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Research

Population Biology/Genetics

Urban Populations of *Aedes aegypti* (Diptera: Culicidae) From Central Argentina: Dispersal Patterns Assessed by Bayesian and Multivariate Methods

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Abstract

Aedes aegypti (L.), the main vector of dengue and other arboviruses, was declared eradicated from Argentina in 1964; however, in 1987, it was detected again and nowadays it occurs in most of the country territory. To understand the transmission of vector-borne diseases, knowledge of the dispersal of vector populations is essential to evaluate the risk of pathogen transmission. We conducted a population genetic analysis of *Ae. aegypti* in 20 neighborhoods from Córdoba, the second largest city in Argentina, using 10 microsatellite loci. High genetic differentiation and the absence of an isolation by distance pattern was found using Weir and Cockerham's θ. Bayesian and multivariate clustering analyses showed that the studied sites included individuals with high membership coefficients (Q) in their populations, individuals with membership in another cluster, and admixed individuals. Individuals with high Q in clusters different from the population in which they were collected strongly suggests that passive transport is important in shaping the *Ae. aegypti* dispersal pattern in Córdoba city. Knowing the genetic structure of *Ae. aegypti* populations and their dispersal patterns would contribute to the implementation of vector control programs.

Key words: *Aedes aegypti*, microsatellites, population genetic structure, Córdoba city (Argentina)

Aedes aegypti (L.) is a highly domestic and anthropophilic mosquito species native to Africa introduced in the New World by shipping trade routes, most likely during the 17th century (Powell et al. 2018). Females oviposit mostly in artificial containers inside and around human dwellings; therefore, the species' dispersal range is strongly conditioned by both the availability of oviposition sites and blood sources, which contribute to its efficiency as vector of different arboviruses (Gubler 2014). *Aedes aegypti* is the primary urban vector of several emerging mosquito-borne pathogens throughout all tropical and subtropical areas, with over 3.6 billion people currently living in infested areas (Reiter 2014, Higgs and Vanlandingham 2015). Although dengue is the most common virosis transmitted by this mosquito, in recent years, other arboviruses such as zika and chikungunya have spread rapidly in the Americas, also gaining epidemiological relevance (Mayer et al. 2017).

In the 1940s, the Pan American Health Organization initiated an *Ae. aegypti* eradication program, which resulted in its elimination from 19 countries (Gubler 2014). Unfortunately, in Argentina, this mosquito was probably never fully eliminated (Rondan Dueñas et al. 2009), and in 1987, the vector was detected in the north of the country (Curto et al. 2002). The expansion continued in the subsequent years (Schweigmann and Boffi 1998), and nowadays, the species occurs in most Argentine provinces (Domínguez and Lagos 2001, Grech et al. 2012, Zanotti et al. 2015, Díaz Nieto et al. 2016). In mosquito-borne diseases, the levels and patterns of transmission among human populations are determined by multiple and complex factors; for this reason, vector control remains to be one of the most viable strategies to prevent epidemics (Gubler 2014). To evaluate the risk of pathogen transfer and to develop effective control strategies, knowledge of the dispersal patterns of vector populations is essential (McCoy 2008). Using mark-release-recapture methods, it was found that individuals of this species typically fly only a few hundred meters (Gubler 2014). While these methods are a direct way of measuring *Ae. aegypti* movement

distances because they involve tracking individual organisms, they do not quantify effective dispersal, i.e., they do not reflect the levels of gene flow, which depend on the reproductive success of migrants in the receiving population (Broquet and Petit 2009). Data obtained from highly variable loci provide knowledge of the distribution of genetic variation among populations; thus, more accurate inferences of effective dispersal can be made and, therefore, the genetic relationships and patterns of gene flow among populations can be assessed.

Throughout the world, the genetic structure of *Ae. aegypti* populations was estimated at different spatial scales, using different molecular markers. In Argentina, the first estimates of the species' genetic structure after its reemergence were made by de Sousa et al. (2001) using Random Amplified Polymorphic DNA markers (RAPDs). They found significant genetic differentiation among populations from central and northern Argentina. In later phylogeographic studies based on three mitochondrial genes, the coexistence of three divergent haplogroups with a well-defined geographical distribution was reported. This pattern would have resulted from the persistence of relictual populations that had not been eradicated by the continental control campaign, combined with multiple introductions of different lineages of the species from neighboring countries, probably facilitated by passive transport (Rondan Dueñas et al. 2009; Albrieu Llinás and Gardenal 2011, 2012). Soliani et al. (2010) suggested passive transport of larvae and eggs from Argentina as the main origin source of populations of Uruguay.

