POPULATION GENOMICS OF 2 MAJOR WHITEFLY PREDATORS: *ORIUS INSIDIOSUS* AND *GEOCORIS PUNCTIPES*

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(Under the Direction of William E. Snyder)

ABSTRACT

Biocontrol is the process of using predators, parasitoids, or pathogens of pest species for population suppression. For optimal biocontrol, these natural enemies would persist in the local environment and quickly recolonize areas after disturbance. Failure to recolonize can have significant consequences on pest suppression, allowing pests to proliferate before natural enemies can take hold. Understanding spatial relationships between natural enemies and their environment can inform management decisions to preserve natural enemy populations. We analyzed the population genomics of two major whitefly predators (*Geocoris punctipes* and *Orius insidiosus),* using relatedness of their populations across geographic distance to better understand their movement patterns and dispersal capabilities. Both *G. punctipes* and *O. insidiosus* were found to have little to no genetic differentiation across all populations examined, providing support for long-distance recolonization post-disturbance for more effective biocontrol.

INDEX WORDS: Biocontrol, Natural enemies, Pest suppression, Population genomics, Whitefly, Dispersal

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TABLE OF CONTENTS

Page

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW

Biocontrol is the process of using natural enemies of pest species for population suppression. Parasitism, predation, and pathogen spread can all be utilized to reduce populations of pests to a more manageable size (Hokkanen and Sailer 1985). Within biocontrol, there are three broad categories: classical, augmentative, and conservation control.

Classical biocontrol involves the release of an organism outside of its native range for the purpose of pest control (Howarth 1991). Oftentimes this is used for management of nonnative pest species and seeks to reestablish control through importation of natural enemies from the pest's native range (Howarth 1991). Ideally, the biocontrol agent will have high host specificity, and research on the potential effects on nontarget organisms is critical prior to release (Barratt et al. 2010). Due to the need for high host specificity, most classical biocontrol agents are parasitoids, many of which specialize on a single host genus or even species (Stiling and Cornelissen 2005). As the global spread of crop pests increases, classical biocontrol continues to play a critical role in pest control (Bebber et al. 2014).

Augmentative biocontrol involves the mass production and release of a natural enemy to supplement local populations and increase biocontrol effectiveness (Collier and van Steenwyk 2004). This is often used for microbial and nematode control agents, as these organisms can be mass produced and applied through spraying (Copping and Menn 2000). Other forms of augmentative control involve the sale and release of insect biocontrol agents such as ladybugs or lacewings, although evidence for the effectiveness of this is mixed (Collier and van Steenwyk 2004).

Conservation biocontrol does not involve the release of any organism, but rather focuses on land management techniques for preserving and increasing natural populations of biocontrol agents (Shields et al. 2019). This often involves the use of non-crop vegetation such as floral resources (Ramsden et al. 2015) or beetle banks (dense grasses for ground predator overwintering) (Collins et al. 2002). Alternative prey is also an important factor in conservation of natural enemies by providing consistent food resources for predators and potentially reducing intraguild predation (Snyder 2019). Providing a diversity of these resources can increase natural enemy diversity and consequently pest suppression (Ramsden et al. 2015, Snyder 2019).

However, trophic interactions in ecological systems are complex and numerous, making it difficult to predict and characterize the full effects of biocontrol measures (Thomas and Willis 1998). Potential biocontrol agents may feed on the pest species of interest, but aspects of their feeding may make them less efficient control agents. Natural enemies may engage in intraguild predation (Müller and Brodeur 2002), or the feeding strategies they engage in may be redundant to a predator already present in the environment (Roubinet et al. 2018).

Another important aspect is whether the biocontrol agent is a specialist or a generalist. Specialist natural enemies are focused one family or even one genus or species of insect; generalist natural enemies feed across multiple groups. While specialist natural enemies can exert higher predation pressure (Diehl et al. 2013), generalists are able to persist in the environment even when the pest is not present (Symondson et al. 2002). These factors can have a significant impact on the success of a biocontrol agent. Consequently, gathering extensive information on pest and biocontrol organisms alike is crucial to pinpoint optimal and effective control (Thomas and Willis 1998).

For optimal biocontrol, natural enemies would persist in the local environment and quickly recolonize areas after disturbance (Rand et al. 2006). Failure of natural enemies to recolonize early can have significant consequences on pest suppression, allowing pest species to proliferate before natural enemies can take hold (Costamagna et al. 2015). For this reason, maintaining stable natural enemy presence is critical.

Landscape predator conservation techniques can help to keep consistent natural enemy populations. However, natural enemy responses to different land management techniques can also vary. While beetle banks may be greatly beneficial to ground beetles, predatory flies such as Syrphidae and Empididae often require floral resources to establish within an agricultural plot (Holland et al. 2016). Different forms of refuge habitat can provide shelter for predators allowing quick recolonization, but some studies show a decrease in natural enemy density with the presence of refuge crop stands (Liman et al. 2016). Understanding specific interactions of target natural enemies and landscape characteristics can be important in their conservation and maintenance on agricultural landscapes (Holland et al. 2016).

Regardless, maintenance of natural enemies can be difficult to achieve consistently, as spatial patterns between insect populations are difficult to monitor with conventional techniques due to their small size and potential dispersal (Hassell et al. 1991). Patches of habitat can improve the likelihood of natural enemy persistence (Hassell et al 1991), but it is important to know the specifics of how large and nearby patches must be for effective natural enemy colonization (With et al. 2002; Visser et al. 2009). To evaluate this, we need to understand the movement patterns of natural enemies.

Different natural enemies can be influenced by different landscape factors and spatial scales, even within closely related taxa. For example, Aviron et al. (2016) found that two predatory Miridae were differentially affected by specific landscape factors and operated at different spatial scales, with *Macrolophus* seeming to operate at a scale over twice the size of *Dicyphus*. Similarly, a study by Hirose et al. (1996) looked at egg parasitoid success of soybean pests, finding that *Ooencyrtus nezarae* spends a considerable amount of time dispersing as compared to the other studied species. These behavioral differences can completely change a species' relationship to its landscape, potentially allowing *O. nezarae* to colonize new habitat patches more easily (Hirose et al. 1996). Understanding these spatial relationships between different natural enemies and their environment can inform landscape management decisions to preserve natural enemy populations.

A variety of methods have been developed and applied to understand insect movement. These methods can be broadly grouped into path tracking (directly evaluating location at specific time intervals), mark-recapture (Marking insects and recording location upon recapture), behavioral study (artificially evaluating movement capability in a lab environment), radar (remotely collecting movement information from diodes attached to specimens), and molecular techniques (using genetic information or molecular traits to categorize populations, examine variation, and infer movement). (Osborne et al. 2002).

Each of these methods have limitations in their application. Some methods are often inefficient in studying long-distance dispersal, such as path-tracking and mark-recapture (Osborne et al. 2002; Mahroof et al. 2010; Showers 1997); some require high costs for precise location data, such as radar (O'Neal et al. 2004); and others take place in highly controlled lab environments that may not accurately represent natural movement, such as behavioral studies (Naranjo 2019, Minter et al. 2018).

For an effective analysis of insect movement and dispersal, the methods should be capable of reaching a large enough scope to encompass relevant movement distance without heavily altering natural behavior (Osborne 2002). One solution to this problem is to broaden research from the individual level to the population level through molecular techniques. Molecular techniques can use genetic differences and molecular markers to infer movement and dispersal rates between populations and draw conclusions about movement patterns and capabilities (Sheikha 2019). Using unique molecular markers has been shown to be quite effective, but identification of these markers is not always easy, and sometimes there is not a reliable marker to differentiate between closely related populations (Sheikha 2019). Contrastingly, population genomics uses variation across a wider swath of an organism's genome, broadening the area where genetic differences between populations might be found.

Population genomics refers to the study of multiple areas of a genome to better understand evolutionary processes within and across populations. This includes genetic variation, natural selection, genetic diversity, etc. (Luikart et al. 2003). Over time this definition has been refined to focus on "genome-wide effects" (Black IV et al. 2001). When individual gene loci are studied, often the focus is identifying loci that serve specific purposes. When focusing on genome-wide effects, neutral loci are targeted. These gene regions are not under local selection, and therefore are better for studying population demography. Since neutral loci are not under local adaptation, they are at the will of demographic events such as genetic drift and gene flow (Black IV et al. 2001). Using neutral loci in an overall genetic analysis allows better identification of demographic processes acting on our populations of interest.

