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SCIENTIFIC NOTE

ORIGIN OF *AEDES AEGYPTI* IN CLARK COUNTY, NEVADA

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ABSTRACT. *Aedes aegypti* is the primary vector for serious diseases, including those caused by chikungunya, dengue, and Zika viruses. In 2017, the Southern Nevada Health District first detected this invasive species in Clark County, NV, including in the city of Las Vegas. We analyzed *Ae. aegypti* from the city of North Las Vegas to determine the likely source of the invasion. We genotyped a sample of *Ae. aegypti* at 12 highly variable microsatellites and analyzed the data in reference to published data from 25 sites in the southern USA. We found that the *Ae. aegypti* in Las Vegas most likely invaded from southern California. Knowing the source of new invasions may provide information about the invading population (e.g., previous insecticide exposure) and can help prevent future invasions from the region.

KEY WORDS *Aedes aegypti*, invasion, Las Vegas, Nevada, population genetics

The introduction of an invasive species is often cause for concern, but few introduced species are as unwelcome as *Aedes* (*Stegomyia*) *aegypti* (L.). Originally named the yellow fever mosquito, today *Ae. aegypti* is most dangerous as the primary vector for dengue, chikungunya, and Zika viruses. Of these arboviral diseases, only dengue has an approved vaccine, and its safety and effectiveness are still in question for seronegative individuals (Sridhar et al. 2018). Therefore, vector control and prevention education are the primary forms of protection in areas where the diseases occur.

Aedes aegypti is mostly found in tropical and subtropical urban areas. Its distribution has been extending into temperate regions, and in the USA it has a patchy distribution below the 33°N line (Hahn et al. 2016). New detections are still made regularly; the species was first detected in northern California in 2013 and in southern California in 2014 (Gloria-Soria et al. 2014, Metzger et al. 2017, Pless et al. 2017). Since 2014, there have been dozens of 1st detections of *Ae. aegypti* in California counties and cities, including the 1st detection of this species in Merced County in 2017 (California Department of Public Health 2018, Merced County Mosquito Abatement District 2018). Before 2017, however, there were no known established populations of *Ae. aegypti* in Nevada.

Southern Nevada Health District (SNHD) is the local governmental public health authority for southern Nevada, serving a population of >2 million residents. Southern Nevada Health District has maintained a mosquito surveillance program since 2005 and has been preparing and surveying for *Aedes* mosquitoes in Clark County since 2014. Clark County is the southernmost county in Nevada and is composed of 5 municipalities and 1 county government, the city of Las Vegas, city of North Las Vegas, city of Henderson, city of Mesquite, Boulder City, and unincorporated Clark County. In May of 2017, SNHD detected *Ae. aegypti* for the 1st time in the city of North Las Vegas. Ongoing surveillance over the course of the summer detected additional *Ae. aegypti* in this municipality as well as in the city of Las Vegas.

Aedes aegypti has a short lifetime natural dispersal—often <200 m—so new introductions of the species are generally a result of human-mediated dispersal (Russel et al. 2005, Reiter 2007). *Aedes aegypti* populations are genetically differentiated across the species' distribution, allowing us to track new introductions to their original source. We have successfully used these genetic signatures to determine the origins of introductions into the Netherlands (Brown et al. 2011b), California (Gloria-Soria et al. 2014, Pless et al. 2017), and Washington, DC (Gloria-Soria et al. 2018). One reason these results are important is that the species' vector competence and insecticide resistance can vary across regions (Hemingway and Ranson 2000, Bennet et al. 2002, Vontas et al. 2012). In addition, knowing the origin of a new introduction provides clues into how the introduction occurred and thus how to prevent further introductions. Our objective in this study was to determine the origin of the new invasion into Clark County, NV.

Samples were collected in the city of North Las Vegas and sent to Yale University for processing. The DNA was extracted from 31 samples using the Qiagen DNeasy Blood and Tissue kit (QIAGEN, Hilden, Germany). All samples were genotyped at 12 highly variable microsatellites, as in Brown et al. (2011a). Data from 25 other North American sites included in analyses are published elsewhere (Gloria-

