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Life-history stage and the population genetics of the tiger mosquito *Aedes albopictus* at a fine spatial scale

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Abstract

As a widespread vector of disease with an expanding range, the mosquito *Aedes albopictus* Skuse (Diptera: Culicidae) is a high priority for research and management. *A. albopictus* has a complex life history with aquatic egg, larval and pupal stages, and a terrestrial adult stage. This requires targeted management strategies for each life stage, coordinated across time and space. Population genetics can aid in *A. albopictus* control by evaluating patterns of genetic diversity and dispersal. However, how life stage impacts population genetic characteristics is unknown. We examined whether patterns of *A. albopictus* genetic diversity and differentiation changed with life stage at a spatial scale relevant to management efforts. We first conducted a literature review of field-caught *A. albopictus* population genetic papers and identified 101 peer-reviewed publications, none of which compared results between life stages. Our study uniquely examines population genomic patterns of egg and adult *A. albopictus* at five sites in Wake County, North Carolina, USA, using 8425 single nucleotide polymorphisms. We found that the level of genetic diversity and connectivity between sites varied between adults and eggs. This warrants further study and is critical for research aimed at informing local management.

KEYWORDS

Aedes albopictus, invasive species, life stage, mosquito, population genetics

INTRODUCTION

The tiger mosquito, *Aedes albopictus*, a vector of zoonotic and human diseases, is pervasive across human-inhabited landscapes around the globe (Paupy et al., 2009; Pereira-Dos-Santos et al., 2020). Native to eastern Asia, *A. albopictus* became globally invasive through intercontinental trade (Hawley et al., 1987). The invasion success of *A. albopictus* is likely due to its oviposition ecology (Bonizzoni et al., 2013). Females lay eggs in containers with ephemeral water sources, and eggs require a wet-dry-wet cycle before hatching. Eggs are desiccant-resistant and can survive for long periods before hatching, including through winters, dry seasons and intercontinental travel (Bentley & Day, 1989; Lounibos, 2002). This behaviour, which likely evolved as a form of predator avoidance for larvae, preadapted *A. albopictus* to thrive in human-dominated areas where artificial containers are abundant (Bonizzoni et al., 2013). This anthropophilic tendency combined with opportunistic, aggressive biting behaviours and competence to transmit viruses makes the tiger mosquito one of the most abundant human pests and disease vectors in the world (Gratz, 2004; Hawley, 1988; Hawley et al., 1987).

Due to its global distribution and threat to public health, *A. albopictus* has been a priority for mosquito control (Benedict et al., 2007; Bonizzoni et al., 2013). Control of *A. albopictus* has included conventional approaches like larval habitat removal and adult insecticidal spraying, as well as the use of the symbiont *Wolbachia* and genetically modified (GM) mosquito releases to suppress population growth (Hollingsworth et al., 2020; Mains et al., 2016; Roiz et al., 2018). Informed decisions about mosquito control rely on accurate assessments of population genetic structure and gene flow (Takken & Scott, 2003). 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 & 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, researchers can use population genetic analyses to identify strategic areas for modified mosquito releases, track and interrupt the spread of insecticide resistance and evaluate programme success (Goubert et al., 2016; 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 a few populations is appropriate for detecting genetic bottlenecks, but may not be suitable in landscape genetics studies where it is 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 & 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. This can underestimate within-population genetic diversity and overestimate between-population differentiation (Goldberg & Waits, 2010). An important question for container mosquitoes is what life history stage best represents an unbiased, random sample.

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 mosquitoes, 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 & 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 sampled at a single time point may be misguided if gene flow and genetic structure differ between immature and adult mosquitoes. Several processes could result in incongruent population genetic patterns between life stages. For example, instability of local populations could mean that adult *A. albopictus* are not the progenitors of eggs sampled simultaneously. In addition, dispersal mechanisms vary between life stages. Research suggests that anthropogenic transport networks facilitate long distance dispersal of eggs and larvae (Ibáñez-Justicia, 2020; Medley et al., 2015), while adult movement tends to be unaided or via passive dispersal, primarily at smaller spatial scales (Eritja et al., 2017; Flacio et al., 2015; Ibáñez-Justicia, 2020). The impacts of adult, egg and larval dispersal on other life stages further complicate our ability to interpret population structure and connectivity. To account for variation in migration patterns and selection between mosquito life stages, continuous monitoring paired with temporal patterns of gene flow and genetic structure would be more appropriate and informative for management.

In this study, we first conducted a literature review of *A. albopictus* population genetic studies to evaluate the life stages sampled to assess genetic diversity and structure. We then used field-collected adult and egg *A. albopictus* at five sites in Wake County, NC to directly compare population genetic patterns between life stages. We measured genetic diversity, differentiation and structure among these sites for adults, eggs and combined using methods commonly used in population genetic research. Based on these results, we identified avenues for continued research and offered recommendations for study design to inform mosquito control.

MATERIALS AND METHODS

Literature review

To identify peer-reviewed articles on the population genetics of *A. albopictus*, we conducted a systematic literature review following the PRISMA 2020 flowchart (Page et al., 2021). We extracted study citations on 1 September 2022 from the Web of Science Core Collection database using three criteria, separated by the "AND" Boolean operator: (1) "*Aedes albopictus*"; (2) at least one term matching genetic, genomic, gene flow, or population structure; and (3) at least one term matching structure, variation, diversity, or population. With the resulting articles, we screened and extracted metadata using Covidence, an online systematic review software (Covidence, 2022). We conducted two levels of screening, one for titles and abstracts and one for full texts. For the former, we excluded papers that did not include the species name "*Aedes albopictus*" and a reference to genetic or genomic analysis, review articles, experimental research and research conducted on laboratory populations in the title or abstract. During the full text screening, we further removed studies that (1) did not use field-caught *A. albopictus* genetic data, (2) did not include original data and (3) did not state the life stage sampled. For each article, we recorded the year published, type(s) of genetic markers used, the life-stage(s) sampled, the number of individuals and locations sampled, the spatial extent sampled (local, regional, national, continental, global) and the primary population genetic goal(s) of the study. For research goals, we extracted terms used by the authors based on titles, abstracts and the objectives paragraph in the introduction. We then grouped these terms into seven broad categories: genetic structure, genetic variation, invasion origin, gene flow, phylogenetics and phylogeographic, natural selection and species identification. We examined associations between these factors and choice of

