Population Genetics of *Aedes vexans* (Diptera: Culicidae) from New Orleans

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ABSTRACT

Understanding genetic variation among populations of medically significant pest insects is important in studying vector transmission, disease epidemiology, and disease control. For this study, 99 adult mosquitoes representing 11 sampled populations from 3 Louisiana parishes were subjected to genetic analysis using PCR to amplify and sequence a region of the mitochondrial NADH dehydrogenase subunit 5 (ND5) gene. High levels of heterozygosity were observed within the sampled populations, indicating high levels of gene exchange among populations. This is not typical of insects under selection pressure from insecticides and may be due to *Aedes vexans* breeding in marshes outside of New Orleans and migrating into the city.

INTRODUCTION

Aedes vexans (Diptera: Culicidae) (Meigen), vexans mosquito, is a very widespread pest mosquito; its distribution includes Africa and Asia (Horsfall 1972), southern Canada, and continental United States (Means 1979). *Ae. vexans* is an important pest species due to its abundance, attraction to lights, its ability to migrate long distances to urban areas, and its preference for mammalian hosts. *Ae. vexans* is also able to vector St. Louis encephalitis (SLE) and eastern equine encephalitis (EEE) (Cupp et al. 2004). Knowledge of genetic variation within medically important insect species is important for understanding vector transmission, disease epidemiology and disease control (Tabachnick and Black 1995).

The objective of this study was to determine the extent of genetic variation within and among populations and regions of *Ae. vexans* in the New Orleans area.

MATERIALS AND METHODS

Adult specimens were collected from 3 Louisiana parishes in 2005 (Table 1, Fig. 1). DNA was extracted from individual mosquitoes using the Puregene DNA extraction kit (Gentra, Minneapolis, MN). Polymerase chain reaction on the mtDNA marker was conducted using the primers 6500 and 7398 (Birungi and Munstermann 2002). These PCR primers amplify a 423 bp region of the mtDNA ND5 gene. PCR reactions were conducted per Szalanski et al. (2000). Amplified DNA from individual mosquitoes was purified and concentrated, and sent to UAMS DNA Sequencing Core Facility (Little Rock, AR) for direct sequencing in both directions. DNA sequences were aligned using CLUSTAL W (Thompson et al. 1994). Haplotype distribution between populations, number of haplotypes, number of unique haplotypes, haplotype diversity, and average number of pairwise differences were calculated using DNAsp v3.51 (Rozas and Rozas 1999). To test for neutral mutation, the *D* statistics of Tajima (1989) and Fu and Li (1993) was calculated using DnaSP. Genealogical relationships among haplotypes were constructed using TCS (Clement et al. 2000). Tests for differentiation were conducted using AMOVA as implemented in Arlequin v. 2.0 (Schneider et al. 2000)

RESULTS AND DISCUSSION

A portion of the mtDNA ND5 gene were sequenced for 99 individual *Ae. vexans* from 3 Louisiana parishes (Table 1). From the DNA sequences, 67 unique haplotypes were observed. A total of 39 nucleotide sites were polymorphic with 15 being parsimony informative. Forty one haplotypes were observed among the sampled mosquitoes with 26 (63%) occurring only once and two haplotypes, 1 and 4, were found from 43% of the mosquitoes. High levels of heterozygosity were observed within the sampled populations indicating high levels of gene exchange among populations. The strongly negative values for Fu's F+ and Tajimas D suggest population growth (Table 2).

The amount of genetic variation in these populations is surprising, because of the existence of mosquito abatement programs in New Orleans. Normally, insects that experience high insecticide pressure undergo a genetic bottleneck. Our research provides genetic evidence that *Ae. vexans* may be breeding in other areas, such as wetlands surrounding the city, and adults are migrating into the city.

Table 1. Collection localities and locality codes for all *Ae. vexans* examined.

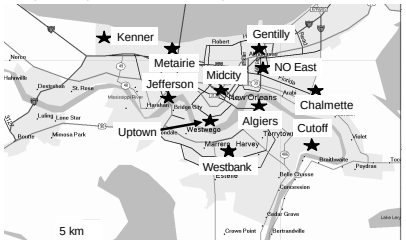
Locality (code)	Parish	*N*	Haplotypes (frequency)
Metairie	Jefferson	7	4(4),38,46,53
Jefferson	Jefferson	7	1,4,25,38,41,43,63
Kenner	Jefferson	9	4(3),11,38,43,47,55,59
Westbank	Jefferson	9	1(2),4(3),25,37,38,57,58,61,67
Chalmette	St. Bernard	9	1,4(2),35,38(2)58,61,67
New Orleans, Midcity	Orleans	10	1(3),4,31(2),45,46,48,49
New Orleans, East	Orleans	13	1(3),2,4(6),50,51,62
Algiers	Orleans	6	4,7,19,25,31,51
New Orleans, Uptown	Orleans	13	4(3),19,25(2),37,52,57,60,64,65,66
Gentilly	Orleans	8	1,2,4(2),37,38,43,54
Cutoff	Orleans	8	1,4(2),25,31,37,42,55

Table 2. Summary statistics for mtDNA polymorphisms.

Region	*n*	NS + S	*h*	*p (k)*	?$_s$	?$_g$	D+	F+	D
Jefferson Parish	35	4 + 18	18 (0.887 ± 0.04)	0.006 (2.47)	0.010	4.371	-1.97 NS	-2.12 NS	-1.45 NS
Orleans Parish	55	6 + 24	26 (0.903 ± 0.03)	0.006 (2.68)	0.015	6.557	-2.58 S*	-2.80 S*	-1.94 S*
Total	99	8 + 30	41 (0.898 ± 0.02)	0.006 (2.63)	0.017	7.548	-4.44 S**	-4.17 S**	-2.03 S*

Note. *n* = number of sequences; NS +S = number of nonsynonymous and synonymous mutations; *h* = number of haplotypes (haplotype diversity ± SD shown in parentheses); p = nucleotide diversity; *k* = mean number of pairwise nucleotide differences; ?s = theta per site; ?*g* = theta per gene; D+ and F+ statistics (Fu and Li 1993); D = Tajima's (1989) statistic. * $P < 0.05$, ** $P < 0.02$.

Fig. 1. Map of *Ae. vexans* sample sites



Ae. vexans adult
(image by M.M. Cutwa)

Ae. vexans larvae
(image by M.M. Cutwa)

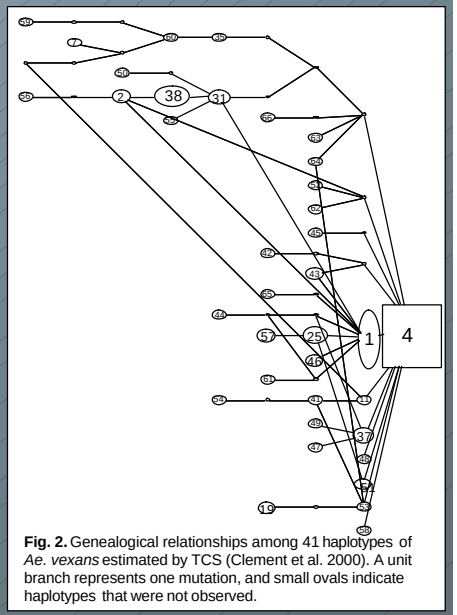


Fig. 2. Genealogical relationships among 41 haplotypes of *Ae. vexans* estimated by TCS (Clement et al. 2000). A unit branch represents one mutation, and small ovals indicate haplotypes that were not observed.

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