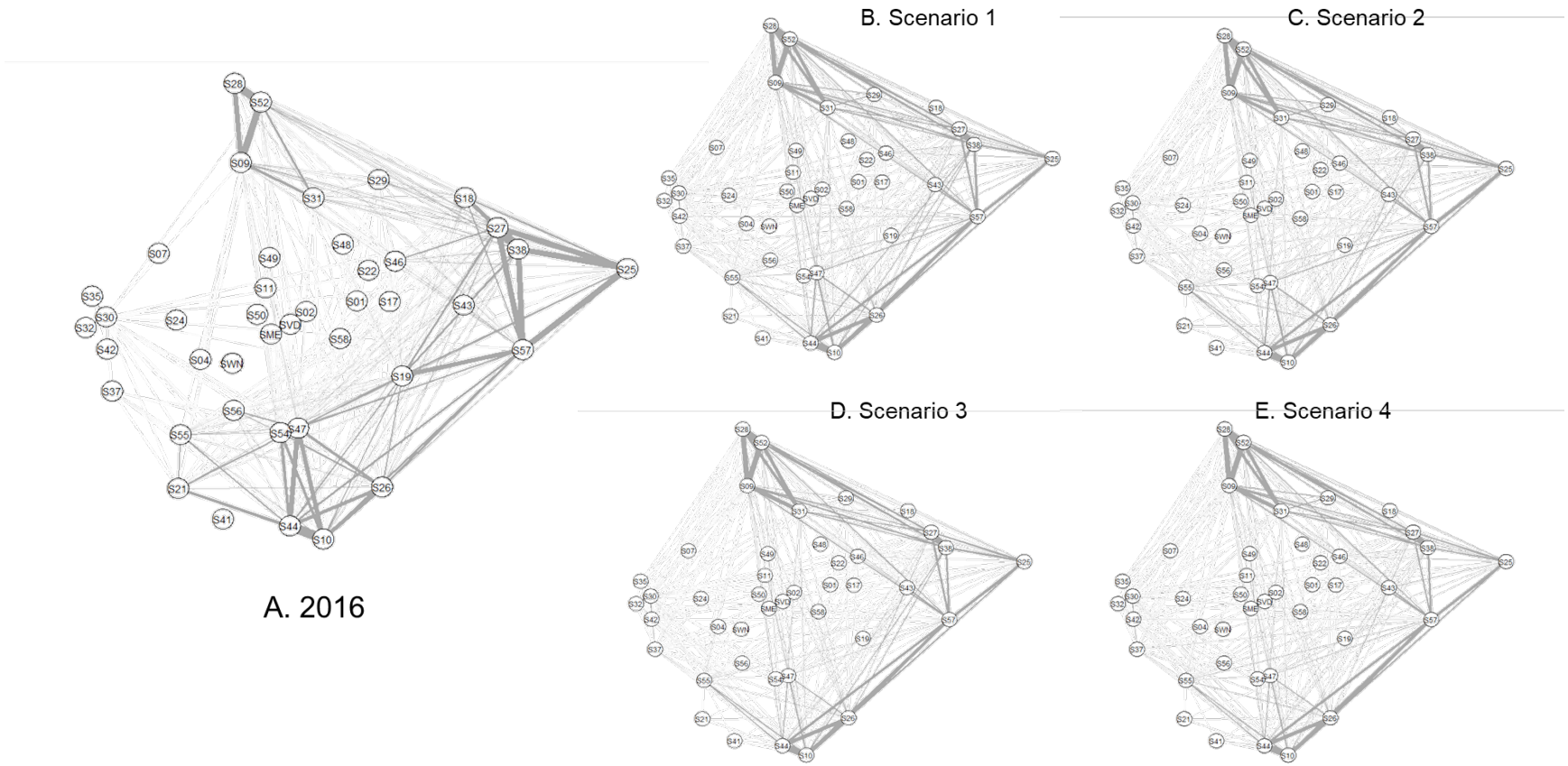


**Figure 4.6** Relative connectivity between sites for (A) current land use and (B-E) four future land use scenarios based on 2016 NLCD data. In all four scenarios, the same area of land transitioned from undeveloped to developed, but the proportion of medium and high intensity developed by 50, 100, and 200% for scenarios 2-4 from 2016 proportions (scenario 1). Line thickness is proportional to the inverse of the log-transformed resistance cost between connected sample locations. I retained edges between sites if their inverse distances were in the highest 75$^{th}$ percentile for the given scenario.