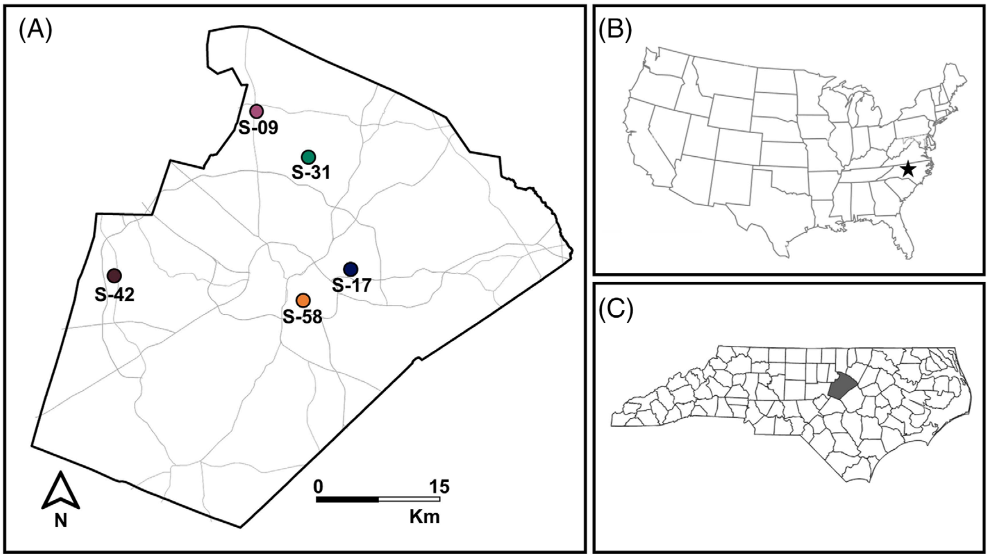

FIGURE 1 Map of (A) Wake County, North Carolina and the five sites for *Aedes albopictus* adult and egg samples. We sequenced DNA from 6 to 10 individuals of each life stage per site. (B) and (C) show the location of Wake County in the United States and North Carolina, respectively.

life stage using a chi-squared test, and we simulated *p* values using a Monte Carlo test with 10,000 replicates (Hope, 1968). For this statistical test and subsequent analyses, we used R v.4.0.3 in RStudio v.1.3.1093 RStudio Team (2021).

Egg and adult sampling

For this study, we used adult and egg *A. albopictus* collected between 7 June and 25 June 2018 from five sites in Wake County, North Carolina, USA. These individuals were sampled as part of a larger project, where we sampled *A. albopictus* adults from 61 locations across the county. We selected sites by randomly generating points across Wake County using the r.random.cells function in GRASS GIS (GRASS Development Team, 2018). We collected *A. albopictus* eggs from 20 of these sites, determined by a random number generator. We sampled adults using BG sentinels baited with BG Lures, a chemical attractant targeted for *Aedes* species (Biogents GmbH, Regensburg, Germany). We sampled each location once per week for 3 weeks and left traps in the field for 24 h each. After the 24-h period, we collected trapped mosquitoes and euthanized them by placing the collection bag in a -20°C freezer for 8–12 h. We then counted and identified the trapped mosquitoes and retained *A. albopictus* individuals.

We sampled mosquito eggs using ovitraps following the methods of Reed et al. (2019). We made ovitraps using black plastic cups filled with $\sim$350 ml of tap water and lined with seed germination paper. We placed three ovitraps in a triangle around the BG Sentinel trap at the selected locations. Each ovitrap was within 100 m of the BG Sentinel and at least 25 m away from other ovitraps to decrease the probability of an *A. albopictus* female laying eggs in multiple traps. We left ovitraps in the field for 2 weeks and collected egg papers once a week (six egg papers total per site). We stored egg papers in a sealed plastic bag with a damp paper towel until they were ready to hatch. We then counted the number of eggs on the paper before placing it in a nutrient broth to facilitate hatching. We identified mosquitoes as fourth instar larvae and placed *A. albopictus* larvae in microcentrifuge tubes with 90% ethanol for DNA extraction.

DNA extraction, sequencing and processing

For a site to be considered for genomic sequencing, it required at least 10 preserved *A. albopictus* adults and 10 *A. albopictus* larvae, with a maximum of three larvae per ovitrap. This reduced potential bias from sampling siblings (Goldberg & Waits, 2010) and was consistent with previous population genetic studies using container-breeding *Aedes* larvae (Schmidt et al., 2018). Of the 20 locations where we sampled both eggs and adults, five sites met these criteria. We 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). We included all five sites in the final genomic libraries (Figure 1) and extracted DNA concentrations of 8 ng DNA/μl or greater for at least eight adults and eight larvae. We built genomic libraries of 48 individuals each using double-digest restriction enzyme associated DNA sequencing (*ddRADseq*) following Burford Reiskind et al. (2016). We used *MluCI* and *SphI* restriction enzymes to fragment extracted DNA. We sized selected fragments between 350 and 475 base pairs using the BluePippin™ gel cassette (BLF7510, Sage Science) at the North Carolina State University Genomic Sciences Laboratory (Raleigh, NC, USA) and amplified using PCR. The amplified libraries were sequenced using single-end reads of 100 base pairs on the Illumina HiSeq 4000 at the University of Oregon Genomics & Cell Characterization Core Facility (GC3F; Eugene, OR, USA).

We used STACKS version 1.09 (Catchen et al., 2011) to process samples post-sequencing. Using the *process_radtags* command, we demultiplexed individual barcodes, trimmed sequences to

