

# Genetic Structure of *Aedes vexans* (Diptera: Culicidae) Populations from Central United States Based on Mitochondrial ND5 Sequences

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Ann. Entomol. Soc. Am. 99(1): 157–163 (2006)

**ABSTRACT** *Aedes vexans* (Meigen), the vexans mosquito, is a species that prefers mammalian hosts and is a vector of West Nile virus (family *Flaviviridae*, genus *Flavivirus*). It is one of the most widespread pest mosquitoes in the world and North America, and it is commonly found in southern Canada and continental United States. Population structure of this species in Kansas was examined using DNA sequences of a 423-bp region of the mitochondrial NADH dehydrogenase subunit 5 (ND5) gene, relative to three other states. From the 54 Kansas samples, a total of 39 nucleotide positions were polymorphic, with 34 haplotypes. Of the 34 haplotypes, 22 (79%) were not shared among populations. The average haplotype diversity (0.953) from 11 Kansas populations indicated a high level of genetic diversity in *Ae. vexans*. Among the Kansas, South Dakota, Texas, and Louisiana samples, a total of 40 haplotypes were observed. Analysis of molecular variance was conducted on the resulting haplotypes for all populations and no geographical structure was observed among populations by using isolation-by-distance tests. This first genetic study of *Ae. vexans* provides evidence that there is a large amount of haplotype variation within and among populations, and gene flow occurs across broad geographical areas in this species.

**KEY WORDS** *Aedes vexans*, mitochondrial DNA, genetic variation, population structure

*Aedes vexans* (Meigen), the vexans mosquito, is one of the most widespread pest mosquitoes in the world. Its distribution includes Nearctic and Palearctic regions, the African west coast, and Oriental regions south and east to Samoa (Horsfall 1972). In North America, it is common in southern Canada and is found throughout the United States, with the exception of Hawaii. *Ae. vexans* is one of the most important pest mosquito species because of its abundance, attraction to lights, ability to migrate long distances to urban areas, and attraction to humans. This mosquito also readily enters houses to feed (Means 1979). *Ae. vexans* is often the most abundant mosquito encountered in surveillance studies, accounting for 74% of the mosquitoes collected from Nebraska (Janousek and Kramer 1999), 39% in Arkansas (Kent et al. 2003), and 49% in Kansas (A.B.B., unpublished data). In addition to being a widespread pest species, *Ae. vexans* also has been implicated in the transmission of several important diseases. Lewandowski et al. (1980) determined that *Ae. vexans* is a suitable host for *Dirofilaria immitis* (Leidy), and it seems to be one of the primary vectors of dog heartworm in central Michigan. Viruses vectored by *Ae. vexans* include St. Louis encephalitis

(family *Flaviviridae*, genus *Flavivirus*) and eastern equine encephalitis (family *Togaviridae*, genus *Alphavirus*) (Cupp et al. 2004). Recently, *Ae. vexans* has been demonstrated to have the potential of vectoring West Nile virus (Turell et al. 2001a, Goddard et al. 2002); and in 1999 and 2000, *Ae. vexans* from New York tested positive for West Nile virus (family *Flaviviridae*, genus *Flavivirus*) (Turell et al. 2001b). In 2002 and 2003, West Nile surveillance programs in Rhode Island (Anonymous 2002), Pennsylvania (Anonymous 2003b), and Louisiana (Anonymous 2003a) have all reported adult *Ae. vexans* that tested positive for West Nile virus.

An important factor in the ecology of West Nile virus is the dispersal capacity of its mosquito vectors. The movement of mosquitoes from one area to another is often poorly understood. Knowledge of genetic variation within medically important insect species is important for understanding vector transmission, disease epidemiology, and disease control (Tabachnick and Black 1995). Genetic studies could provide insight into the dispersal and population structure of *Ae. vexans*. For example, if genetically distinct mosquito populations are detected, gene flow and dispersal of mosquitoes between populations may be limited. However, if genetic analysis reveals a lack of genetic differentiation among *Ae. vexans* populations, this high level of gene flow indicates that mosquitoes are frequently migrating among populations and could