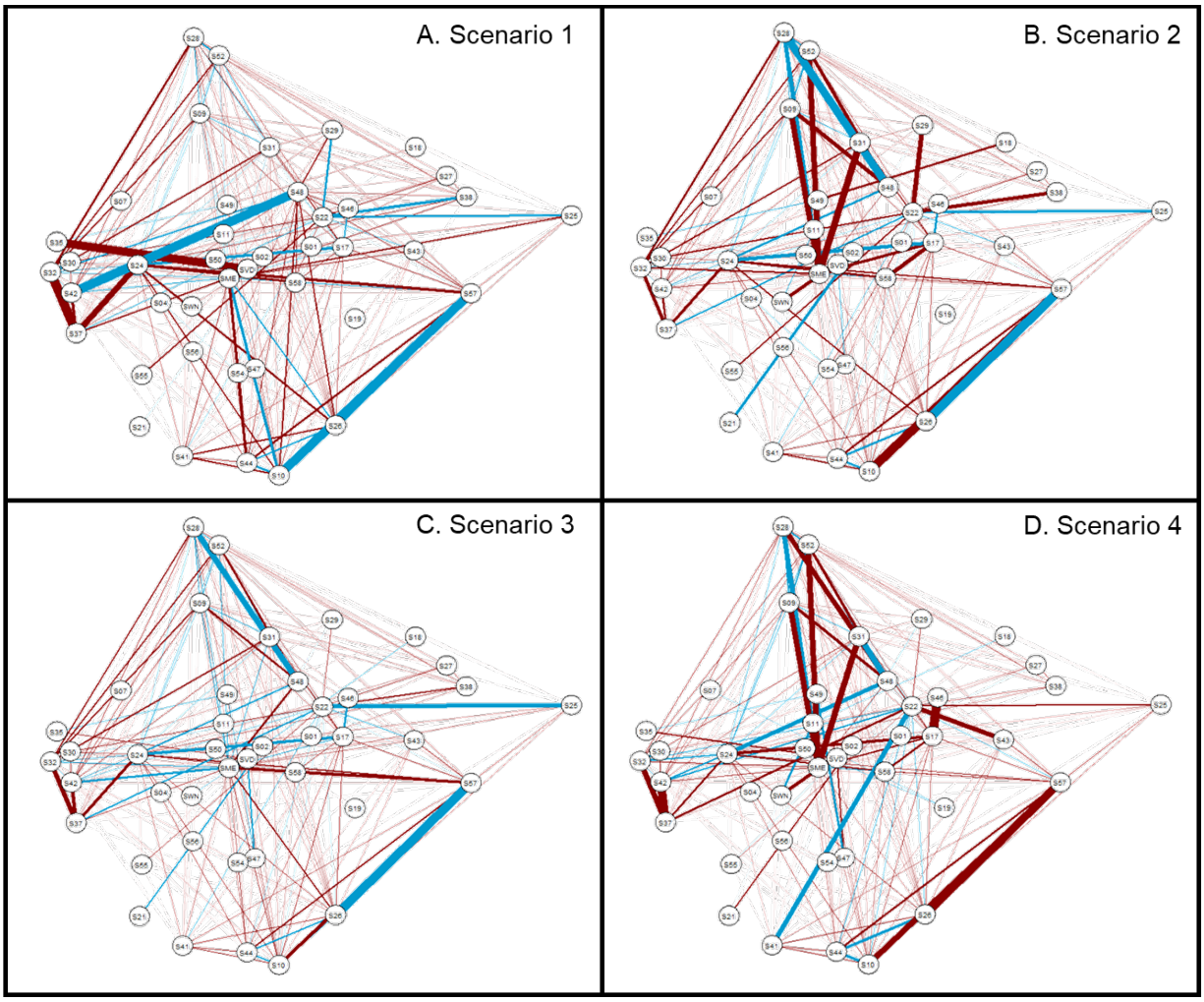


Figure 4.7 Differences in predicted future connectivity and 2016 connectivity based on four future land use scenarios. Red lines represent pairwise connections where connectivity decreased (increased genetic distance, decreased gene flow), while blue lines show sites where connectivity increased. In all four scenarios, the same areas transitioned from undeveloped to developed land uses. In scenario 1 (A), development classification (open, low, medium, and high-intensity development) was proportional to current levels in Wake County, while in scenarios 2-4, the proportion of medium- and high-intensity development increased by (B) 50%, (C) 100%, and (D) 200%. Line width is proportional to the inverse log difference between resistance distances. I retained edges between sites if the absolute value of the difference in resistance distance from 2016 was in the highest 75th percentile for the given scenario.

# CHAPTER 5

## *Aedes albopictus* life stage affects population genetic patterns at fine spatial scales

### Introduction

The tiger mosquito *Aedes albopictus* has come to dominate human-inhabited landscapes around the globe. Native to eastern Asia, *A. albopictus* became globally invasive through intercontinental trade (Hawley et al. 1987). The oviposition ecology of *A. albopictus* predisposed the species to invasion success. Females lay eggs in containers with ephemeral water sources, and eggs require a wet-dry-wet cycle before hatching. This behavior, likely evolved as a form of predator avoidance for larvae, preadapted *A. albopictus* to thrive in human-dominated landscapes where artificial containers are abundant (Bonizzoni et al. 2013; Albeny-Simoes et al. 2014). *Aedes albopictus* are also opportunistic feeders characterized by aggressive biting behaviors (Hawley 1988). As a result, the species is both a nuisance for humans and a vector of zoonotic disease (Hawley et al. 1987; Gratz 2004).