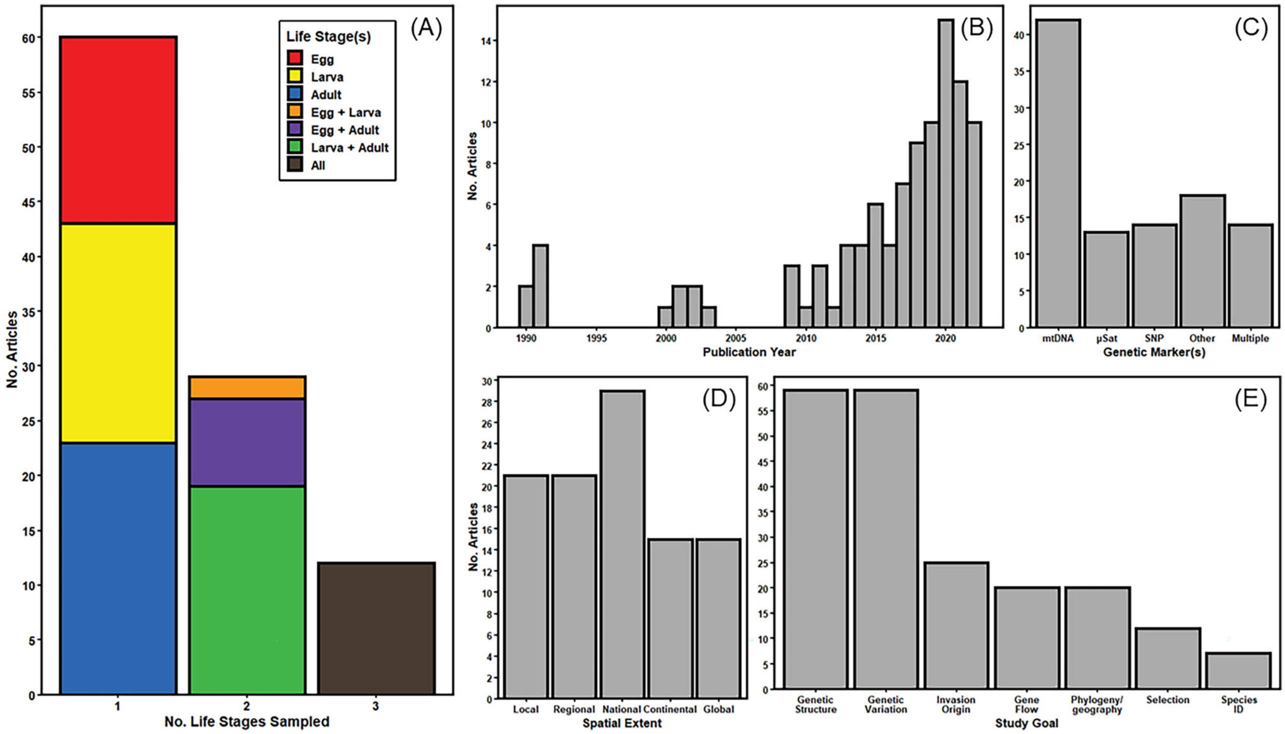

FIGURE 2 Results of literature search for population genetics studies on field-caught *Aedes albopictus*. We identified 101 studies that involved sampling wild populations of *A. albopictus* and conducted population genetics analyses. (A) Shows the number of articles that sampled different life stage(s) of *A. albopictus*. (B–E) show histograms of (B) publication year, (C) genetic markers used in the studies (mtDNA, mitochondrial DNA sequencing; μSat = microsatellite loci; SNP, single nucleotide polymorphisms), (D) spatial extent and (E) study goal.

90 base pairs, and filtered reads with a phred score of below 33. We generated a SNP catalogue using the *denovo* pipeline with a minimum read depth of size (−*m* flag), a maximum of three mismatches between loci within an individual (−*M* flag), and a maximum of two mismatches between loci within the catalogue (−*n* flag). To filter SNPS, we first ran the *populations* pipeline in STACKS with the adult and larval samples from sites where we collected *A. albopictus* eggs (five sites, 80 individuals). We included SNPs that were present in at least 75% of individuals (−*r* flag) from at least two sampling locations (−*p* flag). We treated the two life stages as separate groups for 10 separate 'populations' in the pipeline. We further filtered SNPs in PLINK v1.19 (Purcell et al., 2007) to remove SNPs with a minimum allele frequency of less than 0.01 and a genotyping rate of less than 75% and individuals with over 25% missing data. We used a more stringent genotyping rate to accommodate the sensitivity to missing data in connectivity analyses (Arnold et al., 2013; Gautier et al., 2013). Finally, we removed SNPs significantly out of Hardy–Weinberg Equilibrium after applying a sequential Bonferroni correction using the hw.test function in the R package *pegas* v0.14 (Paradis, 2010).

Genetic diversity and differentiation

For population genetic analyses, we made comparisons between sampling locations for (a) egg samples, (b) adult samples, and (c) combined adult and egg samples. We 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, 2021). We 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: 5000.

We also directly compared genetic variation between adult and egg sampled from the same location using discriminant analysis of principal components (DAPC) in the R package adegenet (Jombart, 2008; Jombart & 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, and then a discriminant analysis (DA) is used on the retained PCs to maximize differences between populations (Liu et al., 2019). For these comparisons, we retained five principal components. This was equivalent to roughly one-third of individuals sampled at a site, following the best practices of discriminant function analysis (Huberty, 1975; Williams & Titus, 1988).

Genetic structure

We used two approaches to evaluate the genetic structure between sampled individuals. For the first, we used *fastStructure* v.1.0 (Raj et al., 2014), a Bayesian *k* clustering algorithm adapted from STRUCTURE (Pritchard et al., 2000) for large SNP datasets. We used a logistic prior for values of *k* ranging from 1 to 6 with 10 iterations per value of *k*. We used the web-based software *StructureSelect* (Li & Liu, 2018) to evaluate the optimal *k* value using maximum marginal likelihood. We also evaluated genetic structure using principal components analysis (PCA) implemented with the *prcomp* function in RStudio. We found the percent variance explained by each principal component and plotted the first two PCs for each of the three groups (adults only, eggs only and combined) to visualize potential genetic clusters.

Genetic connectivity

We investigated site-level patterns of genetic structure and connectivity for adults, eggs and combined. We first ran DAPC using the maximum number of principal components per group (the number of individuals −1) and generated minimum-spanning trees to compare patterns of connectivity between the three groups. We also looked at connectivity between sites by generating population graphs, which we created using the R package *popgraph* (Dyer & Nason, 2004). Population graphs use a multivariate network model to define relationships among groups of populations simultaneously. An algorithm identifies the minimum number of connections between populations while still retaining enough information to accurately describe patterns of among-population genetic variation. We compared the population graphs to the minimum spanning trees generated in DAPC to look at the level of congruence between the methods for each group of individuals.

RESULTS

Literature review

We identified 604 unique peer-reviewed papers during our literature review. We excluded 447 studies during the title and abstract screening and an additional 28 when reviewing full texts, leaving 101 studies that fulfilled all criteria for inclusion (Data S1). Of these papers, 59% ($n = 60$) sampled one life stage, 29% ($n = 29$) sampled two life stages, and 12 sampled all three life stages (Figure 2A). For the studies that used one life stage, we found roughly equal representation of sampling choice (eggs: $n = 17$; larvae: $n = 20$; adults: $n = 23$). None of the studies that used more than one life stage separated genetic analyses by stage.

Publication years ranged from 1990 to 2021, though 85% have been published since 2010 (Figure 2B). The most common genetic marker used to address the study objectives was mitochondrial DNA sequencing (e.g., COI), followed by SNPS and nuclear microsatellite loci (Figure 2C). In addition, 14 studies used multiple marker types. The spatial extent of the studies ranged from local (e.g., within a city or county) to global. Studies at the country level were the most common, but we found a relatively even distribution across scales

TABLE 1 Metrics of genetic diversity, observed heterozygosity (H_O), expected heterozygosity (H_E) and inbreeding coefficient ($F_{IS} = 1 - H_O/H_E$) for different life stages of *A. albopictus* at five locations in Wake County, North Carolina.

Site	H_O	H_E	F_{IS}
Adults			
S-09	0.1072	0.1249	0.1415
S-17	0.1083	0.1243	0.1286
S-31	0.1167	0.1287	0.0934
S-42	0.1092	0.1258	0.1316
S-58	0.1112	0.1261	0.1180
Eggs			
S-09	0.1131	0.1252	0.0968
S-17	0.1064	0.1221	0.1285
S-31	0.1063	0.1212	0.1224
S-42	0.1092	0.1224	0.1081
S-58	0.1207	0.1289	0.1218
Combined			
S-09	0.1088	0.125	0.1296
S-17	0.1073	0.1241	0.1357
S-31	0.1105	0.1247	0.1139
S-42	0.1093	0.1245	0.1218
S-58	0.1146	0.1274	0.1009

Note: The life stages measured were adults, eggs and adults + eggs combined.