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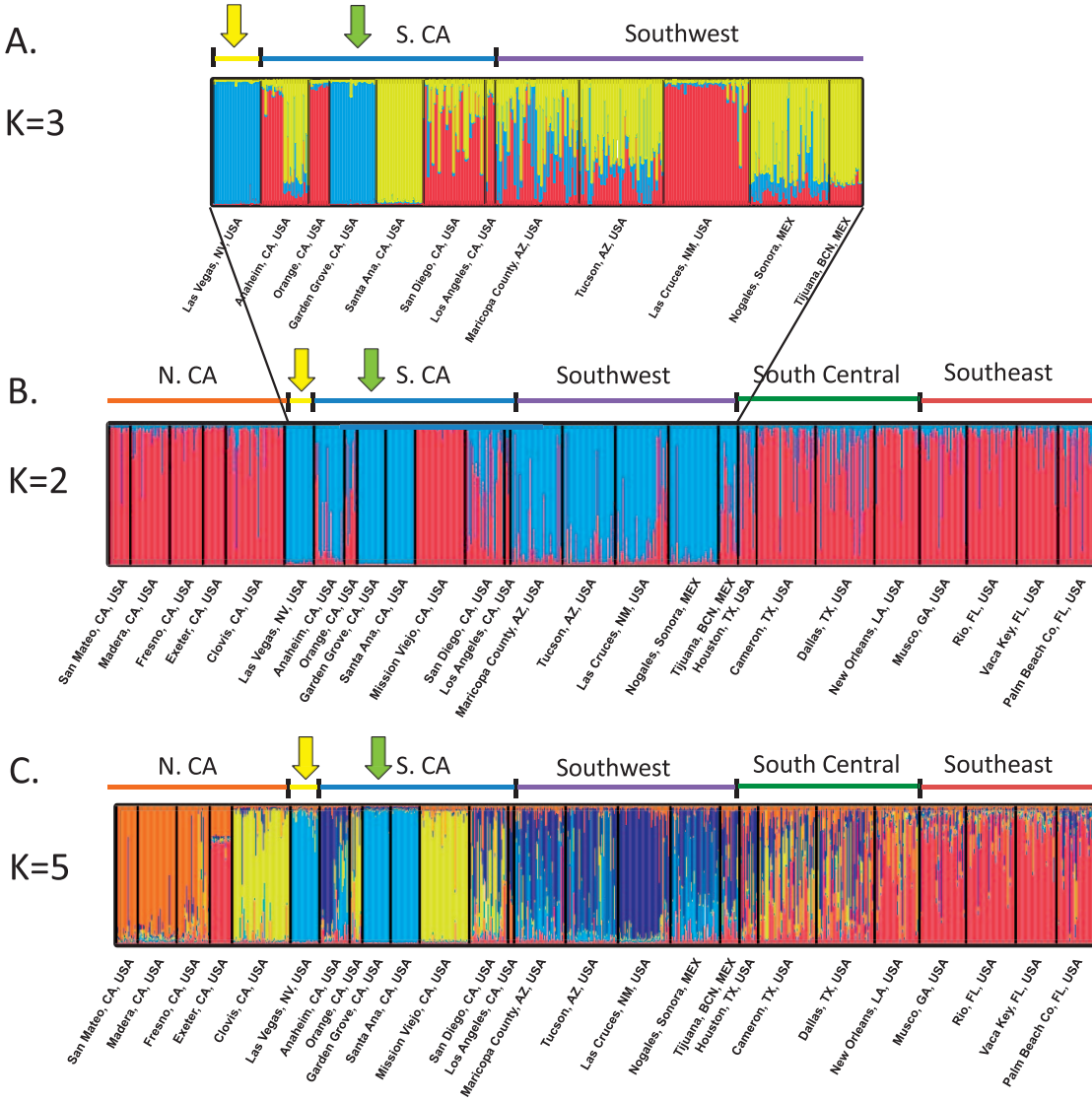

Fig. 1. Genetic structure of North American populations. Each vertical bar represents an individual, and the proportion of each color assigned to an individual represents the proportion of the individual's ancestry attributable to each of K theoretical genetic clusters. The yellow arrows indicate Las Vegas, and the green arrows indicate Garden Grove. (A) Southern California and Southwest USA (K = 3), (B) North America (K = 2), and (C) North America (K = 5).

Soria et al. 2016, Pless et al. 2017). The mean number of samples was 39, and the range was 6–54. For convenience, we have grouped the samples other than Las Vegas into 5 broad geographic regions referred to as southern California, northern California, Southwest USA, South Central USA, and Southeast USA (see Fig. 1).

Observed heterozygosity and expected heterozygosity were calculated using the software GenAlEx 6.5 (Peakall and Smouse 2006, 2012), and allelic richness was estimated by rarefaction ($N = 30$) using the software HPRARE (Kalinowski 2005). The Adegenet package v. 2.0.2 was used for principal component analysis (PCA), discriminant analysis of principal components (DAPC), and the calculation of F_{ST} values among populations (Jombart 2008).

To identify likely genetic clusters and possible origins of the Las Vegas population, we used a Bayesian clustering method implemented by the software STRUCTURE v. 2.3.4 (Pritchard et al. 2000). STRUCTURE identifies K genetic clusters and estimates what proportion of each individual's ancestry is attributable to each cluster, with no a priori location information about the individuals. Twenty independent runs were conducted at K = 1–15 for the full set of 26 North American populations.

Table 1. Genetic diversity of Las Vegas compared with regions in the USA.