Due to its global distribution and threat to public health, *A. albopictus* has been a priority for mosquito control (Benedict et al. 2007). This includes *Wolbachia*-infected and genetically modified (GM) mosquito releases to suppress population growth (Mains et al. 2016; Versteeg et al. 2016). Informed decisions about mosquito control rely on accurate assessments of population genetic structure and gene flow (Takken and Scott 2003; Alphey and Bonsall 2014). Population structure and dispersal are especially important for genetic control programs, as these rely on the controlled spread of population-limiting genes or, in the case of *Wolbachia* infection, bacteria (Takken and Scott 2003). For example, a gene will spread more slowly in a highly fragmented and genetically differentiated *A. albopictus* metapopulation than in a panmictic, interbreeding population. In addition to providing insight into the ecological and evolutionary dynamics of the targeted mosquito population (Scott et al. 2002), researchers can use population genetic analyses to identify strategic areas for modified mosquito releases and to continuously evaluate program success (Harris et al. 2012).

Because of the important role of population genetics in *A. albopictus* vector control decisions, sampling methods for these studies should be thorough, unbiased, and reflect the specific questions and needs of practitioners. There are numerous reviews and simulation studies that examine how population genetic results are affected by the number of sampling locations,

individuals, and genetic markers (e.g., Hoban et al. 2013; Meirmans 2015; Peterman et al. 2016). For example, sampling many individuals from few populations is appropriate for detecting genetic bottlenecks, but may not be suitable in landscape genetics studies where is it necessary to capture a wide range of environmental variation (Meirmans 2015). Failing to capture environmental variation can lead to erroneous estimates about the effect of landscape features on populations (Lotterhos and Whitlock 2014). To avoid bias within sampled populations, researchers should randomize which individuals are genotyped in a way that is appropriate to the study system and research questions. Randomized sampling decreases the probability of genotyping related individuals, such as siblings. If relatives are overrepresented in the population, estimates of population level allele frequencies will be inaccurate and likely result in underestimating within population genetic diversity and overestimating between population genetic differentiation (Allendorf and Phelps 1981; Goldberg and Waits 2010).

There is less clear guidance in the literature about how sampling different mosquito life stages affects population genetics results. Researchers regularly collect eggs and larvae for genetic studies on container-breeding mosquitos such as *A. albopictus* because these methods are inexpensive, time efficient, and do not require specialized equipment (Reed et al. 2019). To minimize bias from sampling related individuals, researchers will either pool or subsample immature mosquitoes caught in the same trap for genotyping. This approach has been adequate to correct for bias from sampling related larvae in several amphibian species (Goldberg and Waits 2010; Peterman et al. 2016). However, this does not address whether genetic variation and structure in immature *A. albopictus* reflects that of the adult population.

Genetically modified mosquito releases generally target adult populations, particularly those that involve the release of sterile males. Therefore, decisions based on the population genetics of field-collected mosquito eggs may be misguided if patterns of gene flow and genetic structure differ between immature and adult mosquitoes. In this study, I compared population genetic patterns of field-collected adult and egg *A. albopictus* at five locations over the same time period in Wake County, NC. I evaluated genetic diversity, differentiation, and structure among these sites for adults, eggs, and combined life stages using methods commonly used in population genetic research. Based on these results, I identified avenues for continued research and offer preliminary recommendations for study design to inform mosquito control.

## Methods

### Ovitrap Sampling

During 2018 sampling (Reed 2021, Chapter 2-3), I also placed ovitraps at 20 out of 61 sample sites. I used a random number generator to select the 20 sites, and at each of these locations, I set three ovitraps in a triangle around the adult BG Sentinel trap to capture *A. albopictus* eggs. Following the methods of Reed et al. (2019), I made ovitraps with 473 mL black plastic cups lined with 8.9 x 25.4 cm 76# seed germination paper (Anchor Paper Co., Plymouth, MN). I filled each cup with ~350 mL of tap water and positioned the germination paper to be half above and half below the water line. Each cup had a hole to prevent overflow from rainfall. I placed each ovitrap within 100m of the adult trap, with a minimum distance of 25m between ovitraps to decrease the probability of an *A. albopictus* female laying eggs in multiple traps. I left ovitraps in the field for two weeks and collected egg papers once a week (six egg papers total per site). I store egg papers in a sealed plastic bag with a damp paper towel until they were ready to hatch. I then counted the number of eggs on the paper before placing it in a nutrient broth to facilitate hatching. I identified mosquitoes as fourth instar larvae and placed *A. albopictus* larvae in microcentrifuge tubes with 90% ethanol for DNA extraction.