TABLE 2 Pairwise Wright's F_{ST} for *A. albopictus* individuals at five sites in Wake County, North Carolina for different life stages: Adults only, eggs only and adults and eggs combined.

	S-09	S-17	S-31	S-42
Adults				
S-17	0.0000			
S-31	0.0000	0.0108		
S-42	0.0000	0.0043	0.0000	
S-58	0.0000	0.0024	0.0000	0.0000
Eggs				
S-17	0.0015			
S-31	0.0000	0.0000		
S-42	0.0000	0.0000	0.0000	
S-58	0.0030	0.0000	0.0000	0.0000
Combined				
S-17	0.0014			
S-31	0.0000	0.0039		
S-42	0.0000	0.0022	0.0000	
S-58	0.0000	0.0002	0.0000	0.0000

(Figure 2D). Most studies (65%) had multiple goals. The majority of papers included characterization of the genetic structure and/or genetic variation of *A. albopictus* populations in their objectives

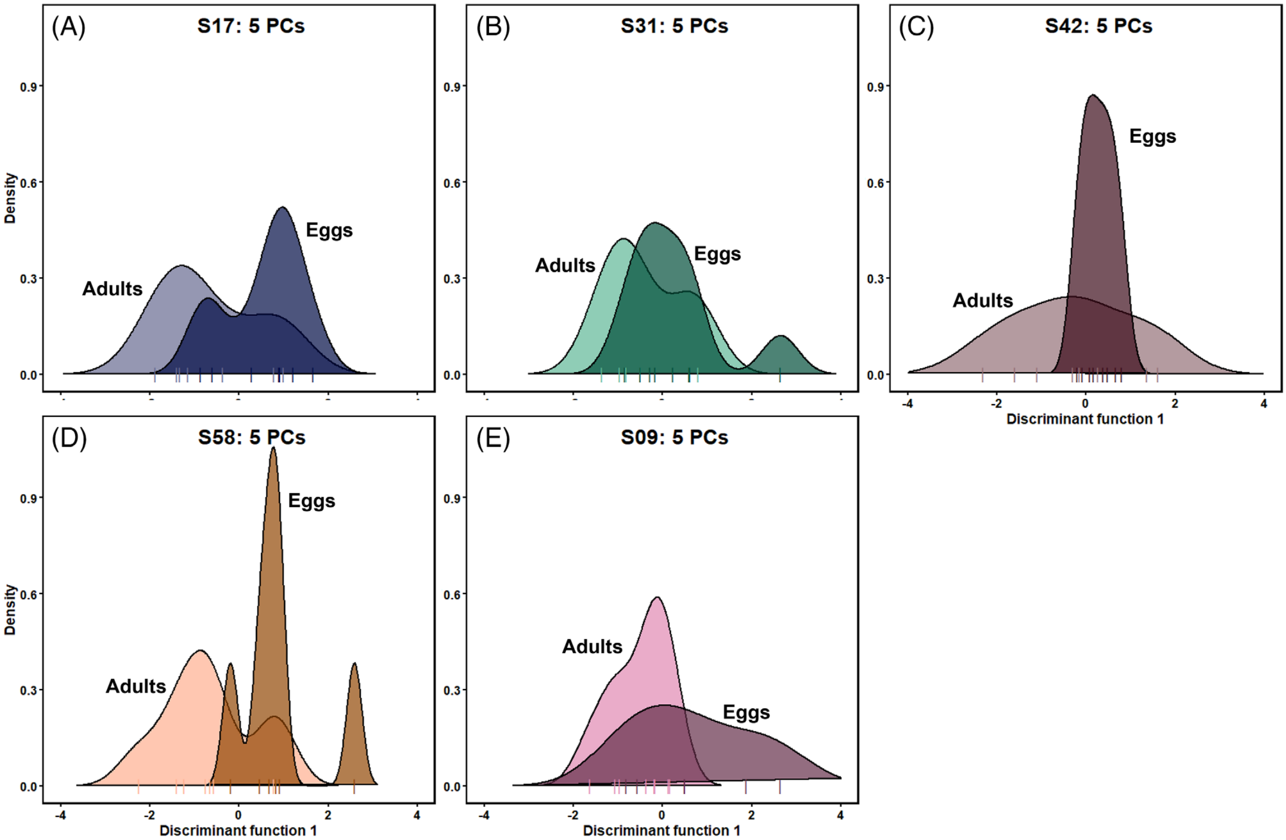

FIGURE 3 Discriminant analysis of principal components (DAPC) of the genetic variation between eggs and adult *Aedes albopictus* within sampling locations (adults: Lighter colour, eggs: Darker colour). Density plots are shown within the plot space, and location of individuals along discriminant function 1 are represented as tic marks along the x-axis. The number of individuals per sampling site ranged from 14 to 16, and we used 5 principal components for each site to facilitate direct comparison without overfitting the data.

(n = 59 for each; Figure 2E). Other common objectives were to identify the geographic origins of *A. albopictus* introductions outside its native range (n = 25), to investigate patterns of gene flow and connectivity (n = 20), and to analyse phylogenetic or phylogeographic patterns (n = 20).

We found a statistically significant pattern of association between decade of study and life stage (χ^2 = 50.19, simulated p = 0.0006). The six studies published in the 1990s only used *A. albopictus* eggs, while adult sampling was more common after 2010. Similarly, eggs were more likely to be used in global studies. No other variables were associated with the life stage.

Genomic sequencing

The STACKS *Populations* pipeline retained 77,996 SNPs. PLINK filtering removed 4228 SNPs and 63,894 SNPs for not meeting the minimum allele frequency and genotyping rate thresholds, respectively. Seven individuals were removed for missing data. Finally, we found 1449 SNPs violated HWE, leaving 8425 SNPs and 73 individuals for further analysis.

Genetic diversity and differentiation

We found that heterozygosity and F_{IS} were similar between sites regardless of life stage (ΔH_O = 0.0144; ΔH_E = 0.0077; ΔF_{IS} = 0.0481). Mean observed heterozygosity among sites was highest for egg populations, while adults had the highest expected heterozygosity and F_{IS} on average (Table 1). This indicates that adult *A. albopictus* had higher rates of homozygosity than eggs compared to than expected with HWE. The sites with the highest and lowest genetic diversity varied depending on the life stage we sampled (adults, eggs, or combined). For example, the site with the lowest inbreeding coefficient was S-31 for adults only, S-09 for eggs only and S-58 when we combined all individuals (Table 1).