Analyzing population genomics can be done using multiple methods, including the use of microsatellites. Microsatellites are small snippets of a genome that are highly repetitive. Because of their neutral nature, they are popular as genetic markers for studying population demography (Edwards and Bensch 2009, Zink et al. 2013, Hodel et al. 2017). They also show high levels of polymorphism, requiring little time to show significant genetic divergence from a parent population (Ellegren 2004). Originally, one of the major hurdles in using microsatellites as genetic markers was the difficulty in locating even relatively small numbers of microsatellites (Hodel et al. 2017). New sequencing methods have helped alleviate this, as more genetic polymorphisms are now able to be located through next-generation sequencing (NGS), making analysis more efficient and cost-effective (Schuster 2008).

As genome sequencing continues to be refined, new methods of sequencing are being developed and popularized. One category of analytical methods growing in application is restriction site associated DNA sequencing (RADseq). As opposed to microsatellites, the loci analyzed by RADseq tend to have relatively low mutation rates. Notably, RADseq is an improvement in the sheer number of polymorphic loci it can identify. One RADseq analysis can examine thousands of different loci across a genome (Andrews et al. 2016). RADseq targets snippets of DNA within these loci. These can be compared to a reference genome or another sample to identify single nucleotide polymorphisms (SNPs). These are small base pair changes used to quantify genetic difference. RADseq has become a useful tool in identifying these differences between populations and identifying the effects of demographic evolutionary processes (Andrews et al. 2016). These methods can be applied in biocontrol to monitor invasive species spread, understand natural enemy management, reveal seasonal pest movements, etc. (Schmidt et al. 2020; Sethuraman et al. 2020; Fu et al. 2020).

This is well shown in a study by Schmidt et al. (2020) on the movement of two invasive mosquitos, *Aedes aegypti* and *Aedes albopictus*. The study analyzed 480 different mosquitos from 27 locations across the Indo-Pacific and compared their genetics to make inferences about their movement patterns using RADseq. They found that the most differentiated populations of *A. aegypti* were those separated by large geographic distances. Contrastingly, *A. albopictus* was found to have multiple accounts of long-distance colonization, with access to human transportation pathways being a primary factor in genetic similarity. Although both species are believed to utilize human transportation pathways, this suggests differences in the dispersal behavior of these two species. With this new information, stronger and more precise methods for controlling the spread of these invasive mosquitos can be developed (Schmidt et al. 2020).

Similarly, a study by Swaegers et al. (2015) used population genomics to examine the range expansion of the damselfly *Coenagrion scitulum*. By examining the genomes of populations near the edge of their range with those toward the center, they were able to find large amounts of genetic differentiation in edge populations. Edge populations were significantly different from core populations, and edge populations were greatly differentiated from each other. This seems to suggest multiple range expansion events, prompted by evolutionary adaptation by the damselfly to tolerate conditions outside of its usual range (Swaegers et al. 2015). This same idea can be applied to pest species. Understanding range expansion of a pest can be used to inform biocontrol decisions and identify areas in danger of invasion.

Movement of invasive species is not just long-term range expansion. Understanding the short-term movement patterns of invasive insects is also critical to effective control. In Fu et. al (2020), population genomics were used to identify refuge habitat of potato psyllid, *Bactericera cockerelli* (Šulc), an important crop pest. These psyllids were using non-crop hosts to preserve their populations during the off-season of their summer host, potato. RADseq was used to confirm that the same populations of psyllid that were feeding on potato crops were also utilizing *Lycium spp.* during the off-season. Other populations of potato psyllid were found to only feed on *Solanum dulcamara* year-round and were not as often found on potato crops. Although these two genetic clusters are visually identical, one is an important crop pest, and the other is not. The differences between them were only able to be identified through genetic methods.

By working on controlling *Lycium spp*. (an invasive plant), potato psyllid populations may not be able to preserve their populations into the next season. This would be a huge step forward in the control of potato psyllid (Fu et al. 2020).

In addition to learning more about invasive insects, population genomics can help us to better understand biocontrol agents. Understanding the movement capabilities of a biocontrol agent can inform researchers of the species' ability to independently disperse, and therefore the importance of reintroduction and refuge. Fu et al. (2021) used this concept in a genetic study of the beneficial entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema feltiae*. Using RADseq, Fu et al. studied the population genomics of these nematodes and mapped their relatedness across geographic distance. Geographically close populations tended to be more similar than distant ones, but many distant populations did appear closely related. This could be due to the occasional long-distance dispersal of nematodes through movement of infected soil by farm equipment or animals (Shapiro et al. 1999). By understanding the movement patterns of these nematodes, we can better cater farm management practices toward their preservation as a biocontrol agent (Fu et. al 2021).

Population genomics is not without limitations. After identifying genetic differences between populations, it can be hard to account for all the possible roots of this variation. Not only are time and space significant factors, but agricultural lands also can differ widely from one another in terms of management practice and landscape structure, potentially affecting genetic divergence (Pelissie 2018). To account for this would be difficult, and many genomic studies will just focus on one or a couple factors (Pelissie 2018). Regardless, even with these limitations, population genomics holds great potential.

Understanding movement of species is important for any biocontrol endeavor, but gain urgency when pests begin to evade current control efforts. This is the case for the silverleaf whitefly, *Bemisia tabaci*. *B. tabaci* is a major pest that can feed on over 600 plant species, transmitting a multitude of plant viruses, and causing additional economic loss through honeydew excretion (Navas-Castillo et al. 2011, Oliveira et al. 2001). Overall, they are estimated to cause hundreds of millions of dollars in damage annually in the southern United States alone (Li et al. 2021). In Georgia, whiteflies are especially dangerous to tomatoes, snap peas, and cucurbits due to diseases such as leaf crumple virus (snap beans and cucurbits), yellow stunting disorder (cucurbits), tomato yellow leaf curl virus (Solanaceous plants), and tomato chlorosis (Solanaceous plants) (Dutta et al. 2018).

As global temperatures increase, the effects of whitefly infestation could heighten, as increased temperatures were found to decrease whitefly development time (Chandi et al. 2021). Additionally, some plant viruses vectored by *B. tabaci* may be able to increase their range and virulence under warmer climatic conditions (Varma et al. 2011, Ramos et al. 2019). Insecticide control is often used to suppress *B. tabaci*, but large genetic diversity and short lifecycles make *B. tabaci* capable of quickly generating insecticide resistance (Horowitz et al. 2020, Horowitz et al. 2007). B. tabaci is believed to be a complex of species or biotypes, with some having high resistance to multiple pesticide groups including neonicotinoids and pyrethroids (Horowitz et al. 2020). Since these species and biotypes are cryptic, it can be difficult to predict which pesticide is the best fit for an infested field (Horowitz et al. 2020). This makes biocontrol of *B. tabaci* critical, and multiple effective natural enemies of whitefly have been shown to already be present in the United States (Kheirodin et al. 2020). The hemipteran families Geocoridae and Anthocoridae are considered particularly effective in whitefly control (Kheirodin et al. 2020; Vandervoet et al. 2018).

Maintenance of Anthocoridae and Geocoridae populations in agriculture have been shown to benefit from the use of cover cropping, presence of refuge habitat, and decreased agrochemical use (Fernandez et al. 2008, Olson et al. 2018, Abbate et al. 2022). However, knowledge on movement behaviors and capabilities of these predators is limited. Depending on the movement patterns of these groups, recolonization of agricultural lands after disturbance may be more or less likely to occur. If Geocoridae and Anthocoridae are limited in their movement across landscapes, maintenance of strong populations within agricultural sites becomes more critical for sustained biocontrol.