Given the anthropophilic habits of the species and its short flight range, many studies of small-scale genetic structure have been conducted within cities around the world (e.g., Huber et al. 2002, Mousson et al. 2002, da Costa-Fraga et al. 2003, Endersby et al. 2011). In Argentina, the only study of *Ae. aegypti* genetic structure at micro-geographical scale was carried out by Julio et al. (2009) using Random Amplified Polymorphic DNA markers (RAPDs) in seven neighborhoods from Córdoba city, the second largest metropolitan area in Argentina and an important commercial and industrial center located at the crossroads of many national and international routes. Populations formed three clusters by genetic similarity. Each cluster comprised populations connected by, or near to, main roads. The authors suggested that the observed genetic structure would be determined by a combination of low to moderate levels of gene flow (mediated mainly by passive transport along main roads) and significant genetic drift events every winter that differentiate the populations at random. The authors proposed that a more extensive sampling would be necessary to estimate dispersal levels within the city. In addition, RAPD markers are dominantly inherited (which prevents to estimate mating system), have a low reproducibility (the technique is sensitive to minor changes in reaction conditions), and present problems of co-migration of bands (a visible band may contain amplified fragments of similar molecular weight that differ in nucleotide sequence). Thereby, they are not as efficient as co-dominant markers like microsatellite loci for population genetics studies (Kumar and Gurusubramanian 2011). In the present work, we improved the sampling strategy, molecular markers and statistical methodologies used, to deepen the knowledge of the genetic structure of *Ae. aegypti* populations from Córdoba. We used several approaches (classical population genetics methods, and Bayesian and multivariate genetic clustering) to estimate the genetic differentiation among 20 neighborhoods and to infer dispersal patters within the city.

Methods

Study Area and Sample Collection

Córdoba city (64°12′W 31°22′S), with a surface area of 576 km^2 and about 1.4 million inhabitants, is an important commercial and industrial center located at the crossroads of several national and international routes. The city is surrounded by a beltway and crossed by the Suquía River and one of its tributaries, partially channeled into a waterway (La Cañada creek). The region presents a temperate semidry climate, with a mean annual precipitation of 800 mm concentrated in the summer, and markedly dry winters.

Samples were obtained from 20 neighborhoods (sampling sites) distributed evenly throughout the city (Fig. 1 and Table 1). In each site, a single household was randomly selected to place a georeferenced ovitrap with the consent of its residents. Each ovitrap was replaced weekly from February to April 2012, yielding 12 ovitraps per sampling site. Ovitraps were transported to the laboratory and the eggs were reared to fourth-instar larvae. A mean of 425 eggs (range 99–1,149) were obtained for each sampling site (Table 1). Twenty larvae per neighborhood were randomly chosen from the pool of fourth-instar larvae obtained for each location and identified according to the key of Rossi and Almirón (2004). Taking into account the 'skip oviposition' behavior of the species (Reiter 2007), this procedure decreases the probability of having full sibs in the analyzed sample.

Molecular Methods and Genotyping

Genomic DNA was extracted from each larva using the phenol–chloroform procedure (Ballinger-Crabtree et al. 1992). The quality of the DNA obtained was analyzed by running a 5-µl aliquot on a 1% agarose gel stained with ethidium bromide. DNA was eluted to a final concentration of 10 ng/µl and stored at −20°C.

Ten microsatellite loci (Chambers et al. 2007, Slotman et al. 2007) were amplified by polymerase chain reaction (PCR) using fluorescently labeled forward primers. The PCRs were performed in a total volume of 10 µl, containing 1× reaction buffer (10 mM Tris–HCl, pH 8.8; 50 mM KCl, 0.08 % Nonidet P40), $MgCl_2$ (1.20–1.75 mM depending on each primer, Supp Table 1 [online only]), forward and reverse primers (0.5 µM each), dNTPs (0.2 mM), genomic DNA (10 ng), and 1 U of Taq polymerase (Fermentas Life Sciences). The amplification proceeded through an initial denaturation at 92°C for 5 min, followed by 28 cycles of 92°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension of 5 min at 72°C. The molecular size of the PCR products was determined using an automatic sequencer ABI3730XL (Macrogen Korea).

Fragments were scored using the software Peak Scanner v0.1.1 (Applied Biosystems 2006) and the bin limits of fragment sizes were defined with the program MsatAllele (Alberto 2009).