We did not find evidence of genetic differentiation between sites for all life stages, with pairwise F_{ST} values ranging from 0.000 to 0.0108 (Table 2). However, the sites that were most differentiated varied depending on life stage. For example, the adult sample at S-17 had greater genetic differentiation among other adult sites, while eggs at S-09 showed greater differentiation among other egg sites. However, there was no evidence of significant genetic differentiation between populations for any life stage or for combined life stages (exact G test, p > 0.05 for all pairwise comparisons; Table 2).

We did not find significant genetic differentiation between adults and eggs sampled from the same location. DAPC correctly assigned 68.755% to 85.71% of individuals to their corresponding life stage, depending on the site. We observed overlap in adult and egg density curves along the discriminant axis (Figure 3). If individuals from different life stages were more genetically differentiated than those in the same age class, we would expect to see higher assignment rates and isolated clusters of density. While this was not the case, we did find differences in genetic variance between life stages, seen as contrasting widths of the density curves between life stages along the discriminant axis. The life stage with greater variance differed among sites. We found that genetic variance was higher in adults than in eggs at

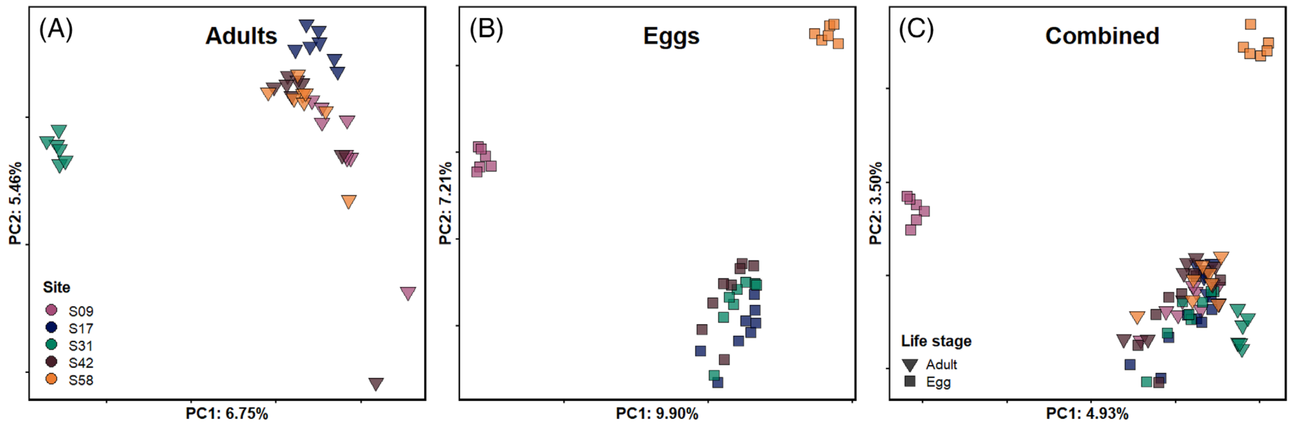

FIGURE 4 Principal component analysis plots for *Aedes albopictus* (A) adults, (B) eggs and (C) adults and eggs combined at five sampling sites. Adults are represented by upside-down triangles and eggs by squares. Shape colours show the different sampling locations.

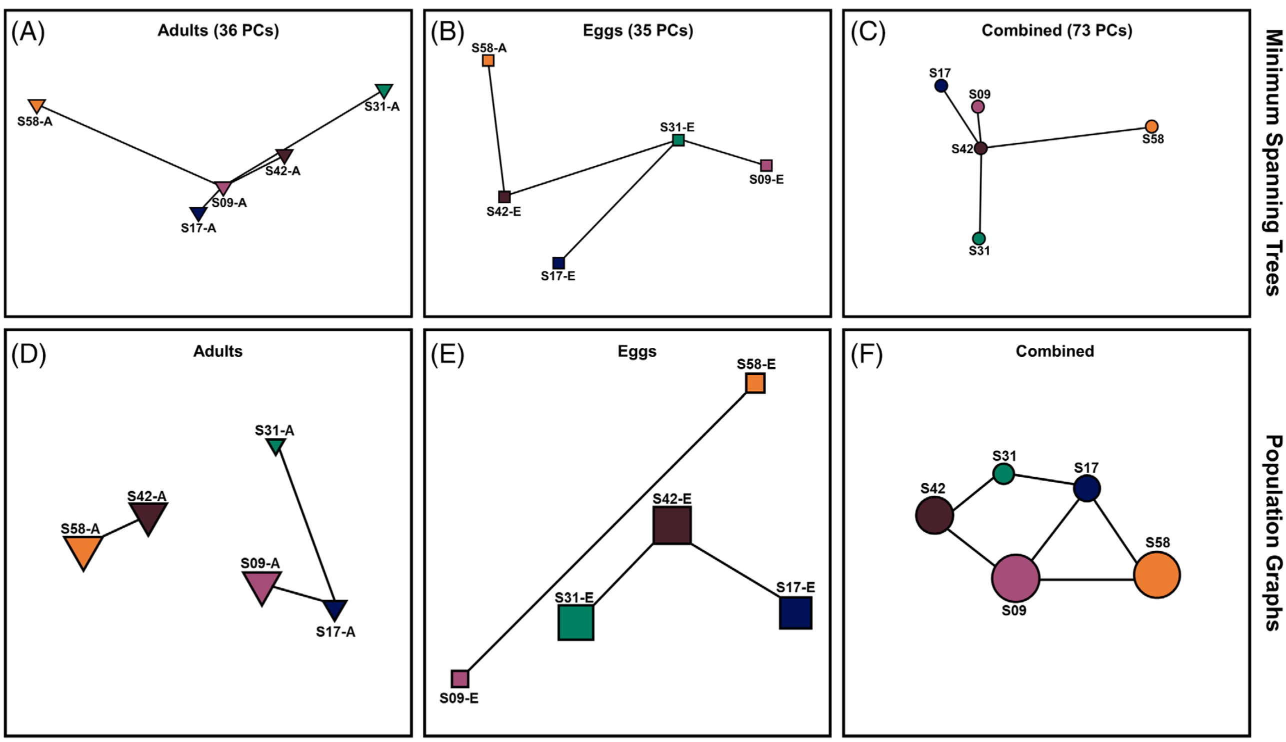

FIGURE 5 Minimum spanning trees (MST; a–c) and population graphs (D–F) of *Aedes albopictus* across the five sites, where each point represents one site. We generated minimum spanning trees using discriminant analysis of principal components and population graphs from the *gstudio* R package. The location of points in (A–C) are the centroid of the individuals at a sampling location along the first two principal components. To understand how the individuals sampled were related to one another, we used the maximum number of PCs possible for each scatterplot (max PCs = #individuals −1). In the population graphs, size of shapes shows the relative amount of total genetic variation captured within a sampling location. Points are arranged based on edge length, where longer edges indicate more genetic variation between the two connected sites. (A) and (D) used adult mosquitoes, represented by triangles; (B) and (E) used mosquito eggs, represented by squares; and (C) and (F) used all individuals combined, represented by circles.