Using population genomics, we can identify these movement patterns of both Anthocoridae and Geocoridae. This can inform landscape management decisions to sustain consistent biocontrol of *B. tabaci* on agricultural land pre- and post-disturbance. If populations of Anthocoridae and Geocoridae have similar genetic diversity across geographic distance, it can be inferred that populations disperse regularly. Should even small distances create large genetic divergences, dispersal may be limited. Similar methods have been used to evaluate movement of entomopathogenic nematodes (Fu et al. 2021) and the potato psyllid, *Bactericera cockerelli* (Fu et al. 2020). This study focuses on cotton crop systems due to the economic importance of *B. tabaci* to the cotton industry as well as the historical use of cotton by *B. tabaci* as source habitat for dispersal to other crops (Oliviera et al. 2001).

CHAPTER 2

SCIENTIFIC MANUSCRIPT

Introduction

Agricultural fields are highly disturbed habitats, with frequent tillage, insecticide sprays, and other disruptions that are harmful to natural enemies. Conservation biological control seeks to mitigate this harm by providing shelter and supplemental food for predators and parasitoids. For example, at the local scale, growers might practice cover cropping (Fernandez et al. 2008), supplementation of floral resources (Ramsden et al. 2015), or beetle banks for predator shelter and overwintering (Collins et al. 2002). At the landscape scale, presence of natural habitat and increased landscape complexity in the areas surrounding cropland are believed to increase natural enemy abundance and persistence (Thies et al. 2003; Bianchi et al. 2006). While conceptually simple, it is perhaps surprising how often conservation biological control schemes fail to measurably enhance natural pest control. At the landscape scale, a long list of reasons have been proposed for why biocontrol might fail to improve with growing diversity of regional habitats. These include the lack of efficient predators in the region, cropland providing more important resources than natural habitat to predators and parasitoids, and insufficient proximity of natural habitat to the cropland (Tscharntke et al. 2016). At either local or landscape scales, it is clear that predator movement patterns will play a key role in determining whether conservation biological control will be effective.

Unfortunately, tracking movement of predators, as with other insects, can be challenging. In rare cases, predators are large enough to effectively use mark-recapture methods, such as the marking of mantids with UV-resistant ink **(**Linn and Griebeler 2016). Other methods use fluorescent dust to mark large numbers of insects (Zolubas and Byers 199**5**) or through distinct antibodies as immunomarkers (Hagler 1997). Regardless, the small size of insects makes sufficient recapture difficult (Osborne et al. 2002). Often studies that have found success either marked huge numbers of individuals (Schneider 1999), or focused their recapture efforts only a short distance from the initial capture site (Zolubas and Byers 1995). Molecular approaches can also be used to study insect movement (Osborne et al. 2002). Some species can be traced in their geographic origin using stable isotopes, as is the case with some syrphid flies (Clem et al. 2022). Microsatellites have also been used to study long-distance movement of some insects such as lady beetles (Sethuraman et al. 2015). However, it can be difficult to track fine-scale differences through these methods, and identifying appropriate molecular markers and microsatellites for analysis can be time-consuming (Osborne 2002; Zane et al. 2002). Population genomic methods utilizing broader comparisons in overall genetic differentiation can remove the need for specific markers (Fu et al. 2020; Schmidt et al. 2020). For example, in Fu et al. (2021), restriction site-associated DNA sequencing (RADseq) was used to compare genetic differentiation in entomopathogenic nematodes. Through this study, a clear relationship between geographic distance and genetic differentiation was found, along with evidence suggesting that long-distance dispersal of these nematodes might have occurred through infested farm equipment.

Geocoris and *Orius* spp. are broad generalists that feed on a diversity of soft-bodied arthropod prey in addition to doing some plant feeding (Crocker and Whitcomb 1980; Hernandez 2010). These predators can be remarkably abundant in agricultural fields, suggesting that they readily recolonize following tillage or insecticide sprays (Ramachandran et al. 2002; Sivakoff 2012). This in turn infers ready movement from other habitats, but the scale and frequency of dispersal by these predators have rarely been studied. *Geocoris spp*. have been shown to disperse up to 4000m, though recapture rates at this distance were very low and it can be hard to draw strong conclusions based on these low rates (Sivakoff 2012). Most movement studies on *Orius spp.* focus more on their movement between cropping systems than over significant distance, with each of these studies only spanning a few hundred meters (Ramachandran et al. 2002; Hayashi et al. 2020). Genetic approaches to tracking predator movement allow study of movement across much larger distances (Osborne 2002). By comparing the genetic differentiation between populations, we can infer whether populations across large distances are regularly interbreeding.

Here, we used population genomics to study genetic differentiation between populations of *Geocoris punctipes* (Say) and *Orius insidiosus* (Say) to better understand their dispersal capabilities and movement behaviors across the Southeastern United States. Our core hypothesis was that, because these predators are relatively small with no known migration patterns, predators collected from geographically distant sites would be genetically different.

Methods

Sample Collection

Cotton plots were sampled from 10 university research centers, chosen as sampling locations due to their managers' willingness to participate in the research. These sites were located across Georgia and into Florida, Alabama, and South Carolina (Figure 1). Geocoridae and Anthocoridae were collected at each location, with *Geocoris punctipes* and *Orius insidiosus* as representative species due to their prominence within test sites. Sampling was capped at 3 hours per location using a combination of sweep netting and beat sheeting, with a goal of collecting at least 10 individuals of each species. Specimens were aspirated into collection tubes and transported in ice boxes to prevent DNA degradation. Samples were then stored in 70% ethanol and kept in a freezer at -20^{o}C. Sampling was done from September to early October before cotton harvest.

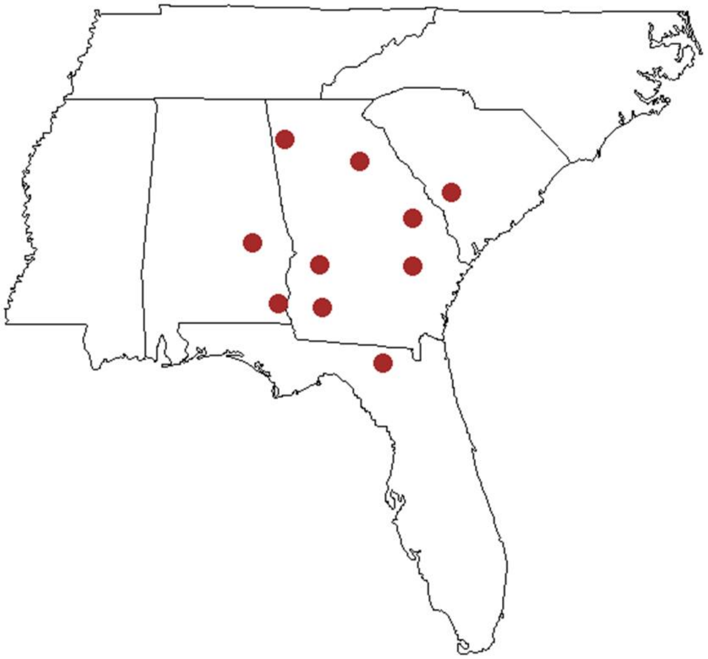

Figure 1. *A map of the 10 research and education centers used for collection of* O. insidiosus *and* G. punctipes.

DNA Extraction

DNA was extracted for up to 10 individuals from each site for both *G. punctipes* eocoridae (N = 100) and *O. insidiosus* (N = 86). Extractions were performed using the DNeasy Blood & Tissue kit (Qiagen, Germantown, MD). Individual specimens were placed in 1.5ml microcentrifuge tubes with 180 µl ATL buffer. For *G. punctipes,* samples were crushed using sterilized pestles until sufficiently pulverized. For *O. insidiosus*, specimens were removed from the tube and dissected in 15 µl of the ATL buffer to better fragment the sample, as the small size of *O. insidiosus* rendered pestling ineffective. This mixture was then added back to 165 µl of ATL remaining in each sample tube. Extraction followed kit protocol, with an additional elution to increase DNA yield. DNA yield was measured using a Qubit 4.0 Flourometer (Thermo Fisher Scientific). *Geocoris punctipes* extractions were largely successful, and concentrations averaged around 20ng/µl with a 50µl elution. *Orius insidiosus* averaged around 3ng/µl in a 50µl elution. Sequencing is most successful with at least 100ng of DNA, roughly what the *O. insidiosus* extractions produced.