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

Locality (code)	State	n	Latitude, longitude
Garnett, Anderson Co. (GAR)	KS	5	38° 16' 50" N, 95° 14' 30" W
Atchison, Atchison Co. (ATC)	KS	7	39° 33' 47" N, 95° 07' 17" W
Hiawatha, Brown Co. (HIA)	KS	6	39° 51' 09" N, 95° 32' 08" W
Beloit, Mitchell Co. (BEL)	KS	6	39° 27' 22" N, 98° 06' 21" W
Concordia, Cloud Co. (CON)	KS	4	39° 34' 15" N, 97° 39' 44" W
Council Grove, Morris Co. (CGR)	KS	5	38° 39' 40" N, 96° 29' 30" W
Fort Riley, Geary Co. (FTR)	KS	4	39° 06' 29" N, 96° 48' 52" W
Emporia, Lyon Co. (EMP)	KS	5	38° 24' 14" N, 96° 10' 53" W
Manhattan, Riley Co. (MAN)	KS	4	39° 11' 01" N, 96° 34' 17" W
Hays, Ellis Co. (HAY)	KS	4	38° 52' 45" N, 99° 19' 35" W
Pratt, Pratt Co. (PRA)	KS	4	37° 38' 38" N, 98° 44' 14" W
Brookings, Brookings Co. (BRO)	SD	4	44° 18' 36" N, 96° 47' 36" W
Britton, Marshall Co. (BRI)	SD	2	45° 47' 36" N, 97° 45' 06" W
New Orleans, Orleans Pa. (ORL)	LA	8	29° 57' 16" N, 90° 04' 30" W
Metairie, Jefferson Pa. (MET)	LA	4	29° 59' 02" N, 90° 09' 10" W
College Station, Brazos Co. (CST)	TX	5	30° 37' 40" N, 96° 20' 03" W
Hidalgo, Hidalgo Co. (HID)	TX	2	26° 06' 00" N, 98° 15' 46" W

contribute to the spread of West Nile virus. This information would likely have a direct bearing on the nature and implementation of management or control programs of *Ae. vexans*. Insect DNA extractions used for insect genetics studies can be used for identification of pathogens carried by insects (Szalanski et al. 2004), and *Ae. vexans* DNA extractions could be used to identify bloodmeal sources by using polymerase chain reaction (PCR) (Ngo and Kramer 2003).

Molecular genetic studies have been conducted on other *Aedes* species (Wu and Fallon 1998, Ginnig and Eldridge 1999, Ginnig 2000), but to date, none have been performed on *Ae. vexans*. The objectives of this study were to determine the extent of genetic variation within and among populations of *Ae. vexans* in Kansas, and to determine if there is any geographical component to its genetic structure among four states by using isolation-by-distance tests.

### Materials and Methods

Adult specimens were collected from 11 counties in Kansas (Table 1; Fig. 1) in 2002–2004 as part of a West Nile virus surveillance contract with the Kansas Department of Health and Environment and the Centers for Disease Control (CDC). Mosquitoes were collected with CDC traps baited with dry ice. Additional adult samples collected during 2004 were obtained from South Dakota, Texas, and Louisiana (Table 1). Mosquitoes were identified to species using keys of Means (1979) and stored in 95% ethanol or frozen at  $-80^{\circ}\text{C}$ . Voucher specimens are deposited in the Arthropod Museum (Department of Entomology, University of Arkansas, Fayetteville, AR) and at the Kansas State University Museum of Entomology and Prairie Arthropod Research (Manhattan, KS). Collection of mosquitoes in Kansas was conducted under a contract with the Kansas Department of Health and Environment for the surveillance of West Nile virus.

DNA was extracted from individual mosquitoes using either the DNeasy tissue kit (QIAGEN, Valencia, CA) or the Puregene DNA extraction kit (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50–150  $\mu\text{l}$  of Tris-EDTA and stored at  $-20^{\circ}\text{C}$ . PCR on the mitochondrial (mt)DNA marker was conducted using the primers 6500 (5'-TCCTTAGAATA-