S17, S42 and S58 (ratio of variances for adults: eggs = 1.6377, 15.1277 and 1.2886, respectively; Figure 3A,C,D), while the opposite was true at the remaining sites (ratio of variances for adults: eggs = 0.6518 at S31 and 0.2254 at S09; Figure 3B,E).

Genetic structure

fastSTRUCTURE identified an optimal *K* of 1 for all groups (eggs, adults and combined) with maximum marginal likelihood, indicating one panmictic population. We also investigated results from non-optimal *K* values, which did not reveal any potential sub-structuring of populations and supported panmixia. However, when we explored patterns using PCA, we found several groups of individuals that clustered separately along the first two principal components (Figure 4). Among adults, individuals from S17 and S31 formed unique groups (Figure 4A). Among eggs, S09 and S58 were distinct (Figure 4B), and this remained the dominant pattern when all individuals were combined (Figure 4C).

Genetic connectivity

We found that patterns of genetic connectivity were inconsistent between life stages and between the minimum spanning trees and population graphs (Figure 5). We evaluated connectivity using minimum spanning trees (Figure 5A–C) and using population graphs (Figure 5D–F). Minimum spanning trees were necessarily composed of five nodes representing the sampled sites and four connecting edges. In contrast, population graphs allowed the number of edges to vary and did not necessitate all nodes to be connected. Using the minimum spanning method, adult and combination trees showed all four allowed edges connected to a single site, which acted as a hub connecting the remaining sites (Figure 5A,C). However, the identity of the central site differed between adult and combined trees (S09 and S42, respectively). The minimum spanning tree based on eggs was more complex and had two sites with multiple connections (Figure 5B). No edges were shared between all three trees. We found S09-S3 was shared between adult and egg trees, S09-S42 was shared between adult and combined trees, and S31-S42 and S42-S58 were shared between egg and combined trees. Population graphs for adult, egg and combined individuals ranged from four to six edges (Figure 5D–F). Two node pairs, S09-S17 and S31-S42, shared edges in all three population graphs. The latter was also present in two of the three minimum spanning trees, missing only in the adult tree.

DISCUSSION

Overall, we found evidence that life stage did affect population genetic results for *A. albopictus* in our study. Comparisons of site-level genetic diversity and between-site genetic connectivity changed depending on the life stage sampled, which led to inconsistent results between mosquitoes trapped as eggs versus adults. For example, site S-58 had the highest expected heterozygosity for eggs, versus site S-31 for adults (Table 2). We also found that S-58 eggs and S-31 adults formed distinct clusters in the principal component analysis (Figure 4). Though we only had five sample sites, these results demonstrate a need for further investigation.

One way to assess the observed differences between egg and adult population genetic patterns is to consider the expected results in a demographically stable population not under selection, with consistent birth rates, death rates and population size over time. In this scenario, we would expect to see similar levels of genetic diversity and genetic variance between adult and egg life stages. The genetic diversity within an individual mosquito would reflect the overall diversity of the population, so even though only a subset of adults contribute to the gene pool at a given point in time, rates of heterozygosity would remain consistent between generations. This was true for some, but not all, of the sites we sampled. One reason for this pattern may be due to the fitness of mosquitoes at different life stages. In a scenario where different selective pressures are acting on immature and adult mosquitoes, we would expect mortality-induced bottlenecks between life stages to impact genetic diversity between eggs and adults. In this case, we would posit that expected heterozygosity in adults would be lower than that of eggs if selection favours homozygous alleles and vice versa if selection favours heterozygotes. Because we hatched collected eggs in a laboratory environment, we changed potential selective pressures that may influence which eggs hatch into larvae, and we removed the bottleneck effect for mortality before emergence by extracting DNA from larvae.

In addition, we may expect to see differences in genetic structure between adults and eggs depending on the primary mode of dispersal and the spatial scale. Natural dispersal occurs during adulthood at fine scales; most *A. albopictus* adults disperse within 200 m of their larval habitat (Honório et al., 2003). Passive dispersal mediated by human transportation networks tends to occur at the egg stage and is more commonly observed at regional or larger scales (Lounibos, 2002; Medley et al., 2015). If the major mode of dispersal is at the adult stage and migration rates are relatively low, we would expect to see higher levels of genetic connectivity among adults, while eggs may appear more differentiated if migrants are not immediately contributing to the gene pool. Our results are consistent with this prediction, indicating that adult dispersal may be more important than human-mediated spread of eggs at local scales. However, we would also expect between-site patterns of genetic structure to be consistent between life stages. For example, the most genetically distinct adult population would also have the most genetically distinct eggs, which we did not observe in this study. This suggests that mosquito populations have high rates of turnover and temporal variability within one location. This makes it harder to predict patterns of connectivity using a single time point.

In this study, we found eggs had lower expected heterozygosity and F_{IS} on average than adults. While these are both measures of genetic diversity, they can be inversely related. Higher expected heterozygosity can indicate that a population has many loci with more than one allele and that the frequencies of these alleles are similar across the population. In comparison, F_{IS} measures the difference between the number of individuals expected to be heterozygous at a locus under HW expectations compared to the observed number of heterozygotes. *A. albopictus* eggs have lower heterozygosity and F_{IS} suggests there were fewer biallelic loci and/or alleles at lower frequencies than in adults. In contrast, 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 were 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 1).

We were surprised to find inconsistent patterns of genetic variance between adults and eggs across locations using DAPC. We expected to see higher variance among adults, as eggs represent only a fraction of the adult gene pool, but this was the case at only three of five sites. One of these sites, S42, had much higher variance among adults, with an approximate 15:1 ratio to eggs (Figure 3C). At the remaining two sites, one to two individuals contributed to elevated variance among eggs (Figure 3B,E). This could indicate that these individuals were unrelated to the adult population. One explanation is that we failed to sample enough individuals at each life stage to fully capture the range of genetic variation in the population. While next generation sequencing can capture the genetic patterns of a population with fewer individuals, this advantage is somewhat tempered by reoccurring introductions of *A. albopictus* from different source populations. Therefore, the genetic variation of adults at a location may be similar to that of eggs but was not reflected in the individuals we sequenced. Alternatively, the adult population could be highly ephemeral, possibly due to high mortality or emigration rates. The two sites with higher variance among eggs were the most rural sites, with the lowest percent of impervious surface and the highest percent of forested land within a 1 km radius (Figure 1). In contrast, the site where we observed 15 times more genetic variance among adults was highly suburban, characterized by low density, large single family residences and manicured green spaces. These trends warrant further investigation using more sampling sites and highlight the value of examining population genetic characteristics of different life stages for *A. albopictus*.