Sequencing and Analysis

Samples were sent to SNPsaurus (Eugene, OR) for sequencing. SNPsaurus performed NextRAD sequencing for *G. punctipes* because of its relatively larger genome, and whole genome sequencing for *O. insidiosus* due to its relatively smaller genome. NextRAD sequencing uses the Nextera reagent (Illumina, San Diego, CA) to fragment DNA and attach adaptors to fragment edges to allow amplification of sequences. Sequences from both species were trimmed using BBTools (http://jgi.doe.gov/data-and-tools/bbtools/) to filter out Nextera adapters and sequences of low quality (Phred quality <20). There were 8 reads sequenced for both *G. punctipes* and *O. insidiosus*. These reads were then aligned and variants called using BBTools. Loci missing >25% of the genotypes were removed from the sample set. A reference genome from NCBI was used for *O. insidiosus (*Bioproject PRJNA633727*)*. Since there was no reference genome available for *G. punctipes*, a de novo reference genome was created from our samples for sequence alignment. Using BBTools, the data was formatted in a ‘variant call format’ (VCF) containing SNPs across samples, as well as the chromosome and position data of each SNP. In total there were 45,979 SNPs identified in *G. punctipes* and 13,675 SNPs across *O. insidiosus* samples.

Since our study is interested in genetic drift between populations caused by geographic isolation, loci under local divergent selection were filtered out of our dataset. Using the R package ‘qvalue’ (Storey et al. 2022), we could identify loci in our VCF that were likely under local selection through a multivariate analysis looking for outlier regions with high rates of variation for specific loci. Once identified in R, these loci were removed from the dataset using VCFtools (Danecek et al. 2011). After filtering there were 43,323 SNPs in *G. punctipes* and 13,497 SNPs in *O. insidiosus*.

Using VCFtools, we calculated the pairwise fixation index (Pairwise FST) for each 2-site comparison, totaling 45 unique combinations. Pairwise FST quantifies genetic differentiation between sites by comparing the number of SNPs between individuals of the same site with SNPs of individuals from different sites. More SNPs between individuals of different sites than the same site supports genetic differences between populations, and a higher FST means greater genetic differentiation between sites. We then calculated euclidean distance between each site using Google Earth satellite imagery (Google 2001). Genetic distance between sites was compared to geographic distance using a Mantel test through the r package ‘ade4’ (Dray and Dufour 2007). We also used a principal components analysis (PCA) in R using the ‘poppr’ package (Kamvar et al. 2014) to find factors in the VCF that could explain some of the variance seen in our data. This would allow us to find potential groupings of populations that may not be directly tied to geographic distance.

Results

Genetic Relatedness and Geographic Distance

We found no significant correlation between genetic relatedness and geographic distance between collection sites for *Geocoris* or *Orius.* Mantel tests showed an observation of .136 and p-value of .217 for *Geocoris* (Figure 2), and an observation of .058 and p-value of .366 for *Orius* (Figure 3).

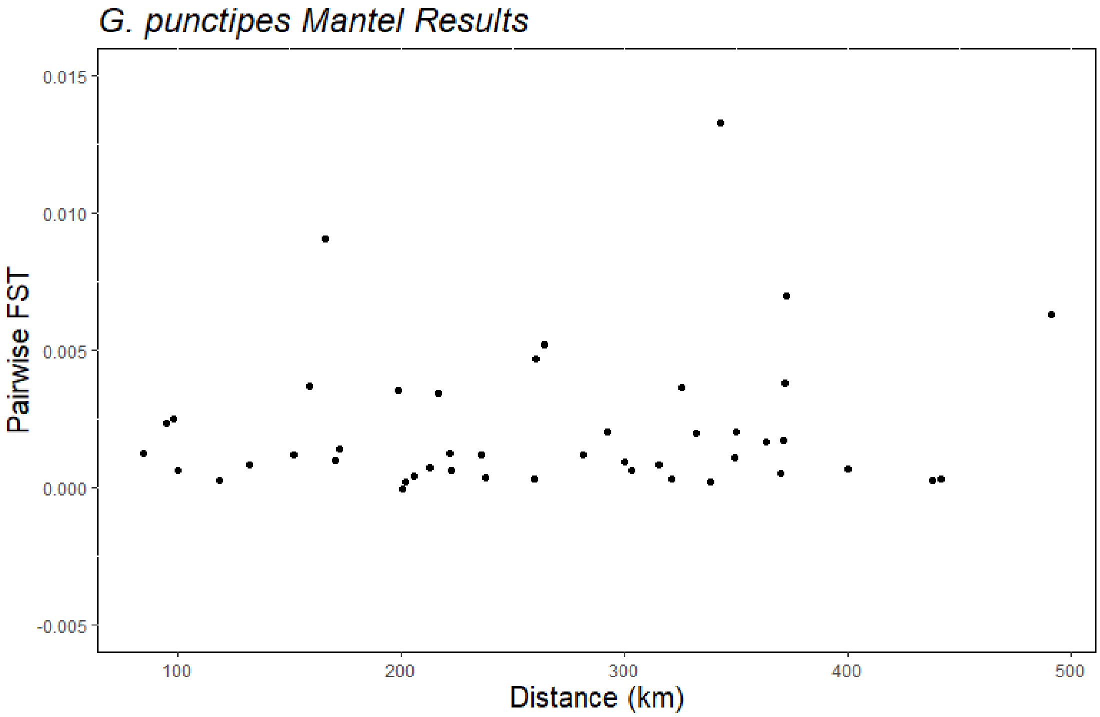

Figure 2. *Results of the Mantel test for* G. punctipes *showing the relationship between genetic relatedness (pairwise FST) and geographic distance (km) between sites.*

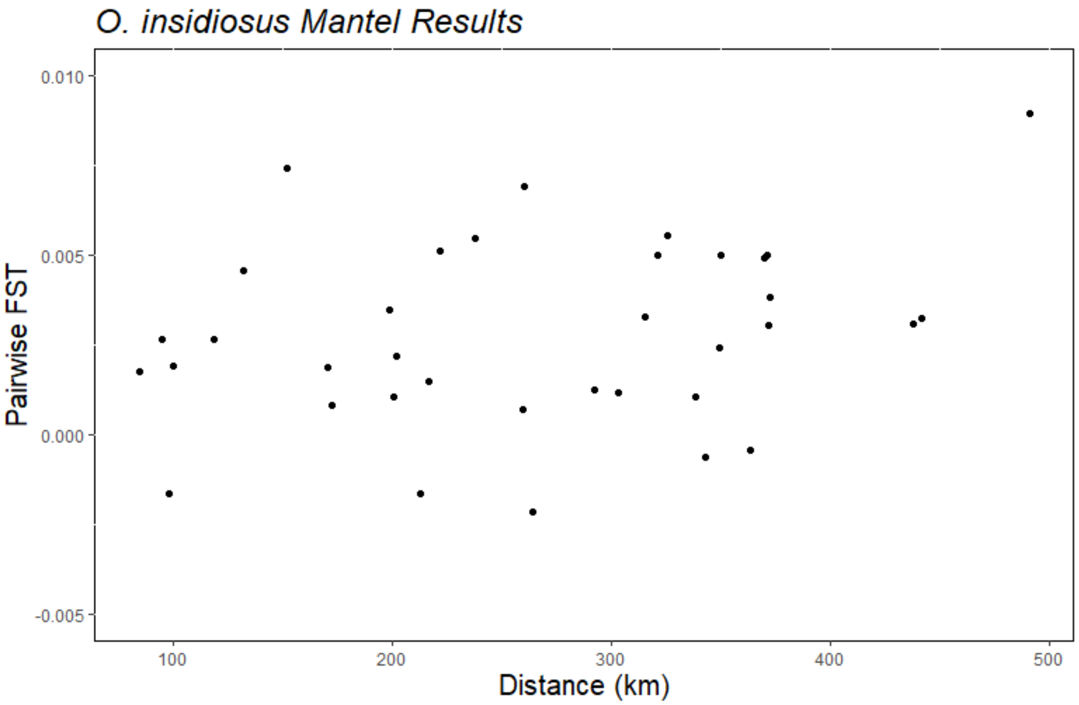

Figure 3. *Results of the Mantel test for* O. insidiosus *showing the relationship between genetic relatedness (pairwise FST) and geographic distance (km) between sites.*

The principal components analysis revealed no significant groupings of populations by genetic similarity for either *G. punctipes* (Figure 4) or *O. insidiosus* (Figure 5). Although some individuals from sites along the edges of our sampling region appear to be slightly more genetically distinct (such as those from Florida (U) and South Carolina (D)), they are not statistically significantly different. Overall, no populations show remarkable differentiation from one another for both *G. punctipes* and *O. insidiosus*.