Region	Expected heterozygosity ± SD	Observed heterozygosity ± SD	Allelic richness ($N = 30$) ± SD
Northern California	0.51 ± 0.028	0.48 ± 0.029	3.4 ± 0.65
Southern California	0.42 ± 0.089	0.44 ± 0.10	2.6 ± 0.42
Southwest	0.55 ± 0.016	0.57 ± 0.038	3.6 ± 0.36
South Central	0.52 ± 0.087	0.51 ± 0.060	3.9 ± 0.61
Southeast	0.59 ± 0.020	0.59 ± 0.055	4.3 ± 0.093
Las Vegas	0.38	0.35	2.6

After finding that Las Vegas clustered with the Southwest USA and southern California, we reran the STRUCTURE analysis on this subset to achieve finer resolution. Each STRUCTURE run was for 600,000 generations with 100,000 discarded as burn-in, assuming an admixture model and correlated allele frequencies. The optimal number of K clusters was chosen using the Delta K method (Evanno et al. 2005, Earl 2012) and guidelines from Pritchard et al. (2000). The results were visualized using the program DISTRUCT v.1.1 (Rosenberg 2004).

The observed heterozygosity of Las Vegas was 0.35, while the mean ± standard deviation (SD) across all sites included in these analyses was 0.51 ± 0.088. The allelic richness of Las Vegas corrected for rarefaction was 2.6, while the US national mean ± SD was 3.4 ± 0.732. The genetic diversity values of Las Vegas are similar to southern California, where observed heterozygosity was 0.44 ± 0.10 SD and allelic richness was 2.6 ± 0.42 SD. Table 1 shows expected heterozygosity, observed heterozygosity, and allelic richness for Las Vegas and each major region.

Bayesian clustering implemented by STRUCTURE for all 26 populations found 2 primary clusters ($K = 2$) (Fig. 1B). One group consisted of Las Vegas, southern California, and the Southwest USA, and the other consisted of northern California, the South Central USA, and the Southeast USA (Fig. 1B). Hierarchical STRUCTURE at higher K values (e.g., $K = 5$) showed that Las Vegas clusters with Garden Grove and Santa Ana (Fig. 1C). After rerunning STRUCTURE with just Las Vegas, the Southwest, and southern California, the most likely number of genetic clusters was 6. At this level, Las Vegas has a distinct genetic signature that separates it from other populations. At lower K values (e.g., $K = 3$), Las Vegas clusters with Garden Grove (Fig. 1A).

The F_{ST} values ranged from 0.021 to 0.35, and the mean ± SD was 0.10 ± 0.061. Just considering population pairs that included Las Vegas, the mean ± SD was 0.17 ± 0.04 SD. The PCA was used to investigate structure among Las Vegas, southern California, and Southwest USA. The 1st axis explained 11% of the variation, and the 2nd axis explained 8% of the variation. Las Vegas clustered most closely with Garden Grove. Using DAPC for the same subset of data, we found Las Vegas formed its own genetic cluster. This cluster was nearest to Garden Grove and to another group which contained individuals from Nogales, Mexico; Tucson, AZ; and Maricopa County, AZ.

The bulk of our results show that the most likely source of invasion for the mosquitoes in Las Vegas is southern California. Of the 26 North American populations included in this analysis, the closest genetic match to Las Vegas is Garden Grove, a town in Orange County, CA. Orange County and Las Vegas are easily connected by Interstate 15, so it is plausible that one or more introductions into Las Vegas occurred from highway traffic (both personal cars and commercial trucks) from the Orange County area.

The Las Vegas population is also closely related to the Southwest USA populations, which include Arizona, New Mexico, and northern Mexico. This makes sense, since we have previously shown that populations in southern California originated from the Southwest USA (Pless et al. 2017). If our understanding of the invasions is correct, we can think of southern California as a stepping-stone between the Southwest USA and Las Vegas.

Las Vegas has low genetic diversity. For example, its observed heterozygosity is 0.35, compared with the North American mean, 0.51 ± 0.088. The only other sites which have similarly low genetic diversity are in southern California, specifically Garden Grove, Santa Ana, and Mission Viejo. This provides some additional evidence that the Las Vegas population is an invasion from southern California.

In general, low genetic diversity is a sign of a population bottleneck, meaning a drastic decrease in population size. This can be due to a small number of mosquitoes founding a new area (called founder effect) or a small number of mosquitoes surviving insecticide use. These causes of bottleneck may have occurred in southern California, Las Vegas, or both regions.

Insecticide resistance and vector competence of *Ae. aegypti* vary regionally (Hemingway and Ranson 2000, Bennet et al. 2002, Vontas et al. 2012), so knowing the source of new invasions can be important for designing local vector control programs. Additionally, understanding gene flow of this important disease vector may help prevent additional introductions or even prevent future invasions to new areas.

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