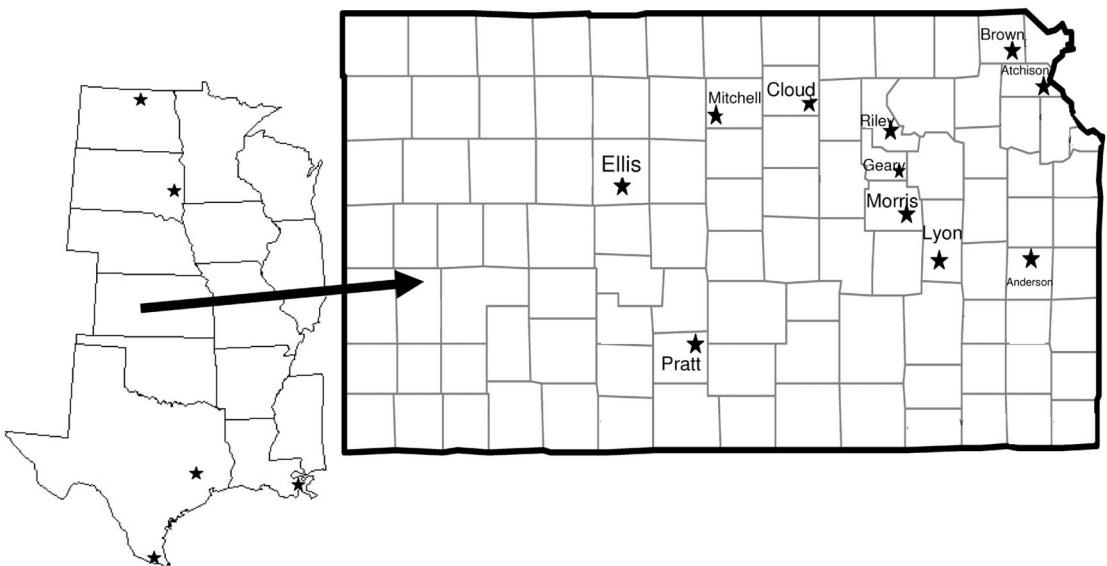


Fig. 1. Map of *Ae. vexans* collection sites (\*).

**Table 2.** Frequency of ND5 haplotypes (Hap) for *Ae. vexans*

Hap	Locality Code															n		
	FTR	CAR	EMP	MAN	BEL	CON	ATC	CGR	HAY	PRA	HIA	BRO	BRI	ORL	MET		CST	HID
1	1	.	.	.	.	1	1	.	.	.	.	.	.	2	1	.	.	6
2	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
3	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
4	1	.	.	.	1	.	3	3	1	1	1	2	.	2	1	2	1	19
5	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	2
6	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	1
7	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1
8	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
9	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
10	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
11	.	1	1	.	1	.	.	.	.	1	.	.	.	.	.	.	.	4
12	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1
13	.	.	.	.	1	.	.	.	.	1	.	.	.	.	.	.	.	2
14	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1
15	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1
16	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1
17	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
18	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	1
19	.	.	.	.	.	1	.	.	.	.	.	.	.	1	.	.	.	2
20	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	1
21	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	1
22	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	1
23	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	1
24	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	1
25	.	.	.	1	.	.	.	.	.	1	1	.	1	.	1	.	5	
26	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1
27	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1
28	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1
29	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
30	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
31	.	.	1	1	.	.	.	.	.	.	.	.	1	1	.	.	.	4
32	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
33	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	1
34	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	1
35	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	1
36	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	1	.	2
37	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	1	.	3
38	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1
39	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1
40	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	1
n	4	5	5	4	6	4	7	5	4	4	6	4	2	8	4	5	2	79

AAATCCCGC-3') and 7398 (5'-GTTTCTGCTT-TAGTTCATTCTTC-3') (Birungi and Munstermann 2002). These PCR primers amplify a 423-bp region of the mtDNA ND5 gene. PCR reactions were conducted using 2–8 µl of the extracted DNA per Szalanski et al. (2000), and the PCR profile consisted of 35 cycles of 94°C for 45 s, 46°C for 45 s, and 72°C for 45 s. Amplified DNA from individual mosquitoes was purified and concentrated using Microcon-PCR Filter Units (Millipore, Bedford, MA). Samples were sent to University of Arkansas Medical Sciences DNA Sequencing Core Facility (Little Rock, AR) for direct sequencing in both directions using an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA). Consensus sequences for each sample were obtained using Bioedit 5.09 (Hall 1999). GenBank accession numbers for the *Ae. vexans* DNA sequence haplotypes found in this study are AY897355–AY897394.