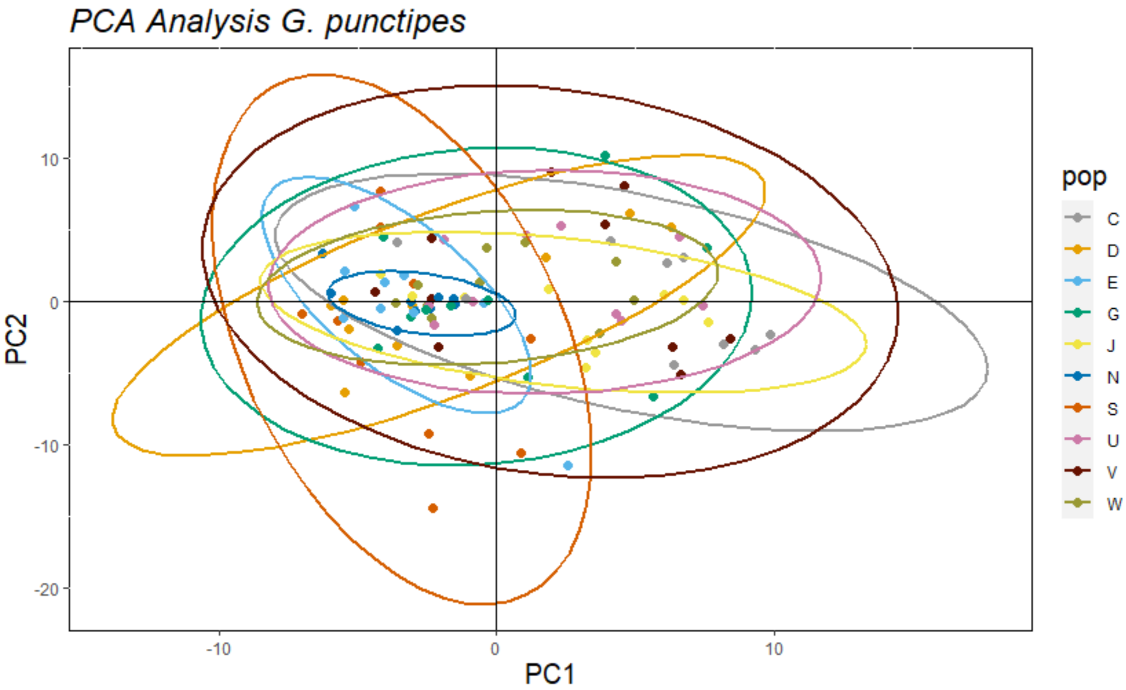

***Figure 3**. Results of the PCA for* G. punctipes *show no differentiation or grouping between populations when analyzing for potential factors influencing variation in the dataset. Each color indicates a different population. Overlapping of individuals across populations indicate little differentiation within principal components.*

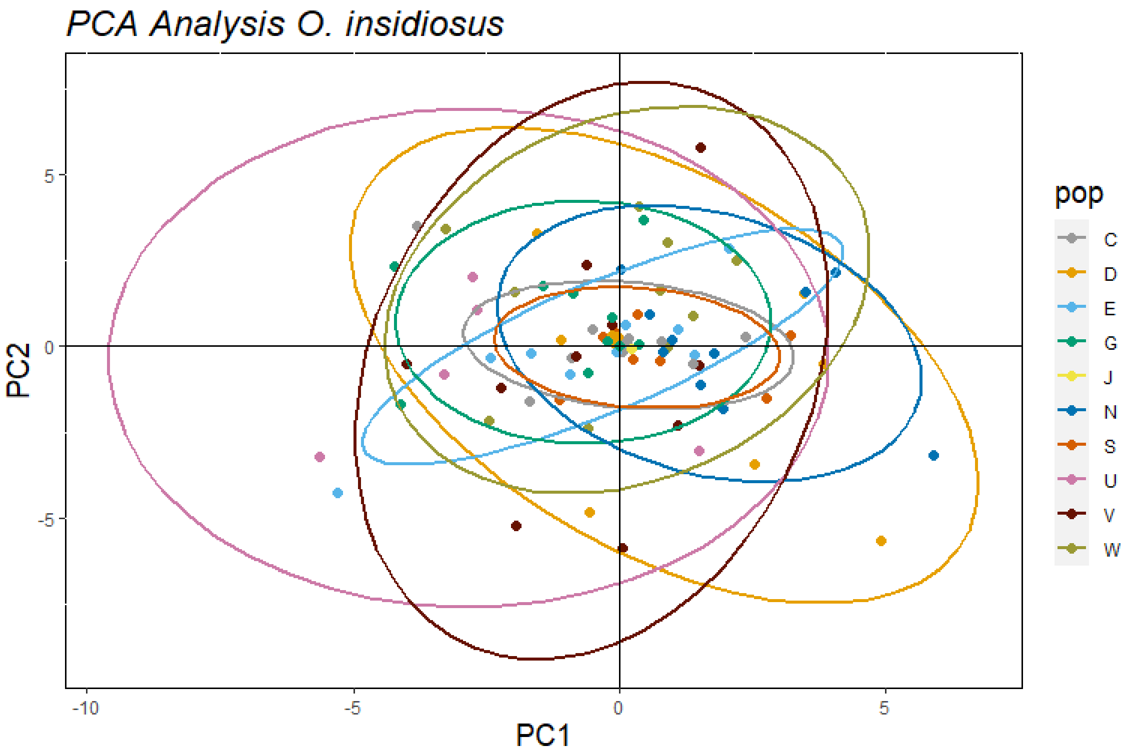

Figure 5. *Results of the PCA for* G. punctipes *show no differentiation or grouping between populations when analyzing for potential factors influencing variation in the dataset. Each color indicates a different population. Overlapping of individuals across populations indicate little differentiation within principal components.*

Discussion

The ability of a biocontrol agent to regularly disperse into new habitats can create increased opportunities for pest suppression (Ives and Settle 1997, Bianchi et al. 2009). Although *O. insidiosus* has previously been shown to move short distances within crop plots (Ramachandran et al. 2002, Hayashi et al. 2020), there is minimal research into their abilities to disperse across longer distances. Due to the small size of *O. insidiosus,* we predicted a lack of long-distance dispersal would create variable structure between populations. Similarly, little information exists on the movement patterns of *Geocoris* species, although they explore their surroundings mainly on foot (Crocker and Whitcomb 1980). Given their terrestrial nature and small size, we again predicted a lack of long-distance interbreeding between *G. punctipes* populations, with a pattern of increasing genetic differentiation as distance between field sites increased.

Both *Orius insidiosus* and *Geocoris punctipes* were found to have little to no genetic drift between populations across our sampling region. The absence of a correlation between these factors suggests a level of interbreeding between populations to maintain some homogeneity in neutral loci. The lack of differentiation between populations in the PCA further support the notion of a well-connected population network for both species within the Southeastern United States. The presence of a few genetically distinct *Orius* individuals in our more distant sites could suggest significant genetic differentiation just outside our sampling region. However, more data would be needed to support these claims.

Due to their small size, it is possible *Orius* achieves long-distance dispersal through air currents, a behavior shown in other insects such as whiteflies (Byrne 1999) and scales(Greathead 2009). Though larger and heavier than *Orius, Geocoris* may also benefit from these air currents during flight. Despite their terrestrial nature, they are surprisingly adept fliers, having been collected at over 1,000 feet in the air (Glick 1957).