Populations were not genetically differentiated between sites regardless of life stage, demonstrated by the lack of significant patterns in both pairwise F_{ST} estimates and *fastSTRUCTURE*. However, in the PCA analysis, we found evidence that eggs were more differentiated than adults, which matched our expectations. Our connectivity analyses were inconsistent between life stages and between minimum spanning trees versus population graphs. However, two node pairs, S09-S17 and S31-S42, were present in the majority of the six connectivity analyses (Figure 5). This suggests that these two sites are more genetically connected than other site pairs, despite being geographically closer to other locations (Figure 1). These results highlight the importance of defining study goals and tailoring sampling and assessment methods to those objectives a priori, as patterns of genetic connectivity may differ depending on life stage at this spatial scale. This is particularly crucial for applied research related to mosquito management. Control methods often target different life stages, which should be informed by research for that stage. For example, a manager may decide to release sterile males at locations with high genetic connectivity. However, those locations may differ with life stage.

Collectively, our results show that choice in life stage to sample can lead to different conclusions about the population genetic patterns of *A. albopictus*. While we found little genetic structure across sampling sites, this is not unusual for *A. albopictus* (Goubert et al., 2016; Maia et al., 2009; Schmidt et al., 2018). None of the studies we identified during our literature search conducted separate analyses based on life stage, and most studies sampled only one life stage. The degree to which results are affected by life stage will depend largely on the specific objectives and spatial or temporal scale of the study in question. For example, there is less likely to be a life-stage effect for research on *A. albopictus* phylogenetics or invasion origin at continental to global scales. However, there may be more pronounced effects for studies seeking to measure genetic diversity and gene flow at local to regional scales, especially at a single time point or when the population is only weakly differentiated.

While the scope of this paper is limited to a small geographic area and a few sampling sites, this is the spatial scale relevant to management questions. The differences we found in population genetic patterns between *A. albopictus* life stages warrant caution when interpreting studies that sample immature *A. albopictus* to infer adult genetic structure. To further illuminate how population genetic characteristics may reflect different ecological and evolutionary processes on egg, immature and adult mosquitoes, we may consider re-analysing data from previously published research that sampled both adult and immature *A. albopictus* (for example, Bibi et al., 2015; Sherpa et al., 2019). Future research could further investigate how conclusions from population genetic studies are influenced by sampling techniques. Because of this potential impact, publications on *A. albopictus* population genetics should provide detailed and precise descriptions of their sampling methods, explicitly discuss how they minimized sampling bias, and justify their decision to collect one or more life stages in the context of their research goals (e.g., Medley et al., 2015; Schmidt et al., 2018).

We recommend that research intended to inform mosquito control consider the context and nuances of the specific study location as it relates to the natural history of *A. albopictus*. In particular, complex management programs, including releases of *Wolbachia*-infected or genetically modified mosquitoes, should not be developed solely on results from mosquitoes sampled at a single life stage or time point. If levels of genetic diversity, differentiation and connectivity constantly 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* (Bibi et al., 2015; Hawley, 1988; Medley et al., 2015). For example, we observed that the percent impervious surface seemed related to within-site genetic variance between life stages, though this was not an explicit hypothesis a priori. Consequently, place-based approaches and knowledge are essential to develop adaptive and effective management programs. Further, investigating population genetic differences between life stages offers new opportunities in the fields of vector ecology and evolution. For example, this approach can be used to gain more detailed information on the sources of new *A. albopictus* invasions. Such research can also elucidate divergent selective pressures on different life stages and provide insight into local adaptation of *A. albopictus* in novel environments, including under different management regimes.

AUTHOR CONTRIBUTIONS

Emily M. X. Reed: Conceptualization, Methodology, Data Collection, Formal analysis, and Writing-original draft. Michael H. Reiskind: Conceptualization, Methodology, Writing-reviewing and editing. Martha O. Burford Reiskind: Conceptualization, Methodology, Formal analysis, Writing-reviewing and editing.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The genomic datasets that support the study are available on DRYAD (https://doi.org/10.5061/dryad.q2bvq83n5).

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REFERENCES

Arnold, B., Corbett-Detig, R. B., Hartl, D. & Bomblies, K. (2013) RADseq underestimates diversity and introduces genealogical biases due to nonrandom haplotype sampling. *Mol Ecol*, 22, 3179–3190.

Benedict, M.Q., Levine, R.S., Hawley, W.A. & Lounibos, L.P. (2007) Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. *Vector-Borne and Zoonotic Diseases*, 7, 76–85.

Bentley, M.D. & Day, J.F. (1989) Chemical ecology and behavioral aspects of mosquito oviposition. *Annual Review of Entomology*, 34, 401–421.

Bibi, M., Zahoor, M.K., Zahoor, M.A., Ashraf, H.M., Naz Majeed, H., Nasir, S. et al. (2015) Genetic analysis of mosquitoes from rural and urban areas of Sialkot, Pakistan. *International Journal of Agriculture and Biology*, 17, 809–814.

Bonizzoni, M., Gasperi, G., Chen, X. & James, A.A. (2013) The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. *Trends in Parasitology*, 29, 460–468.

Burford Reiskind, M.O., Coyle, K., Daniels, H.V., Labadie, P., Reiskind, M. H., Roberts, N.B. et al. (2016) Development of a universal double-digest RAD sequencing approach for a group of nonmodel, ecologically and economically important insect and fish taxa. *Molecular Ecology Resources*, 16, 1303–1314.

Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W. & Postlethwait, J.H. (2011) Stacks: building and genotyping loci de novo from short-read sequences. *G3: Genes, Genomes, Genetics*, 1, 171–182.

Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia. 2022. Available at www.covidence.org.

Dyer, R.J. & Nason, J.D. (2004) Population graphs: the graph theoretic shape of genetic structure. *Molecular Ecology*, 13, 1713–1727.

Dyer, R. J. (2021). gstudio: Tools Related to the Spatial Analysis of Genetic Marker Data. R package version 1.5.3.

Eritja, R., Palmer, J.R.B., Roiz, D., Sanpera-Calbet, I. & Bartumeus, F. (2017) Direct evidence of adult *Aedes albopictus* dispersal by car. *Scientific Reports*, 7, 14399.

Flacio, E., Engeler, L., Tonolla, M., Luthy, P. & Patocchi, N. (2015) Strategies of a thirteen year surveillance programme on *Aedes albopictus* (*Stegomyia albopicta*) in southern Switzerland. *Parasites and Vectors*, 8, 208.

Gautier, M., Gharbi, K., Cezard, T., Foucaud, J., Kerdelhue, C., Pudlo, P., Cornuet, J. M. & Estoup, A. (2013) The effect of RAD allele dropout on the estimation of genetic variation within and between populations. *Mol Ecol*, 22, 3165–3178.

Goldberg, C.S. & Waits, L.P. (2010) Quantification and reduction of bias from sampling larvae to infer population and landscape genetic structure. *Molecular Ecology Resources*, 10, 304–313.