Genetic Variability and Population Differentiation

Hardy–Weinberg (H-W) and linkage equilibrium between pairs of loci were tested using FSTAT (Goudet 2002). Significant deviations from *H–W* proportions were identified and classified according to whether they were consistent with null alleles, short allele dominance or scoring errors associated with stuttering, using the software Microchecker (van Oosterhout et al. 2004).

Observed and expected heterozygosity (H_o and H_e) were calculated using Genalex v0.6.5 (Peakall and Smouse 2012), whereas allelic richness (AR) was assessed applying the rarefaction method of El Mousadik and Petit (1996) with FSTAT. Population genetic structure was examined by means of *F* statistics using the corrected

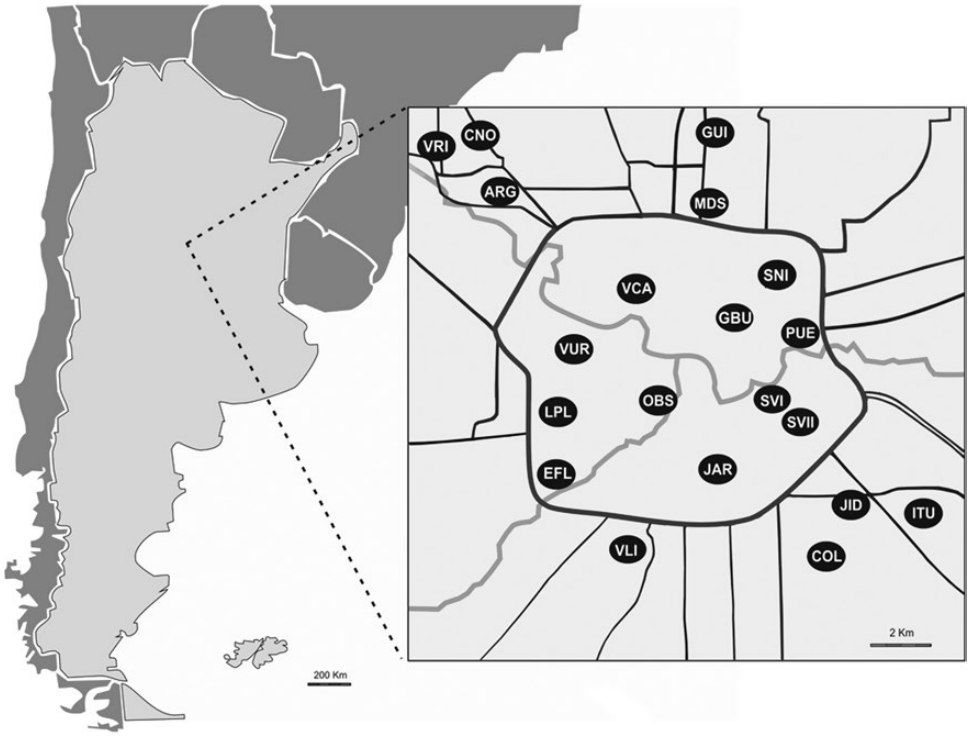

Fig. 1. Geographical location of the 20 neighborhoods in Córdoba city where *Aedes aegypti* samples were collected in February to April 2012. VCA (Villa Cabrera), ARG (Argüello), VRI (Villa Rivera Indarte), CNO (Cerro Norte), MDS (Marqués de Sobremonte), GUI (Guiñazú), SNI (San Nicolás), PUE (Pueyrredón), SVI (San Vicente I), ITU (Ituzaingó), JID (José Ignacio Díaz), COL (Coronel Olmedo), VLI (Villa El Libertador), EFL (Estación Flores), VUR (Villa Urquiza), LPL (Los Plátanos), GBU (General Bustos), SVII (San Vicente II), JAR (Jardín), and OBS (Observatorio).

method of Weir and Cockerham (1984), where θ is an estimator of the parameter F_{ST} measuring the amount of differentiation among populations, and f estimates F_{IS}, the inbreeding coefficient within subpopulations. Significance of θ and f was estimated using 200,000 random permutations. Sequential Bonferroni corrections were applied for all multiple tests. Genetic isolation by geographical distance was analyzed via Mantel tests between the linearized genetic distance (calculated as $F_{ST}/(1 - F_{ST})$ according to Rousset (1997)) and the geographical distances between pairs of neighborhoods using the GenalEx program v0.6.5 (Peakall and Smouse 2012). Significance of the correlation was assessed through 9,999 random permutations.