Consistent long-range dispersal of these predators may allow them to better recolonize agricultural plots after disturbance, such as chemical application. Early recolonization of agricultural fields by natural enemies is critical in suppression of pest species, as long gaps between recolonization allow pest populations to grow unchecked (Costamagna et al. 2015, Ives and Settle 1997). To confirm their ability to recolonize post-disturbance, further research into the short-distance movement and habitat choice behaviors of these species should also be examined. By furthering our understanding of their movement behaviors, we can better educate spraying protocol and local landscape management to maximize control while preserving healthy natural enemy populations.

Another round of analysis is planned to include individuals from states farther north and *Geocoris* from their western range in Utah to examine population genomic structure on a larger scale. Should these populations be reproductively isolated, genetic drift would be expected, with higher pairwise FST as distance increases. Overall, our results to-date suggest a remarkably high frequency of long-distance movement of these two predatory bugs across the southeastern US. This increases support of *G. punctipes* and *O. insidiosus* as important natural enemies that can rapidly colonize agricultural fields after disturbance, even when those fields are quite distant from natural vegetation or other refuge sites. Clearly, our finding of broad panmixis across the southeastern US suggests that additional research into these two predator species' movement is greatly needed.

The results of this study are consistent with predators commonly moving large distances across landscapes, and regularly interbreeding. There are other studies using these methods that have found genetic differentiation across populations for a variety of reasons. Fu et al. (2021) used a similar population genomics approach in their study of entomopathogenic nematodes. Nematodes appear to be so restricted in their movement that even geographically close populations might be expected to have a high degree of genetic differentiation, and while this was generally true the collection of distantly related nematodes at the same site suggested an unexpected ability of the worms to move large distances (albeit rarely). The study by Fu et al. in (2020) on potato psyllid also found genetic differentiation across populations, but these populations appeared to be divided by host plant, not geographic distance. Another study looked at the population genomics of a species of damselfly and found strong genetic differentiation in edge populations (Swaegers et al. 2015). This appeared to be due to a series of range expansions, creating genetic bottlenecks in the newly expanded regions. *Aedes spp*. were also found to be genetically differentiated across geographic distance (Schmidt et al. 2020). Although their sampling region was broader, even some geographically close populations of mosquitoes had levels of genetic differentiation. This could be due to an unknown spatial or behavioral barrier preventing interbreeding, or the lack of significant dispersal behaviors in *Aedes spp*. *G. punctipes* and *O. insidiosus* may disperse greater distances more frequently than these species, creating high levels of homogeneity across populations.

Data syntheses have found inconsistencies in the relationship between regional habitat diversity and enhanced biological control (Ratsimba 2021). While conservation biocontrol orthodoxy suggests that natural enemies should be more abundant and more diverse, and biocontrol thus stronger, in fields embedded in diverse landscapes this link is often not apparent (Karp et al. 2018). Our results suggest this may be due to regular long-distance dispersal of generalist predatory insects. Many insects are well-known to migrate great distances such as the monarch butterfly (Reppert and de Roode 2018). These natural enemies may be moving at such a scale that the surrounding landscape structure around crop fields holds little weight in their abundance and ability to recolonize post-disturbance. Other generalist predators have been shown to move significant distances such as lady beetles (Rankin and Rankin 1980) and syrphid flies (Clem et al. 2022). This behavior may be more common in insect predators than previously thought, thus changing how we think about conservation biocontrol and its relationship to predator populations.

REFERENCES

Abbate, S.L., F. Madeira, O. Bentancur, N. Altier, and X. Pons. 2022. Refuge Areas Favor the Presence of Predators and Herbivores in Bt Soybean: A Landscape Perspective. J Pest Sci

Andrews, K.R., J.M. Good, M.R. Miller, G. Luikart, and P.A. Hohenlohe. 2016. Harnessing the Power of RADseq for Ecological and Evolutionary Genomics. Nat Rev Genet 17:81-92.

Aviron, S., S. Poggi, Y.D. Varennes, and A. Lefevre. 2016. Local Landscape Heterogeneity Affects Crop Colonization by Natural Enemies of Pests in Protected Horticultural Cropping Systems. Agr Ecosyst Environ 227: 1-10.

Barratt, B.I.P., F.G. Howarth, T.M. Withers, J.M. Kean, and G.S. Ridley. 2010. Progress in Risk Assessment for Classical Biological Control. Biol Control 52:245-254.

Bebber, D.P., T. Holmes, and S.J. Gurr. 2014. The Global Spread of Crop Pests and Pathogens. Global Ecol Biogeogr 23:1398-1407.

Bianchi, F.J.J.A., C.J.H. Booij, and T. Tscharntke. 2006. Sustainable Pest Regulation in Agricultural Landscapes: A Review on Landscape Composition, Biodiversity and Natural Pest Control. P R Soc B 273(1595).

Bianchi, F.J.J.A., N.A. Schellhorn, and W. van der Werf. 2009. Predicting the Time to Colonization of the Parasitoid *Diadegma semiclausum*: The Importance of the

Shape of Spatial Dispersal Kernels for Biological Control. Biol Control 50: 267-274.

Black IV, W.C., C.F. Baer, M.F. Antolin, and N.M. DuTeau. 2001. Population Genomics: Genome-wide Sampling of Insect Populations. Annu Rev Entomol 46:441-469.

Byrne, D.N. 1999. Migration and Dispersal by the Sweet Potato Whitefly, *Bemisia tabaci.* Agr Forest Meteorol 97:309-316.

Chandi, R.S., S.K. Kataria, and B.B. Fand. 2021. Effect of Temperature on Biological Parameters of Cotton Whitefly, Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae). Int J Trop Insect Sc 41:1823-1833.

Clem, S.C., K.A. Hobson, and A.N. Harmon-Threatt. 2022. Insights into Natal Origins of Migratory Nearctic Hover Flies (Diptera: Syrphidae): New Evidence from Stable Isotope (δ^2H) Assignment Analyses. Ecography 2023:e06465.

Collier, T. and R.V. Steenwyk. 2004. A Critical Evaluation of Augmentative Biological Control. Biol Control 31:245-256.

Collins, K.L., N.D. Boatman, A. Wilcox, J.M. Hollans, and K. Chaney. Influence of Beetle Banks on Cereal Aphid Predation in Winter Wheat. Agr Ecosyst Environ 93:337-350.

Copping, L.G. and J.J. Menn. 2000. Biopesticides: A Review of their Action, Applications and Efficacy. Pest Manag Sci 56:651-676.

Costamagna, A.C., W.N. Venables, and N.A. Schellhorn. 2015. Landscape-scale Pest Surpression is Mediated by Timing of Predator Arrival. Ecol Appl 25:1114-1130.

Crocker, R.L. and W.H. Whitcomb. 1980. Feeding Niches of the Big-eyed Bugs *Geocoris bullatus*, *G. punctipes*, and *G. uliginosus* (Hemiptera: Lygaeidae: Geocorinae). Environ Entomol 9:508-513.

Danecek, P., A. Auton, G. Abecasis, C.A. Albers, E. Banks, M.A. DePristo, R.E. Handsaker, G. Lunter, G.T. Marth, S.T. Sherry, G. McVean, R. Durbin, and 1000 Genomes Project Analysis Group. 2011. The Variant Call Format and VCFtools. Bioinformatics 27:2156-2158.

Diehl, E., E. Sereda, V. Wolters, and K. Birkhofer. 2013. Effects of Predator Specialization, Host Plant and Climate on Biological Control of Aphids by Natural Enemies: A Meta-analysis. J Appl Ecol 50:262-270.

Dray, S. and A. Dufour. 2007. The ade4 Package: Implementing the Duality Diagram for Ecologists. J Stat Softw 86:1-17.

Dutta, B., B. Myers, T. Coolong, B. Srinivasan, A. Sparks. 2018. Whitefly-transmitted Plant Viruses in South Georgia. UGA Extension, University of Georgia. Retrieved from https://extension.uga.edu/publications/detail.html?number=B1507

Edwards, S. and S. Bensch. 2009. Looking Forwards or Backwards in Avian Phylogeography? A Comment on Zink and Barrowclough 2008. Mol Ecol 18:2930-2933.

Ellegren, H. 2004. Microsatellites: Simple Sequences with Complex Evolution. Nat Rew Genet 5:435-445.

El Sheikha, A.F. 2019. Tracing Insect Pests: Is There New Potential in Molecular Techniques? Insect Mol Biol 28:759-772.