### Genomic bioinformatics

For a site to be considered for genomic sequencing, it required at least 10 preserved *A. albopictus* larvae, with a maximum of three samples per ovitrap. This reduced potential bias from sampling siblings (Goldberg and Waits 2010) and was consistent with previous population genetic studies using container-breeding *Aedes* larvae (Schmidt et al. 2018). I extracted DNA from larvae with the Qiagen DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA, USA) and quantified DNA with a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). I included five sites in the final genomic libraries (**Figure 5.1**), all of which had extracted DNA concentrations of 8 ng DNA/μL or greater for at least eight larvae. I built genomic and sequenced libraries using double-digest restriction enzyme associated DNA sequencing (ddRADseq; see Chapters 2-3, Burford Reiskind et al. 2016).

I created a SNP catalog with the *denovo* pipeline in STACKS version 1.09 (Catchen et al. 2011) with the combined 2018 adult and larval samples. I then ran the *Populations* pipeline in

STACKS with only the adult and larval samples from sites where I collected *A. albopictus* eggs (five sites, 80 individuals). I treated the two life stages as separate groups for 10 separate 'populations' in the pipeline. I retained SNPs that were present in at least 75% of individuals in two or more populations. I further filtered SNPs in PLINK v1.19 (Purcell et al. 2007) to remove SNPs with a minimum allele frequency less than 0.01 and a genotyping rate of less than 0.75 and individuals with over 75% missing data. Finally, I removed SNPs significantly out of Hardy-Weinberg Equilibirum after applying a sequential Bonferroni correction using the hw.test function in the R package *pegas* v0.14 (Paradis 2010).

Population genetic analyses

For population genetic analyses, I made comparisons between populations for (1) egg samples; (2) adult samples; and (3) pooled adult and egg samples. I estimated genetic diversity by calculating expected heterozygosity ($H_E$), observed heterozygosity ($H_O$), and inbreeding coefficient ($F_{IS}$=1-$H_O/H_E$), corrected for small sample sizes, with the *genetic_diversity* function in the R package gStudio (Dyer 2016). I tested for statistically significant differentiation between groups with an exact G test implemented in GENEPOP (Rousset 2008) with the following parameters: dememorization:10,000, batches: 500, iterations per batch: 5,000.

I used two commonly applied approaches to evaluate genetic structure. The first is a Bayesian *k* clustering algorithm implemented in the program STRUCUTRE v.2.3.4 (Pritchard et al. 2000; Hubisz et al. 2009). I ran the admixture ancestry model in STRUCTURE with 10,000 burn-ins, 10,000 MCMC replicates, and a *k* from 1-6 with 10 iterations per value of *k*. I used STRUCTUREharvester (Earl and vonHoldt 2011) to determine the value of *k* with the highest likelihood using the Evanno method. I also used the probability by K method (ln(Pr(X|K)) described in Pritchard et al. (2000) and implemented in CLUMPAK because the Evanno method does not evaluate $k = 1$ and can overestimate the value of *k* (Janes et al. 2017; Cullingham et al. 2020). I used DISTRUCT v.1.1 (Rosenberg 2003) to visualize STRUCTURE plots.

I also ran a discriminant analysis of principal components (DAPC) in the R package adegenet (Jombart 2008; Jombart and Ahmed 2011). DAPC is a multivariate method for assessing population structure. Raw genetic data are first transformed through a principal component analysis (PCA) for individuals, then a discriminant analysis (DA) is used on the retained PCs to maximize differences between populations (Liu et al. 2019). I used cross-validation to identify

the optimal number of principal components to retain with a training set size of 0.95, 1000 replicates, and a maximum of 20 PCs to prevent overfitting the data (Jombart et al. 2008; Plue et al. 2018). I ran DAPC with only adult samples, only egg samples, and all samples to compare patterns of genetic structure and differentiation between the two life stages. I also ran DAPC with only adult samples and only larval samples to compare patterns of genetic structure and differentiation between the two life stages for a range of principal components with a minimum of 1 PC and a maximum of 40 PCs, which is equal to the number of individuals of each life stage. I looked at the rate that individuals were assigned to the correct site (correct assignment rate: CAR) for each set of PCs to evaluate differences in level of genetic differentiation between adults and eggs.

**Results**

Genomic bioinformatics

I collected genomic data for 80 individuals from five locations to compare the population genetic patterns between *A. albopictus* adults and eggs in Wake County 2018, with eight adults and eight eggs sequenced per site. The STACKS *Populations* pipeline retained 77,996 SNPs. PLINK filtering removed 4,228 SNPs and 63,894 SNPs for not meeting the minimum allele frequency and genotyping rate thresholds, respectively. Two individuals, both collected as eggs, were removed for missing data. Finally, 1,487 SNPs were significantly out of HWE, leaving 8,387 SNPs and 78 individuals for further analysis.