Genetic Clustering by Bayesian and Multivariate Approaches

The Bayesian clustering method implemented in the program Structure v0.2.3.3 (Pritchard et al. 2000) was used to estimate the most likely number of populations (K) present in the data set. This software uses a Bayesian approach to estimate the most probable number of clusters of individuals, each characterized by their multilocus genotype, by maximizing H–W equilibrium within loci, and gametic phase equilibrium between loci. The inference of ancestry (Q) of each individual to each inferred cluster was assessed using an admixture model (which allows individuals to exhibit ancestry from multiple clusters), with correlated allele frequencies and without prior information of the populations to which the individuals belonged (POP = USEPOPINFO = 0). Ten independent runs were performed for values of K from 1 to 20. Each run consisted of 5,000,000 MCMC iterations, with a burn-in of 500,000 iterations. The inference of K via the ΔK statistic (the rate of change of the probability of data as a function of K Evanno et al. 2005) was performed employing Structure Harvester (Earl and Von Holdt 2012). The outputs of the 10 independent runs for each K were combined using Clumpp (Jakobsson and Rosenberg 2007) and a visual output for the individual cluster coefficients was generated using the program Distruct (Rosenberg 2004).

A discriminant analysis of principal components (DAPC) was applied, using the Adegenet package (Jombart 2008) for R (R Development Core Team 2007). This multivariate clustering method is based on a discriminant analysis performed on data previously transformed by a principal component analysis. The purpose of this approach was to define groups of genetically similar individuals, by maximizing the intergroup component of the genetic variation. Unlike Bayesian approaches, DAPC does not rely on a particular population genetics model, and is thus free of assumptions about H-W or linkage equilibrium. This allows describing complex structures and generating a visual assessment of between-group structures (Jombart et al. 2010). The number of clusters was assessed by running successive K-means clustering with increasing number of clusters (K), and applying the Bayesian information criterion (BIC) to assess the best supported K-value. DAPC was performed on the PCA transformed data for the optimal K-value. The optimal number of PCs to be retained for DAPC clustering was obtained with the function optim.a.score in Adegenet.

Results

Genetic Variability and H–W equilibrium

Genotypes for the 400 *Ae. aegypti* from Córdoba city can be accessed at https://doi.org/10.6084/m9.figshare.10265630.

In most geographical populations, mean f values were statistically significant according to the Bonferroni correction (f mean = 0.143, ES = 0.054; Table 1). There was no evidence of scoring errors due

Table 1. Geographical location and statistics of 20 populations of *Aedes aegypti* in Cordoba city

Sampling sites*	Latitude	Longitude	Number of eggs	*Ho*	*He*	AR	*f*
VCA	–31.379092	–64.215032	408	0.49	0.61	4.09	*0.23*
ARG	–31.343217	–64.253403	306	0.57	0.71	5.43	*0.23*
VRI	–31.316180	–64.297904	452	0.77	0.72	5.65	–0.04
CNO	–31.323463	–6.427762	391	0.53	0.71	5.83	*0.25*
MDS	–31.368596	–64.189410	315	0.67	0.74	5.79	0.12
GUI	–31.313927	–64.176594	179	0.62	0.72	5.93	*0.17*
GBU	–31.391207	–64.168302	99	0.62	0.66	5.37	0.09
SNI	–31.379152	–64.151052	903	0.67	0.71	5.33	0.09
PUE	–31.407935	–64.162907	827	0.63	0.73	6.24	*0.16*
SVI	–31.421669	–64.138973	282	0.55	0.72	5.69	*0.26*
SVII	–31.420965	–64.154130	323	0.59	0.71	5.32	*0.20*
ITU	–31.464947	–64.087328	454	0.59	0.65	5.61	0.12
JID	–31.455354	–64.128140	273	0.66	0.76	6.20	*0.16*
COL	–31.481008	–64.136469	317	0.62	0.75	6.27	*0.20*
VLI	–31.475372	–64.220960	338	0.62	0.71	5.81	*0.16*
EFL	–31.449382	–64.250986	440	0.53	0.53	4.21	0.04
JAR	–31.444974	–64.179183	373	0.55	0.63	5.46	*0.16*
VUR	–31.391933	–64.235826	1149	0.64	0.76	6.28	*0.19*
LPL	–31.421033	-64.235523	378	0.64	0.60	4.79	–0.04
OBS	–31.421364	–64.200737	288	0.66	0.68	5.80	0.06

H_o: observed heterozygosity; H_e: expected heterozygosity; AR: allelic richness based on a minimum sample size of 16 individuals; *f:* mean value across 10 loci of the F_{IS} estimator; underlined values: statistically significant values after the Bonferroni correction ($P < 0.001$).