Fernandez, D.E., L.I. Cichon, E.E. Sanchez, S.A. Garrido, and C. Gittins. 2008. Effect of Different Cover Crops on the Presence of Arthropods in an Organic Apple (Malus domestica Borkh) Orchard. J Sustain Agri 32:197-211.

Fu, Z., M.S. Crossley, B. Epstein, C. Bates, D.W. Crowder, A.A. Elling, P.A. Hohenlohe, R. Jabbour, R.A. Ramirez, and W.E. Snyder. 2021. Using Fine-scale Relatedness to Infer Natural Enemy Movement. Biol Control 160(104662).

Fu, Z., A.R. Meier, B. Epstein, A.O. Bergland, C.I. Castillo Carillo, W.R. Cooper, R.K. Cruzado, D.R. Horton, A.S. Jensen, J.L. Kelley, A. Rashed, S.R. Reitz, S.I. Rondon, J. Thinakaran, E.J. Wenninger, C.H. Wohleb, D.W. Crowder, and W.E. Snyder. Host Plants and Wolbachia Shape the Population Genetics of Sympatric Herbivore Populations. Evol Appl 13:2740-2753.

Glick, P. A. 1957. Collecting Insects by Airplane in Southern Texas. USDA Tech. Bull. No. 1158. 28 p

Google. 2001. Google Earth: Satellite Imagery and Geographical Information Software. Available Online at https://www.google.com/earth/. Accessed 14 Nov. 22.

Greathead, D.J. 2009. Dispersal of the Sugar-cane Scale *Aulacaspis tegalensis* (Zhnt.) (Hem., Diaspididae) by Air Currents. B Entomol Res 61:547-558.

Hagler, J.R. 1997. Field Retention of a Novel Mark – Release – Recapture Method. Environ Entomol 26:1079-1086.

Hassell, M.P., H.N. Comins, and R.M. Mayt. 1991. Spatial Structure and Chaos in Insect Population Dynamics. Nature 353:255-258.

Hayashi, M., J. Abe, Y. Owashi, and K. Miura. 2020. Estimation of Movement from Insectary Plants to Crop Plants in *Orius* bugs (Heteroptera: Anthocoridae) by Molecular Gut Content Analysis. Appl Entomol Zool 55:361-365.

Hernandez, L.M. 2010. A Review of the Economically Important Species of the Genus *Orius* (Heteroptera: Anthocoridae) in East Africa. J Nat Hist 22:543-568.

Hirose, Y., K. Takasu, and M. Takagi. 1996. Egg Parasitoids of Phytophagous Bugs in Soybean: Mobile Natural Enemies as Naturally Occurring Biological Control Agents of Mobile Pests. Biol Control 7:84-94.

Hivert, V., R. Leblois, E.J. Petit, M. Gautier, and R. Vitalis. 2018. Measuring Genetic Differentiation from Pool-seq Data. Genetics 210:315-330.

Hodel, R.G.J., S. Chen, A.C. Payton, S.F. McDaniel, P. Soltis, and D.E. Soltis. 2017. Adding Loci Improves Phylogeographic Resolution in Red Mangroves Despite Increased Missing Data: Comparing Microsatellites and RAD-Seq and Investigating Loci Filtering. Sci Rep-UK 7(17598).

Hokkanen, H.M.T. and R.I. Sailer. 1985. Success in Classical Biological Control. Crit Rev Plant Sci 3:35-72.

Holland, J.M., F.J.J.A. Bianchi, M.H. Entling, A.C. Moonen, B.M. Smith, and P. Jeanneret. 2016. Structure, Function, and Management of Semi-natural Habitats for Conservation Biological Control: A Review of European Studies. Pest Manag Sci 72:1638-1651.

Horowitz, R., I. Denholm, and S. Morin. 2007. Resistance to Insecticides in the TYLCV Vector, *Bemisia tabaci.* In: Czosnek, H. (eds) Tomato Yellow Leaf Curl Virus Disease. pp 305-325 Springer, Dordrecht.

Horowitz, R., M. Ghanim, E. Roditakis, R. Nauen, and I. Ishaaya. 2020. Insecticide Resistance and its Management in *Bemisia tabaci* Species. J Pest Sci 93:893-910.

Howarth, F.G. 1991. Environmental Impacts of Classical Biological Control. Annu Rev Entomol 36: 485-509.

Ives, A.R. and W.H. Settle. 1997. Metapopulation Dynamics and Pest Control in Agricultural Systems. Am Nat 149: 220-246.

Kamvar, Z.N., J.F. Tabima, and N.J. Grunwald. 2014. Poppr: An R Package for Genetic Analysis of Populations with Clonal, Partially clonal, and/or Sexual Reproduction. PeerJ 2:e281.

Karp, D.S., R. Chaplin-Kramer, T.D. Meehan, E.A. Martin, F. DeClerck, H. Grab, C. Gratton, L. Hunt, A.E. Larsen, A. Martinez-Salinas. *et al.* 2018. Crop Pests and Predators Exhibit Inconsistent Responses to Surrounding Landscape Composition. P Natl Acad Sci USA 115(33):E7863-E7870

Kheirodin, A., A.M. Simmons, J.C. Legaspi, E.E. Grabarcyzk, M.D. Toews, P.M. Roberts, J. Chong, W.E. Snyder, and J.M. Schmidt. 2020. Can Generalist Predators Control Bemisia tabaci? Insects 11(823).

Li, Y., G.N. Mbata, S. Punnuri, A.M. Simmons, and D.I. Shapiro-Ilan. 2021. *Bemisia tabaci* on Vegetables in the Southern United States: Incidence, Impact, and Management. Insects 12:198.

Liman, A.S., K. Eklund, and C. Bjorkman. 2016. Predator Refuges for Conservation Biological Control in an Intermediately Disturbed System: The Rise and Fall of a Simple Solution. J Appl Ecol 53:1823-1830.

Linn, C.A., and E.M. Griebeler. 2016. Habitat Preference of German *Mantis religiosa* Populations (Mantodea: Mantidae) and Implications for Conservation. Environ Entomol 45:829-840.

Luikart, G., P.R. England, D. Tallmon, S. Jordan, and P. Taberlet. 2003. The Power and Pormise of Population Genomics: from Genotyping to Genome Typing. Nat Rev Genet 4:981-994.

Mahroof, R.M., P.A. Edde, B. Robertson, J. A. Puckette, and T.W. Phillips. 2010. Dispersal of Rhyzopertha dominica (Coleoptera: Bostrichidae) in Different Habitats. Environ Entomol 39: 930-938.

Markow, T.A. and P. O’Grady. 2005. Drosophila: A Guide to Species Identification and Use. Elsevier, Amsterdam.

Minter, M., A. Pearson, K.S. Lim, K. Wilson, J.W. Chapman, and C.M. Jones. 2018. The Tethered Flight Technique as a Tool for Studying Life-history Strategies Associated with Migration in Insects. Ecol Entomol 43:397-411.

Müller, C.B. and J. Brodeur. 2002. Intraguild Predation in Biological Control and Conservation Biology. Biol Control 25:216-223.

Naranjo, S.E. 2019. Assessing Insect Flight Behavior in the Laboratory: A Primer on Flight Mill Methodology and What Can Be Learned. An Entomol Soc Am 112:182-199.

Navas-Castillo, J., E. Fiallo-Olive, and S. Sanchez-Campos. 2011. Emerging Virus Diseases Transmitted by Whiteflies. Annu Rev Phytopathol 49:219-248.

Oliviera, M.R.V., T.J. Henneberry, and P. Anderson. 2001. History, Current Status, and Collaborative Research Projects for *Bemisia tabaci*. Crop Prot 20:709-723.

Olson, D.M., A.R. Zeilinger, K.K. Prescott, A.W. Coffin, J.R. Ruberson, and D.A. Andow. 2018. Landscape Effects on *Solenopsis invicta* (Hymenoptera: Formicidae) and *Geocoris* spp. (Hemiptera: Geocoridae), Two Important Omnivorous Arthropod Taxa in Field Crops. Environ Entomol 47:1057-1063.