Genetic Diversity and Differentiation

Adult *A. albopictus* had greater expected heterozygosity than eggs sampled at the same location (**Table 5.1**). Adults also had higher inbreeding coefficients, $F_{IS}$, at all but one site, indicating that the adults at a site had higher rates of homozygosity compared to the expectation under HWE than eggs. Site 58 had the highest expected heterozygosity among both adults and eggs. Otherwise, expected heterozygosity and $F_{IS}$ estimates from eggs were on average lower than those estimated using adults regardless of site. Sites had different relative levels of genetic diversity compared to one other depending on the life stage sampled. For example, expected heterozygosity from lowest to highest based on egg samples was S31, S42, S17, S09, and S58; while in adults, the order was S17, S09, S31, S42, and S58. Estimates for sites with both life

stages produced a combination of these two rankings, with S31, S17, S09, S42, and S58 ordered by increasing expected heterozygosity (**Table 5.1**).

Pairwise genetic distances measured by Wright's $F_{ST}$ were inconsistent between life stages (**Table 5.2**). Pairwise $F_{ST}$ values were low for all groups and ranged from 0.000 to 0.0091. 5, 3, and 4 out of 10 comparisons were greater than zero for adults, eggs, and combined individuals, respectively. All three groups found no differentiation for two comparisons ($F_{ST} = 0$). There were no instances where genetic differentiation was detected between sites for both life stages (i.e., $F_{ST} > 0$). However, I found no statistically significant differentiation between populations for any life stage or when life stages were combined.

Genetic Structure

The Evanno method in STRUCTUREharvester identified an optimal $k = 5$ for adults, $k = 5$ for eggs, and $k = 3$ for combined life stages (**Figure 5.2A, B, D**). In contrast, the probability by K method identified an optimal $k = 1$ for the adults and combined groups and a $k = 2$ for eggs (**Figure 5.2C**). However, there was not a strong pattern of genetic structure between sampled locations for any groups.

Cross validation in DAPC identified an optimal eight principal components (PCs) for the adult and combined groups and 12 PCs for the egg group (**Figure 5.3**). The correct assignment rate for adults was 0.6000, and all populations formed one cluster (**Figure 5.3A**). For eggs, the correct assignment rate was 0.6053. The scatterplot indicated some genetic structure, with site 58 and site 17 somewhat isolated from the remaining locations (**Figure 5.3B**). The correct assignment rate was lowest when all individuals were combined (0.4359), indicating that there was more within-site genetic variance than there was between sites. As in the DAPC scatterplot for eggs, site 17 appeared more isolated than the remaining locations in the scatterplot for combined life stages (**Figure 5.3C**).

To assess differences in level of genetic structure between life stages, I estimated assignment rates for adult and egg samples from 1-40 PCs. I used total number of incorrect assignments for each set of PCs to account for different sample sizes between adults and eggs. DAPC was able to assign all adults and eggs to their correct locations with 28+ and 30+ PCs, respectively. Adults had fewer incorrect assignments than eggs in 10 of 40 comparisons, more

incorrect assignments in 16 of 40 comparisons, and the same number of incorrect assignments in 14 of 40 comparisons.

**Discussion**

Overall, I found evidence that life stage did affect population genetic results for *A. albopictus* in Wake County. Estimates of genetic diversity differed between *A. albopictus* adults and eggs within sample sites, and *A. albopictus* eggs tended to show more genetic structure than adults between sites. Importantly, patterns of genetic differentiation between sites also varied with life stage.

Eggs had lower expected heterozygosity and $F_{IS}$ on average than adults. While these are both measures of genetic diversity, they are often observed to be inversely related. Higher expected heterozygosity can indicate that a population has many loci with more than one allele and that alleles at a locus occur at similar frequencies across the population. $F_{IS}$ measures the difference between the number of individuals expected to be heterozygous at a locus under HWE given the allele frequencies compared to the observed number of heterozygotes. Therefore, *A. albopictus* eggs may have overall had fewer biallelic loci and/or alleles that occurred at lower frequencies than in adults, but gametic frequencies in individual eggs were closer to the estimated number under HWE expectations than for adults. This scenario could arise if unrelated adults fixed for different alleles at a locus bred to produce heterozygous offspring. If this is the case, then sites with the largest differences between adult and egg $F_{IS}$, such as S09 and S58, may have high immigration or turnover rates (**Table 5.1**).