Goubert, C., Minard, G., Vieira, C. & Boulesteix, M. (2016) Population genetics of the Asian tiger mosquito *Aedes albopictus*, an invasive vector of human diseases. *Heredity*, 117, 125–134.

Gratz, N.G. (2004) Critical review of the vector status of *Aedes albopictus*. *Medical and Veterinary Entomology*, 18, 215–227.

Harris, A.F., McKemey, A.R., Nimmo, D., Curtis, Z., Black, I., Morgan, S.A. et al. (2012) Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nature Biotechnology*, 30, 828–830.

Hawley, W.A. (1988) The biology of *Aedes albopictus*. *Journal of the American Mosquito Control Association. Supplement*, 1, 1–39.

Hawley, W.A., Reiter, P., Copeland, R.S., Pumpuni, C.B. & Craig, G.B.J. (1987) Aedes albopictus in North America: probable introduction in used tires from northern Asia. *Science*, 236, 1114–1116.

Hoban, S., Gaggiotti, O., Bertorelle, G. & O'Hara, R.B. (2013) Sample planning optimization tool for conservation and population genetics (SPOTG): a software for choosing the appropriate number of markers and samples. *Methods in Ecology and Evolution*, 4, 299–303.

Hollingsworth, B., Hawkins, P., Lloyd, A.L. & Reiskind, M.H. (2020) Efficacy and spatial extent of yard-scale control of *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae) using barrier sprays and larval habitat management. *Journal of Medical Entomology*, 57, 1104–1110.

Honório, N.A., Silva, W.D.C., Leite, P.J., Gonçalves, J.M., Lounibos, L.P. & Lourenço-de-Oliveira, R. (2003) Dispersal of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in an urban endemic dengue area in the State of Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz*, 98, 191–198.

Hope, A.C.A. (1968) A simplified Monte Carlo significance test procedure. *Journal of the Royal Statistical Society Series B (Methodological)*, 30, 582–598.

Huberty, C.J. (1975) Discriminant analysis. *Review of Educational Research*, 45, 543–598.

Ibáñez-Justicia, A. (2020) Pathways for introduction and dispersal of invasive Aedes mosquito species in Europe: a review. *Journal of the European Mosquito Control Association*, 38, 1–10.

Jombart, T. (2008) Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403–1405.

Jombart, T. & Ahmed, I. (2011) Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics*, 27, 3070–3071.

Li, Y.L. & Liu, J.X. (2018) StructureSelector: a web-based software to select and visualize the optimal number of clusters using multiple methods. *Molecular Ecology Resources*, 18, 176–177.

Liu, Y., Chen, L., Duan, X.Z., Zhao, D.S., Sun, J.T. & Hong, X.Y. (2019) Genome-wide single nucleotide polymorphisms are robust in resolving fine-scale population genetic structure of the small Brown Planthopper, *Laodelphax striatellus* (Fallén) (Hemiptera: Delphacidae). *Journal of Economic Entomology*, 112, 2362–2368.

Lotterhos, K.E. & Whitlock, M.C. (2014) Evaluation of demographic history and neutral parameterization on the performance of F_{ST} outlier tests. *Molecular Ecology*, 23, 2178–2192.

Lounibos, L.P. (2002) Invasions by insect vectors of human disease. *Annual Review of Entomology*, 47, 233–266.

Maia, R. T., Scarpassa, V. M., Maciel-Litaiff, L. H. & Tadei, W. P. (2009) Reduced levels of genetic variation in Aedes albopictus (Diptera: Culicidae) from Manaus, Amazonas State, Brazil, based on analysis of the mitochondrial DNA ND5 gene. *Genetics and Molecular Research*, 8, 998–1007.

Mains, J.W., Brelsfoard, C.L., Rose, R.I. & Dobson, S.L. (2016) Female adult *Aedes albopictus* suppression by Wolbachia-infected male mosquitoes. *Science Reports*, 6, 33846.

Medley, K.A., Jenkins, D.G. & Hoffman, E.A. (2015) Human-aided and natural dispersal drive gene flow across the range of an invasive mosquito. *Molecular Ecology*, 24, 284–295.

Meirmans, P.G. (2015) Seven common mistakes in population genetics and how to avoid them. *Molecular Ecology*, 24, 3223–3231.

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D. et al. (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*, 372, n71.

Paradis, E. (2010) Pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics*, 26, 419–420.

Paupy, C., Delatte, H., Bagny, L., Corbel, V. & Fontenille, D. (2009) Aedes albopictus, an arbovirus vector: from the darkness to the light. *Microbes and Infection*, 11, 1177–1185.

Pereira-Dos-Santos, T., Roiz, D., Lourenco-de-Oliveira, R. & Paupy, C. (2020) A systematic review: is *Aedes albopictus* an efficient bridge vector for zoonotic arboviruses? *Pathogens*, 9, 266.

Peterman, W., Brocato, E.R., Semlitsch, R.D. & Eggert, L.S. (2016) Reducing bias in population and landscape genetic inferences: the effects of sampling related individuals and multiple life stages. *PeerJ*, 4, e1813.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D. et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, 81, 559–575.

Raj, A., Stephens, M. & Pritchard, J.K. (2014) fastSTRUCTURE: variational inference of population structure in large SNP data sets. *Genetics*, 197, 573–589.

Reed, E.M.X., Byrd, B.D., Richards, S.L., Eckardt, M., Williams, C. & Reiskind, M.H. (2019) A statewide survey of container *Aedes* mosquitoes (Diptera: Culicidae) in North Carolina, 2016: a multiagency surveillance response to Zika using Ovitraps. *Journal of Medical Entomology*, 56, 483–490.

Roiz, D., Wilson, A.L., Scott, T.W., Fonseca, D.M., Jourdain, F., Muller, P. et al. (2018) Integrated *Aedes* management for the control of *Aedes*-borne diseases. *PLoS Neglected Tropical Diseases*, 12, e0006845.

Rousset, F. (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, 8, 103–106.

RStudio Team (2021) RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA.. http://www.rstudio.com/

Schmidt, T.L., Filipovic, I., Hoffmann, A.A. & Rasic, G. (2018) Fine-scale landscape genomics helps explain the slow spatial spread of Wolbachia through the *Aedes aegypti* population in Cairns, Australia. *Heredity*, 120, 386–395.

Sherpa, S., Blum, M.G.B., Capblancq, T., Cumer, T., Rioux, D. & Despres, L. (2019) Unravelling the invasion history of the Asian tiger mosquito in Europe. *Molecular Ecology*, 28, 2360–2377.

Takken, W. & Scott, T.W. (2003) *Ecological aspects for application of genetically modified mosquitoes*. Dordrecht, Boston: Kluwer Academic Publishers.

Williams, B.K. & Titus, K. (1988) Assessment of sampling stability in ecological applications of discriminant analysis. *Ecology*, 69, 1275–1285.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1 Supporting information.

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