O'Neal, M.E., D.A. Landis, E. Rothwell, L. Kempel, and D. Reinhard. 2004. Tracking Insects with Harmonic Radar: a Case Study. Am Entomol 50:212-218.

Osborne, J.L., H.D. Loxdale, and I.P. Woiwod. 2002. Monitoring Insect Dispersal: Methods and Approaches. In: Dispersal Ecology: The 42nd Symposium of the British Ecological Society Held at the University of Reading, 2-5 April 2001.

Paradis, E. and K. Schliep. 2019. ape 5.0: An Environment for Modern Phylogneetics and Evolutionary Analyses in R. Bioinformatics 35:526-528.

Pelissie, B., M.S. Crossley, Z.P. Cohen, and S.D. Schoville. 2018. Rapid Evolution in Insect Pests: The Importance of Space and Time in Population Genomics Studies. Curr Opin Insect Sci 26:8-16.

Ramos, R.S., L. Kumar, F. Shabani, and M.C. Picanco. 2019. Risk of Spread of Tomato Yellow Leaf Curl Virus (TYLCV) in Tomato Crops Under Various Climate Change Scenarios. Agr Syst 173:524-535.

Ramsden, M.W., R. Menendez, S.R. Leather, and F. Wackers. Optimizing Field Margins for Biocontrol Services: The Relative Role of Aphid Abundance Annual Floral Resources, and Overwinter Habitat in Enhancing Aphid Natural Enemies. Agr Ecosyst Environ 199:94-104.

Ramachandran, S., J. Funderburk, J. Stavisky, and S. Olson. 2002. Population Abundance and Movement of *Frankliniella* species and *Orius insidiosus* in Field Pepper. Agr Forest Entomol 3: 129-137.

Rand, T.A., J.M. Tylianakis, and T. Tscharntke. 2006. Spillover Edge Effects: The Dispersal of Agriculturally Subsidized Insect Natural Enemies into Adjacent Natural Habitats. Ecol Lett 9:603-614.

Rankin, M.A., and S. Rankin. 1980. Some Factors Affecting Presumed Migratory Flight Activity of the Convergent Lady Beetle, *Hippodamia convergens* (Coccinellidae: Coleoptera). Biol Bull 158:356-369.

Reppert, S.M., and J.C. de Roode. 2018. Demystifying Monarch Butterfly Migration. Curr Biol 28:1009-1022.

Romiguier, J., P. Gayral, M. Ballenghien, A. Bernard, V. Cahais, A. Chenuil, Y. Chiari, R. Dernat, L. Duret, N. Faivre. *et al.* 2014. Comparative Population Genomics in Animals Uncovers the Determinants of Genetic Diversity. Nature 515:261-263.

Roubinet, E., T. Jonsson, G. Malsher, K. Staudacher, M. Traugott, B. Ekbom, and M. Jonsson. 2018. High Redundancy as well as Complementary Prey Choice Characterize Generalist Predator Food Webs in Agroecosystems. Sci Rep-UK 8:8054.

Schmidt, T.L., J. Chung, A.C. Honnen, A.R. Weeks, A.A. Hoffman. 2020. Population Genomics of Two Invasive Mosquitoes (*Aedes aegypti* and *Aedes albopictus*) from the Indo-Pacific. Plos Neglect Trop D 14(7).

Schneider, J.C. 1999. Dispersal of a Highly Vagile Insect in Aheterogeneous Environment. Ecology 80:2740-2749.

Schuster, S.C. 2008. Next-generation Sequencing Transforms Today's Biology. Nat Methods 5:16-18.

Sethuraman, A., F.J. Janzen, and J. Obrycki. 2015. Population Genetics of the Predatory Lady Beetle *Hippodamia convergens*. Biol Control 84:1-10.

Shapiro, D.I., J.J. Obrycki, L.C. Lewis, and J.J. Jackson. 1999. Effects of Crop Residue on the Persistence of *Steinernema carpocapsae*. J Nematol 31:517-519.

Shields, M.W., A.C. Johnson, S. Pandey, R. Cullen, M. Gonzalez-Chang, S.D. Wratten, and G.M. Gurr. 2019. History, Current Situation and Challenges for Conservation Biological Control. Biol Control 131:25-35.

Showers, W.B. 1997. Migratory Ecology of the Black Cutworm. Annu Rev Entomol 42:393-425.

Sivakoff, F.S., J.A. Rosenheim, and J.R. Hagler. 2012. Relative Dispersal Ability of a Key Agricultural Pest and its Predators in an Annual Agroecosystem. Biol Control 63:296-303.

Snyder, W.E. 2019. Give Predators a Complement: Conserving Natural Enemy Biodiversity to Improve Biocontrol. Biol Control 135:73-82.

Swaegers, J., J. Mergeay, A. Van Geystelen, L. Therry, M.H.D. Larmuseau, and R. Stoks. 2015. Neutral and Adaptive Genomic Signatures of Rapid Poleward Range Expansion. Mol Ecol 24:6163-6176.

Stiling, P. and T. Cornelissen. 2005. What Makes a Successful Biocontrol Agent? A Meta-analysis of Biological Control Agent Performance. Biol Control 34:236-246.

Storey, J.D., A.J. Bass, A. Dabney, and D. Robinson. 2022. Qvalue: Q-value Estimation for False Discovery Rate Control. Version 2.30. Current version available online at http://github.com/jdstorey/qvalue.

Symondson, W.O.C., K.D. Sunderland, and M.H. Greenstone. 2002. Can Generalist Predators be Effective Biocontrol Agents? Annu Rev Entomol 47:561-594.

Tang, S., R.A. Cheke, and Y. Xiao. 2009. Effects of Predator and Prey Dispersal on Success or Failure of Biological Control. B Math Biol 71 (2025).

Thies, C., I. Steffan-Dewenter, and T. Tscharntke. 2003. Effects of Landscape Context on Herbivory and Parasitism at Different Spatial Scales. Oikos 101:18-25.

Thomas, M.B. and A.J. Willis. 1998. Biocontrol – Risky but Necessary? Trends Ecol Evol 13:325-329.

Tscharntke, T., D.S. Karp, R. Chaplin-Kramer, P. Batary, F. DeClerck, C. Gratton, L. Hunt, A. Ives, M. Jonsson, A. Larsen. *et al.* 2016. When Natural Habitat Fails to Enhance Biological Pest Control – Five Hypothesis. Biol Control 204:449-458.

Visser, U., K. Wiegand, V. Grimm, and K. Johst. 2009. Conservation Biocontrol in Fragmented Landscapes: Persistence and Parasitation in Host-Parasitoid Model. Open Ecol J 2:52-61.

Varma, A., B. Mandal, M.K. Singh. 2011. Global Emergence and Spread of Whitefly (Bemisia tabaci) Transmitted Geminiviruses. In: Thompson, W. (eds) The Whitefly, Bemisia tabaci (Homoptera: Aleyrodidae) Interaction with Geminivirus-Infected Host Plants. 205-292. Springer, Dordrecht.

Vandervoet, T.F., P.C. Ellsworth, Y. Carriere, and S.E. Naranjo. 2018. Quantifying Conservation Biological Control for Management of Bemisia tabaci (Hemiptera: Aleyrodidae) in Cotton. J Econ Entomol 111:1056-1068.

With, K.A., D.M. Pavuk, J.L. Worchuck, R.K. Oates, and J.L. Fisher. 2002. Threshold Effects of Landscape Structure on Biological Control in Agroecosystems. Ecol Appl 12:52-65.

Zane, L., L. Bargelloni, and T. Patarnello. 2002. Strategies for Microsatellite Isolation: A Review. Mol Ecol 11:1-16.

Zink, R.M., J.G. Growth, H. Vazquez-Miranda, and G.F. Barrowclough. 2013. Phylogeography of the Claifornia Gnatcatcher (*Polioptila californica*) Using Multilocus DNA Sequences and Ecological Niche Modeling: Implications for Conservation. Auk 130:449-458.

Zolubas, P. and J.A. Byers. 1995. Recapture of Dispersing Bark Beetle *Ips typographus* L. (Col., Scolytidae) in Pheromone-baited Traps: Regression Models. J Appl Entomol 119:285-289.