DNA sequences were aligned using ClustalW (Thompson et al. 1994). DNA haplotypes were identified using MacClade version 4 (Sinauer, Sunderland, MA). Haplotype distribution between populations, number of haplotypes, and number of unique haplotypes were calculated using DNAsp version 3.51 (Rozas and Rozas 1999). Analysis of molecular variance (AMOVA), pairwise  $F_{ST}$  values, and a Mantel test for isolation-by-distance were calculated using Arlequin version 2.0 (Schneider et al. 2000). Phylogenetic analysis of  $F_{ST}$  distances was constructed using the NEIGHBOR program incorporated into the PHYLIP package (Felsenstein 1993). The average number of pairwise nucleotide differences (nucleotide diversity,  $\pi$ ) was calculated using the program DnaSP 2.0 (Rozas and Rozas 1999). Genealogical relationships among haplotypes were constructed using TCS (Clement et al. 2000), with the method described by Templeton et al. (1992).

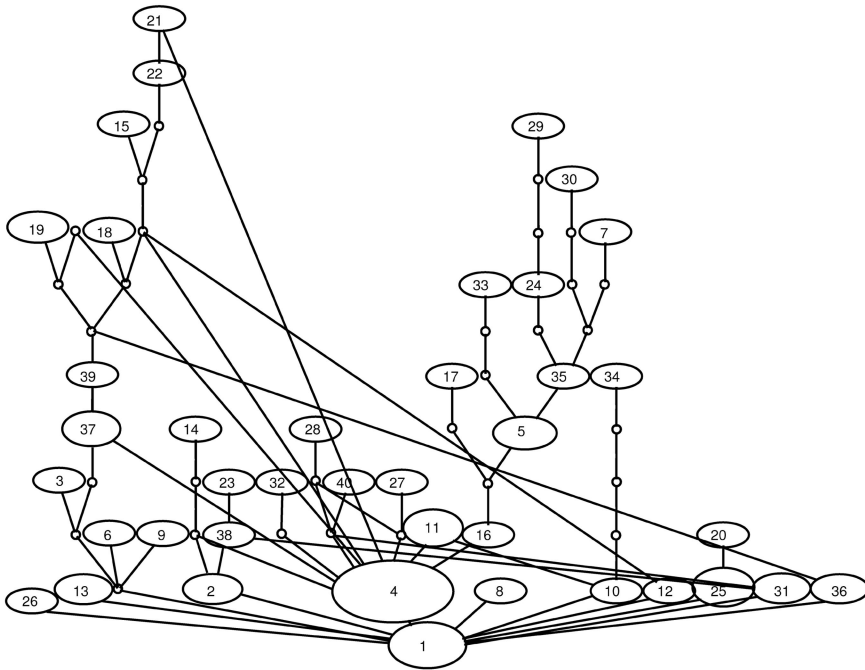


Fig. 2. Genealogical relationships among 40 haplotypes of *Ae. vexans* from four states estimated by TCS (Clement et al. 2000). A unit branch represents one mutation and small ovals indicate haplotypes that were not observed.

### Results

Sequences of a 423-bp fragment of the mtDNA ND5 gene was obtained for 54 individual *Ae. vexans* sampled from 11 locations in Kansas (Table 1). Although at least 10 individuals were obtained from each Kansas sample site, final sample sizes were <10 because initial PCRs did not work consistently, likely because of tissue degradation before samples could be frozen. From the 54 DNA sequences, 34 unique haplotypes were observed (Table 2), and nucleotide diversity,  $\pi$ , was 0.008. Of the 423 nucleotide sites, 39 were variable, consisting of 13 nonsynonymous and 26 synonymous mutations. From 18 additional *Ae. vexans* sampled from South Dakota, Louisiana, and Texas, another six haplotypes were observed (Table 2), and  $\pi$  for all samples was 0.008. The average haplotype diversity was 0.953 for the Kansas populations and 0.937 for all of the 17 studied populations. Figure 2 shows the 95% parsimony network for the 40 haplotypes (Posada and Crandall 2001). Missing haplotypes probably represent sampling gaps. There does not seem to be any structure among the haplotypes with the exception being the lineage of eight haplotypes branching from haplotype 16 (Fig. 2). Haplotype 16, however, is a singleton, and it may have gone extinct elsewhere if it was originally widespread.