I found evidence that eggs were more genetically differentiated between sites in the DAPC analysis, though this was not reflected in pairwise $F_{ST}$ estimates. DAPC was able to assign eggs to the correct location more often and with fewer PCs on average than adults, which indicates that among egg populations, between-group genetic differences are more pronounced than within-group variation. This is consistent with the different levels of genetic diversity I observed between life stages. The genetic variation in eggs reflects only a portion of the adult gene pool, as only a certain number of females will oviposit over a given time interval, but that variation is represented at the individual level. This observation supports previous population genetic patterns of *A. albopictus* in Wake County (Reed 2021, Chapter 2). I found genetic differentiation was more pronounced between sites in 2016, when individuals were collected

with ovitraps, compared to collections in 2018, where I found evidence of an unstructured, panmictic adult population.

While the scope of this paper is limited to a small geographic area and few sampling sites, the differences I found in population genetic patterns between *A. albopictus* life stages are compelling enough to warrant caution when interpreting studies that sample immature *A. albopictus* to infer adult genetic structure. To further understand illuminate how life stages affect the population genetic characteristics of tiger mosquito populations, we can analyze data from previously published research that sampled both adult and immature *A. albopictus* (for example, Beebe et al. 2013; Bibi et al. 2015; Pech-May et al. 2016; Eskildsen et al. 2018; Sherpa et al. 2019). In future studies, I recommend that adult and egg or larval samples be collected in tandem when logistically possible, particularly for research at fine spatial scales. Publications on *A. albopictus* population genetics should provide detailed and precise descriptions of their sampling methods, especially when they use ovitraps, explicitly discuss how they minimized sampling bias, and justify their decision to collect one or more life stages in the context of the research goals (e.g., Medley et al. 2015, Schmidt et al. 2021).

Finally, I recommend that research intended to inform mosquito control, particularly via genetically modified mosquito releases, consider the context and nuances of the specific study location as it relates to the natural history of *A. albopictus.* If levels of genetic diversity, differentiation, and structure do consistently vary between adult and immature life stages, it is likely that the magnitude and direction of those differences will depend on the surrounding landscape. Land use, habitat configuration, and level of urbanization all affect the ecology and evolution of *A. albopictus* (Reed 2021, Chapters 1-4, and relevant citations therein). Consequently, place-based approaches and knowledge are essential to develop adaptive and effective management programs.

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**Table 5.1** Observed heterozygosity, expected heterozygosity, and $F_{IS}$ of immature and adult mosquitoes from five sampled sites.

| **Population** | **Life Stage** | $H_O$ | $H_E$ | $F_{IS}$ |
|---|---|---|---|---|
| S09 | Adult | 0.1065 | 0.1238 | 0.1398 |
| S09 | Egg | 0.1096 | 0.1227 | 0.1070 |
| S09 | Combined | 0.1069 | 0.1233 | 0.1328 |
| S17 | Adult | 0.1056 | 0.1219 | 0.1340 |
| S17 | Egg | 0.1059 | 0.1214 | 0.1271 |
| S17 | Combined | 0.1057 | 0.1226 | 0.1377 |
| S31 | Adult | 0.1109 | 0.1249 | 0.1117 |
| S31 | Egg | 0.1059 | 0.1203 | 0.1194 |
| S31 | Combined | 0.1078 | 0.1226 | 0.1203 |
| S42 | Adult | 0.1089 | 0.1252 | 0.1297 |
| S42 | Egg | 0.1087 | 0.1214 | 0.1046 |
| S42 | Combined | 0.1088 | 0.1237 | 0.1198 |
| S58 | Adult | 0.1106 | 0.1255 | 0.1186 |
| S58 | Egg | 0.1167 | 0.1252 | 0.0684 |
| S58 | Combined | 0.1126 | 0.1256 | 0.1028 |

**Table 5.2** Pairwise $F_{ST}$ values across all loci for five Wake County sites for adults, eggs, and all individuals combined. If estimates were negative, I replaced them with 0.0000. No pairwise differences were statistically significant in the exact G test.

| Adults | | | | |
|---|---|---|---|---|
| | **S09** | **S17** | **S31** | **S42** |
| **S17** | 0.0000 | | | |
| **S31** | 0.0003 | 0.0091 | | |
| **S42** | 0.0000 | 0.0058 | 0.0001 | |
| **S58** | 0.0000 | 0.0034 | 0.0000 | 0.0000 |

| Eggs | | | | |
|---|---|---|---|---|
| | **S09** | **S17** | **S31** | **S42** |
| **S17** | 0.0028 | | | |
| **S31** | 0.0000 | 0.0000 | | |
| **S42** | 0.0000 | 0.0000 | 0.0000 | |
| **S58** | 0.0055 | 0.0000 | 0.0030 | 0.0000 |

| Combined | | | | |
|---|---|---|---|---|
| | **S09** | **S17** | **S31** | **S42** |
| **S17** | 0.0020 | | | |
| **S31** | 0.0000 | 0.0045 | | |
| **S42** | 0.0000 | 0.0027 | 0.0000 | |
| **S58** | 0.0000 | 0.0010 | 0.0000 | 0.0000 |