*VCA (Villa Cabrera), ARG (Argüello), VRI (Villa Rivera Indarte), CNO (Cerro Norte), MDS (Marqués de Sobremonte), GUI (Guiñazú), SNI (San Nicolás), PUE (Pueyrredón), SVI (San Vicente I), ITU (Ituzaingó), JID (José Ignacio Díaz), COL (Coronel Olmedo), VLI (Villa El Libertador), EFL (Estación Flores), VUR (Villa Urquiza), LPL (Los Plátanos), GBU (General Bustos), SVII (San Vicente II), JAR (Jardín), and OBS (Observatorio).

to stuttering or large allele dropouts for any of the loci or populations. The possible presence of null alleles was detected in different loci in some populations, but not in the same locus across samples (Supp Table 2 [online only]). The general probability of linkage between pairs of loci was low, although significant (6.75%, $P > 0.01$). However, no pair was observed systematically correlated across populations; therefore, a physical linkage between loci was rejected (Supp Table 3 [online only]).

Across all loci, the AR values ranged from 4.09 to 6.28 (average AR = 5.56) and the average values of *Ho* and *He* were 0.61 and 0.69, respectively. Locus-by-locus AR, H_o, H_e and *f* values are shown in Supp Table 1 [online only].

Population Genetic Structure

The comparisons between pairs of neighborhoods showed θ values ranging from 0.002 to 0.285 (mean = 0.081, SE = 0.020). The levels of genetic differentiation were statistically significant in 85.26% of the comparisons after the Bonferroni correction (Table 2). The isolation by distance test revealed a nonsignificant correlation ($r = –0.098$, $P = 0.292$, Fig. 2) between normalized genetic and geographical distances between pairs of neighborhoods.

In the Bayesian analysis with STRUCTURE, the best *K* determined by Evanno's Δ*K* statistic was $K = 2$, with $K = 3$ and $K = 5$ also showing high ln[Pr(*X*|*K*)] values (Supp Fig. 1 [online only]). For the two clusters solution (Fig. 3a), most of the individuals from VCA, OBS, VUR, and LPL had a high proportion of ancestry in cluster 1, whereas those from EFL in cluster 2. The $K = 3$ solution (Fig. 3b) partitioned cluster 1: VCA and VUR from OBS and LPL. In both solutions, the other sampling sites consisted in a mixture of individuals having high *Q* in one of the two (or three) clusters and admixed ones.

In the DAPC analysis, the best-supported K-means clustering model, identified by the minimum K value beyond which BIC changes are negligible, was $K = 7$ (Supp Fig. 2a [online only]). The DAPC clustering was performed by retaining 33 PCs (Supp Fig. 2b [online only]) and 6 discriminant functions. The scatterplot of individuals on the two principal components showed that three clusters (2, 4, and 5) differed markedly from the rest (Fig. 4). Individuals from GBU, PUE, and SVII predominated in Cluster 4. All but one individual from EFL were contained in cluster 2, together with half the individuals of VLI and half of those from GUI. The rest of samples from GUI were grouped in cluster 1. The remaining clusters show a considerably degree of overlapping and contained individuals from several populations. However, individuals from southern neighborhoods predominated in cluster 3 (LPL, OBS, JAR, and ITU), and individuals from north/northwestern neighborhoods in cluster 6 (CNO, ARG, VCA, SNI, and PUE, Supp Fig. 3 [online only]).

Discussion

H–W Equilibrium and Genetic Variability

When analyzing the genetic structure of natural populations, one of the main objectives is to delimit breeding units in order to identify discrete populations that, in the case of virus vectors, could present differences in insecticide resistance, vector competence, and other attributes of epidemiological interest. With this aim, the first analysis consists of checking if the genotypic frequencies follow a predictable pattern according to the random mating hypothesis. Some of the geographical populations studied in Córdoba City were close to the *H-W* equilibrium (VRI, EFL, LPL, and OBS), whereas others showed an overall heterozygote deficiency (Table 1). Vidal et al. (2012), Rašić et al. (2015), and Wilke et al. (2017) also reported significant homozygote excess in populations of this species from São Paulo and Rio de Janeiro cities (Brazil). Our results could be explained by the presence of null alleles. However, only a few populations exhibited some loci with evidence of null alleles at low frequencies (Supp Table 2 [online only]). *Aedes aegypti* prefers domestic environments and,