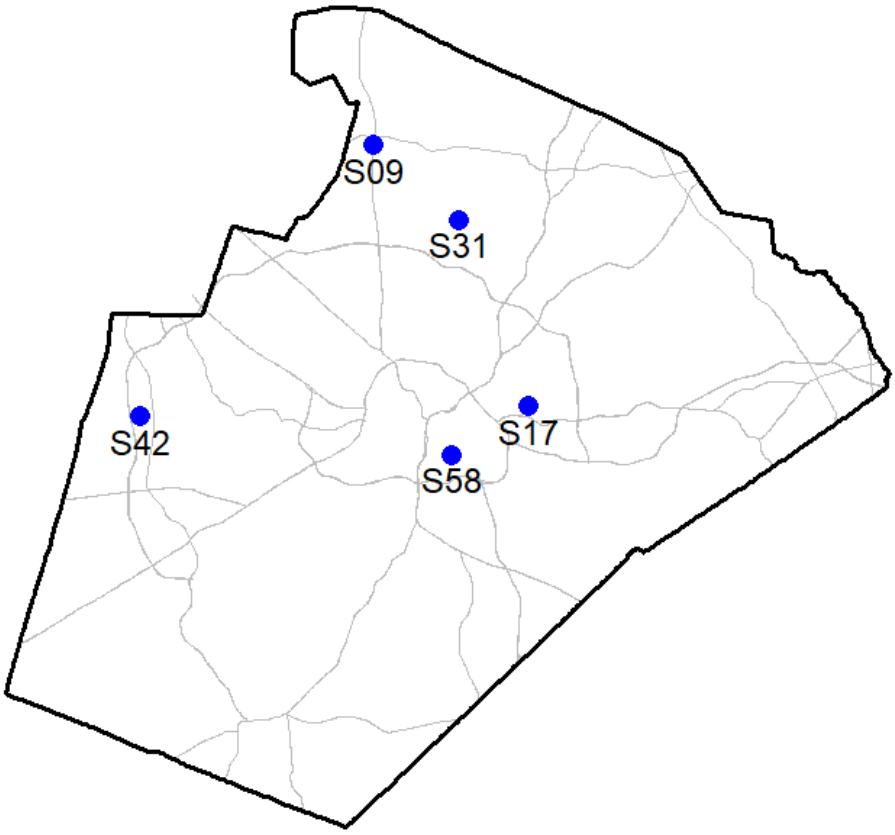


**Figure 5.1** Map of sites with both adult and immature *A. albopictus* genetic data. I sampled five sites total, each with sequence data for eight adults and eight eggs.

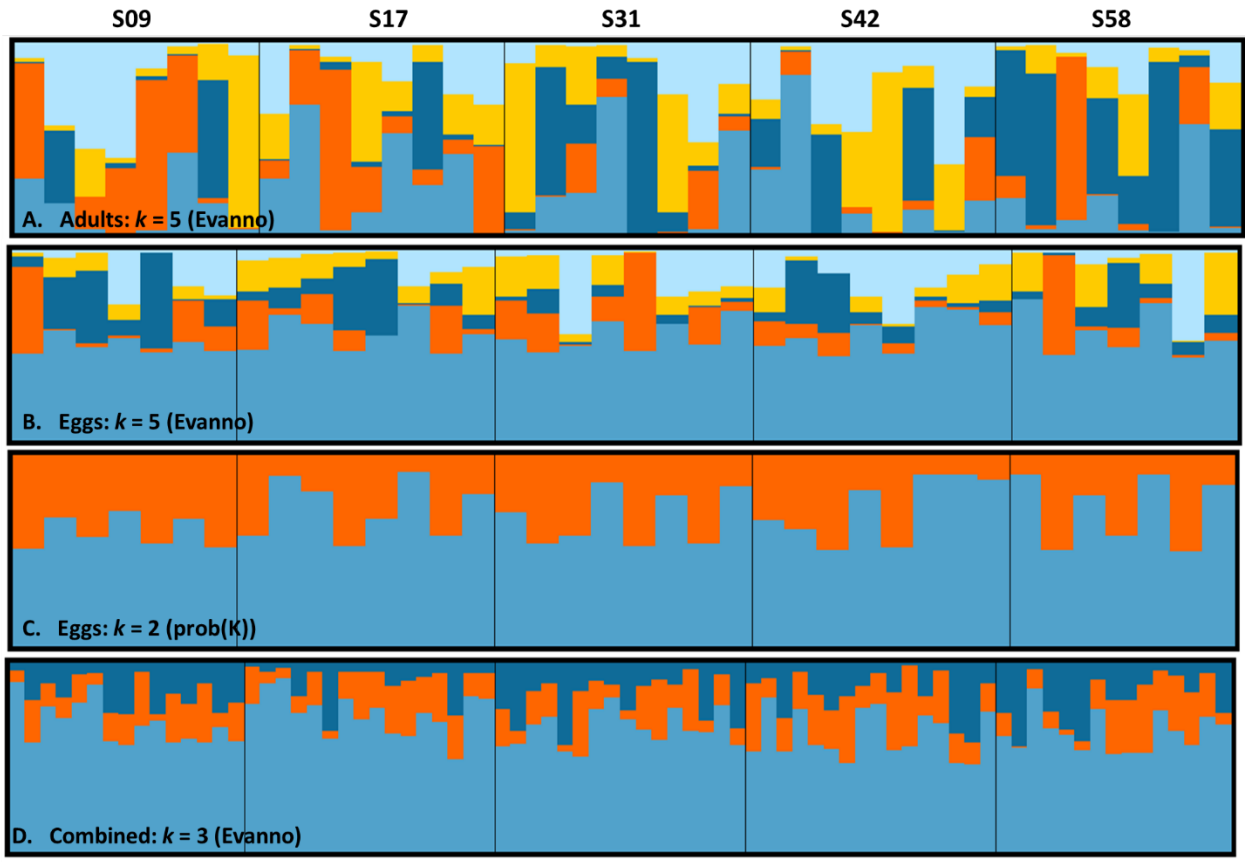


**Figure 5.2** STRUCTURE plots for adults (A), eggs (B-C), and adults + eggs combined (D) at five sites in Wake County. The Evanno method identified an optimal $k = 5$ for the adult group (A) and egg group (B) and an optimal $k = 3$ for the combined group (D). The probability of K (prob(K)) method identified an optimal $k = 1$ for adults and all individuals combined, and a $k = 2$ for the egg group (C).

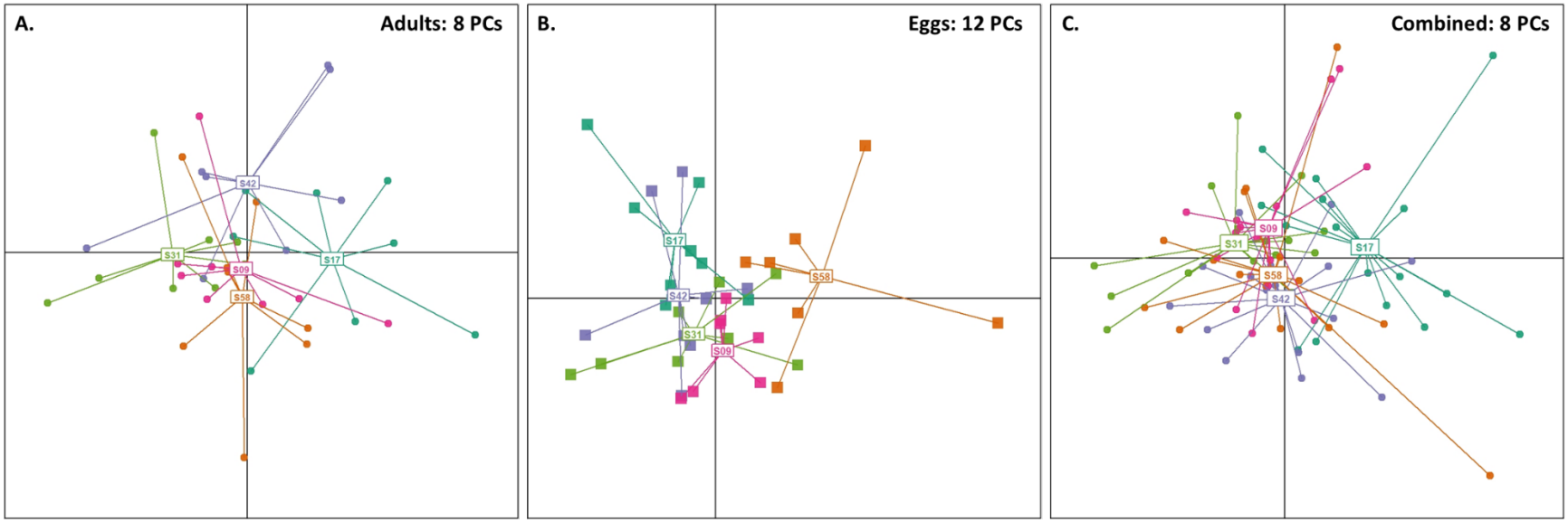


**Figure 5.3** DAPC scatterplots for five sampled sites with genetic data from (A) adults only, (B) eggs only, and (C) adults and eggs combined. Cross-validation identified an optimal eight principal components (PCs) for (A) and (C) with a correct assignment rate (CAR) of 0.6000 and 0.4359, respectively. Cross-validation identified an optimal 12 PCs for (B) with a CAR = 0.6053.