Table 2. Genetic population differentiation of *Aedes aegypti* in Cordoba city

Site	VCA	ARG	VRI	CNO	MDS	GUI	GBU	SNI	PUE	SVI	SVII	ITU	JID	COL	VLI	EFL	JAR	VUR	LPL	OBS
VCA	-	0.119	0.103	0.120	0.105	0.163	0.162	0.119	0.109	0.102	0.154	0.143	0.125	0.142	0.135	0.285	0.200	0.099	0.153	0.141
ARG	**	-	0.050	0.020	0.019	0.067	0.069	0.046	0.016	0.025	0.044	0.037	0.023	0.002	0.058	0.126	0.067	0.032	0.068	0.066
VRI	**	**	-	0.059	0.052	0.086	0.097	0.059	0.039	0.043	0.061	0.072	0.050	0.048	0.073	0.159	0.118	0.040	0.134	0.095
CNO	**	NS	**	-	0.029	0.096	0.085	0.058	0.041	0.033	0.085	0.066	0.034	0.030	0.064	0.158	0.079	0.050	0.084	0.053
MDS	**	NS	**	*	-	0.050	0.076	0.053	0.038	0.024	0.040	0.075	0.018	0.017	0.036	0.120	0.074	0.037	0.095	0.084
GUI	**	**	**	**	**	-	0.129	0.089	0.086	0.054	0.064	0.109	0.046	0.057	0.019	0.121	0.122	0.073	0.153	0.115
GBU	**	**	**	**	**	**	-	0.065	0.039	0.077	0.078	0.096	0.063	0.049	0.095	0.176	0.128	0.077	0.153	0.114
SNI	**	**	**	**	**	**	NS	-	0.023	0.060	0.056	0.079	0.050	0.038	0.056	0.172	0.110	0.048	0.113	0.073
PUE	**	NS	**	**	**	**	NS	*	-	0.020	0.032	0.055	0.023	0.015	0.059	0.130	0.079	0.045	0.093	0.071
SVI	**	**	**	**	NS	**	**	**	NS	-	0.048	0.062	0.013	0.027	0.033	0.111	0.095	0.063	0.103	0.068
SVII	**	**	**	**	*	**	**	**	NS	NS	-	0.087	0.046	0.032	0.064	0.144	0.109	0.066	0.130	0.107
ITU	**	*	**	**	**	**	**	**	*	**	**	-	0.075	0.061	0.109	0.187	0.054	0.078	0.068	0.086
JID	**	**	**	NS	*	*	**	**	*	*	**	NS	-	0.012	0.021	0.116	0.067	0.026	0.093	0.049
COL	**	NS	**	**	**	**	**	**	NS	*	*	**	NS	-	0.033	0.099	0.075	0.029	0.082	0.066
VLI	**	**	**	**	*	**	**	**	**	*	**	**	NS	NS	-	0.093	0.121	0.058	0.134	0.081
EFL	**	**	**	**	**	**	**	**	**	**	**	**	**	**	NS	-	0.205	0.157	0.242	0.219
JAR	**	**	**	**	**	**	**	**	**	NS	**	*	**	**	**	**	-	0.109	0.075	0.096
VUR	**	NS	NS	**	**	NS	**	**	**	**	**	**	*	NS	**	**	**	-	0.124	0.090
LPL	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	NS	**	NS	-	0.080
OBS	**	**	**	NS	**	**	**	**	NS	**	**	**	**	**	NS	**	**	**	**	-

Genetic differentiation (Θ values) between pairs of sampling sites are shown above the diagonal. Below the diagonal, significance of Θ after the Bonferroni correction.

NS: nonsignificant value.

*Significant at α = 0.05

**Significant at α = 0.01.

in the presence of adequate sites for oviposition and feeding, females usually do not move away from their breeding sites (Russell et al. 2005, Reiter 2007). This short range of active dispersal might determine small breeding units. Consistent with this, several works have estimated low effective population size (N_e), regardless of the level of urban development (Endersby et al. 2011, Olanratmanee et al. 2013, Rašić et al. 2015, Saarman et al. 2017). Within these small units, the likelihood of crosses between related individuals would increase, and therefore, the existence of a certain degree of inbreeding cannot be discarded as another factor contributing to the observed departure from the *H*–*W* equilibrium.

Allele richness and mean expected heterozygosities obtained in this work were higher than those reported for several populations from eastern Brazil (Vidal et al. 2012, Monteiro et al. 2014, Rašić et al. 2015, Wilke et al. 2017) and the southern region of the United States (Pless et al. 2017), using different ensembles of the set of microsatellites from Chambers et al. (2007) and Slotman et al. (2007). In *Ae. aegypti* populations from Argentina, Rondan Dueñas et al. (2009) and Albrieu Llinás and Gardenal (2012) reported a high degree of haplotype diversity in two mitochondrial genes in samples from Córdoba, concluding that the convergence of populations of different genetic composition could explain, at least in part, the variability observed. Because of the central location of Córdoba in the country, a similar explanation could be applied to our present results using microsatellites and would help to explain the high levels of genetic variability, despite the evidence for small breeding units.

Population Genetic Structure

The levels of genetic differentiation between pairs of populations were statistically significant in 85.26% of the cases (Table 2) and were random with respect to geographic distance ($r = -0.093$, $P = 0.292$). Other comparable studies of urban settlements using microsatellites reported high levels of genetic differentiation between

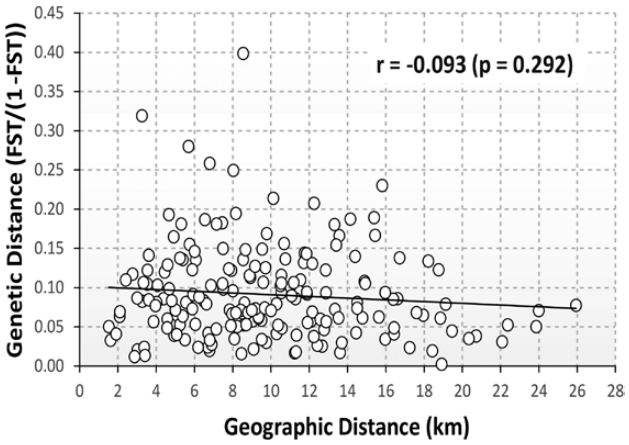

Fig. 2. Genetic isolation by geographical distance. Scatterplot of the linearized genetic distance ($F_{ST}/(1 - F_{ST})$) and geographical distance (km) among pairs of neighborhoods using GenalEx (Peakall and Smouse 2012).

Ae. aegypti populations and lack of isolation by distance. A positive correlation between geographic and genetic distances is observed when the populations reach a balance between genetic drift and gene flow. Different factors were invoked as agents producing genetic differentiation and lack of IBD pattern: rainfall seasonality, high temperatures (causing discontinuity in the water volume of small reservoirs for oviposition) and the periodic application of insecticides, which would originate population bottlenecks, resulting in several independent genetic drift effects that could randomly preserve different combinations of alleles in each population (e.g., Ocampo and Wesson 2004, Paupy et al. 2004, Olanratmanee et al. 2013, Wilke et al. 2017). Wilke et al. (2017) also suggested that, given that breeding sites are widely available in cities, females do

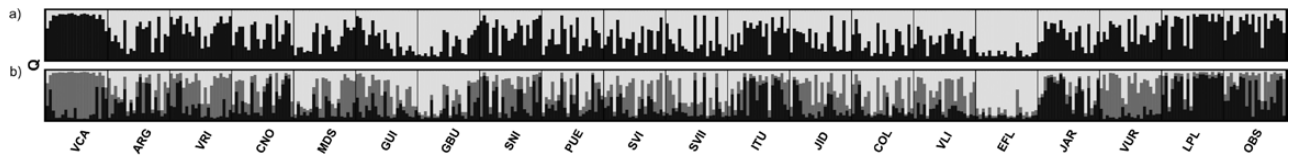

Fig. 3. Genetic structure of *Aedes aegypti* inferred from STRUCTURE analysis based on 10 microsatellite loci. Each individual is represented by a vertical bar divided into sections proportional to its membership (*Q*) to each inferred cluster. (a) $K = 2$, black: cluster 1, white: cluster 2; (b) $K = 3$, black: cluster 1, gray: cluster 2, white: cluster 3.

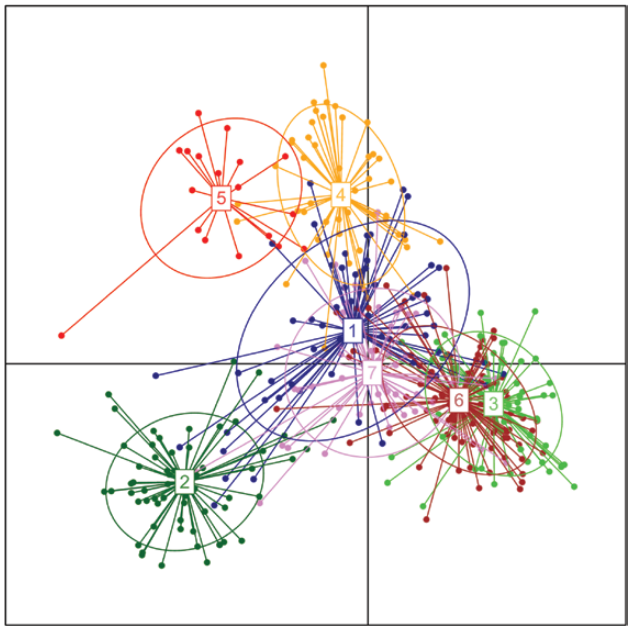

Fig. 4. Multivariate clustering of *Aedes aegypti* obtained with the DAPC method. Clustering was performed using Adegenet (Jombart 2008). The best supported solution was for $K = 7$.

not have to actively seek hosts or breeding sites, discouraging dispersal and which would, in turn, avoid the establishment of an IBD pattern. Besides the within-populations factors discussed above, the establishment of new stocks from bordering countries through passive transport has continued steadily after the first re-introduction of *Ae. aegypti* in Argentina in 1986 (Rondán-Dueñas et al. 2009) and this process would have also prevented the populations from achieving the genetic drift—gene flow balance. Another possible cause of the lack of IBD would be micro-geographic local adaptation in *Ae. aegypti* populations from Córdoba city. However, our current data provide no direct evidence of such process in our study sites.

The Bayesian and multivariate clustering analyses indicated genetic structuring of populations in Córdoba city. Two genetic clusters were detected with STRUCTURE and seven with DAPC. We must consider that the DAPC approach seeks synthetic variables (the discriminant functions), which reflect differences between groups as best as possible, while minimizing variation within clusters. Unlike DAPC, the Bayesian approach infers the most probable *K* by maximizing *H–W* equilibrium within clusters. However, both methods revealed similar trends. Some populations within the city tend to show genetic distinctiveness (like GBU, LPL, OBS, EFL, VUR, and VCA), whereas others present different levels of admixture. In addition, all populations include individuals with high membership in their population, and others highly admixed or with membership in another cluster. The close genetic relationship among geographically distant individuals could be the result of passive transport of eggs, larvae, or adults, since these distances are far beyond the maximum active dispersal distance observed for the species. Moreover, previous works on a wider geographical scale also demonstrated the influence of the passive dispersal of *Ae. aegypti* on its population genetic structure both in Argentina (Julio et al. 2009; Rondan Dueñas et al. 2009; Soliani et al. 2010; Albrieu Llinás and Gardenal 2011, 2012; Díaz-Nieto et al, 2016) and in other parts of the world (Huber et al. 2004, Guagliardo et al. 2019).

The VCA neighborhood presented the lowest AR values (Table 1) and differed genetically from all the other sites with the classical *F* approach (Table 2). In DAPC, it is the only population with a considerable proportion of their individuals in cluster 7 and in STRUCTURE ($K = 3$) almost all of its individuals show high membership to cluster 2 (Fig. 3b). During the re-introduction of *Ae. aegypti* in Córdoba province, one of the first records of the species was documented in this neighborhood (Almirón and Ludueña-Almeida 1998). Accordingly, two hypotheses may be assumed: first, that the individuals sampled in this site are representatives of a small initial foreign population, and second, that to deal with this first detection, an important vector control campaign was conducted in the area. The different genetic constitution and low polymorphism level detected here would be a consequence of an initial founder effect, i.e., the loss of genetic variation that occurs when a new population is established by a minimal number of individuals, which was reinforced by a subsequent bottleneck event generated during the drastic reduction of the vector population in this site.

Final Considerations

The knowledge of population genetic structure provides critical information for the implementation of alternative control strategies (McCoy 2008) like the introduction of sterile adult mosquitoes to natural vector populations (Wilke et al. 2009) and/or the treatment of larvae with insect growth regulators (Ahmed and Vogel 2016), since it will indicate the potential range of spread/effectiveness of the treatment.

Several studies documented that the susceptibility to arboviruses infection (Tabachnick 2013), the efficiency of disease transmission (Failloux et al. 2002), and insecticide resistance (Faucon et al. 2015) depend on the genetic and geographical background of mosquito populations (Faucon et al. 2015). High levels of genetic diversity and the admixed ancestry detected in some individuals in almost all neighborhoods of Córdoba city would be an indicator of a confluence of individuals from different geographical sources, which could also imply polymorphism in the viral susceptibility, vector competence or resistance to insecticides.

Regarding control programs, in the presence of a fever case in a neighborhood with low admixed ancestry, a focal treatment both in the patient's home and in his immediate environment is, in fact, a

useful tool to control the spread of the disease. However, if the detection of a fever case occurs in a highly admixed neighborhood, i.e., with multiple contributions of different genetic pools, more complex measures would be necessary to prevent the virus propagation.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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