The relative haplotype frequencies for each Kansas population were low with the exception of Atchison and Council Grove populations, which share haplotype 4 at a high frequency (Table 2). Haplotype 4 was also found in five other Kansas populations as well as from South Dakota, Louisiana, and Texas. Other shared

haplotypes among states included haplotypes 1, 25, 31, 36 and 37. The majority of the haplotypes were rare, with 26 (74%) of the Kansas haplotypes occurring in single populations. The overall estimates of molecular diversity for the Kansas *Ae. vexans* populations are  $k = 3.72 \pm 1.29$  and  $\theta_g = 8.56 \pm 1.88$ .

The Mantel test indicated that there was no significant correlation between geographical distance and genetic distance (as pairwise  $F_{ST}$  values) (Fig. 3), and a neighbor-joining tree based on Kansas  $F_{ST}$  distances

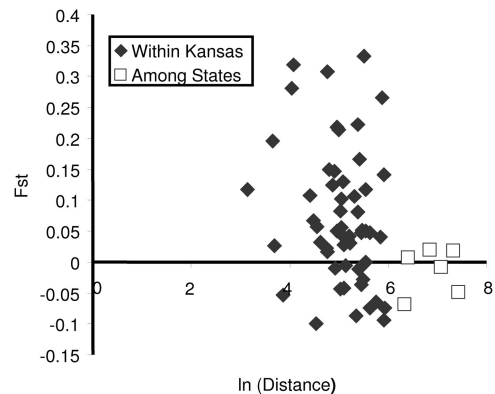


Fig. 3. Pairwise  $F_{ST}$  versus geographic distances (km). Distances were log transformed to allow plotting all pairwise comparisons on a single graph. There was no significant correlation between genetic distances (as  $F_{ST}$ ) and geographical distance ( $p \gg 0.05$  for Mantel test with 1,000 permutations).

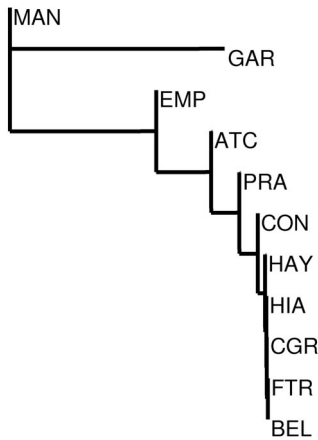


Fig. 4. Phylogenetic relationships among Kansas *Ae. vexans* populations constructed using pairwise  $F_{ST}$  distances and the neighbor-joining method.

was uninformative (Fig. 4). Hierarchical AMOVA analysis of *Ae. vexans* populations among the four states revealed a significant variance component "among populations within groups" ( $F_{ST} = 0.056$ ,  $P = 0.039$ ). This indicates that significant geographical structure exists among populations among the other sampled states.

### Discussion

This genetic analysis of *Ae. vexans* revealed high levels of diversity within this species, suggesting a large effective population size despite the small number of individuals used for this study. Similar levels of genetic diversity have been observed in other mosquitoes. Chen et al. (2004), using DNA sequencing of a 685-bp region of the mtDNA COII gene, found 50 different haplotypes from 76 *Anopheles jeyporiensis* James from southern China and northern Vietnam, and  $F_{ST}$  values ranged up to 0.089 between population groups. High levels of mtDNA haplotype diversity also have been documented in *Anopheles punctipennis* (Say) (Fairley et al. 2000), *Anopheles gambiae* Giles (Besansky et al. 1997, Donnelly et al. 2004), and *Anopheles arabiensis* Patton (Besansky et al. 1997).

The occurrence of three haplotypes (1, 4, and 25) among three of the four sampled states indicates some current or historical gene flow in this species. However, the majority of the observed haplotypes were restricted to individual populations. Within Kansas, many of the observed haplotypes were not shared among populations. This provides evidence for geographical boundaries and differences in habitat acting as potential barriers to dispersal and gene flow. This has been documented for other mosquito species (Conn et al. 1993, 1998; Fairley et al. 2000). It is

interesting that a large number of the lineages from the TCS analysis (Fig. 2) were descendents from haplotype 16, a singleton. This haplotype may have been originally widespread but may have gone extinct in many populations.

In the hierarchical analysis of the four studied states, most of the genetic variation was found within populations. Similar results have been reported for *An. punctipennis* (Fairley et al. 2000), *An. jeyporiensis* (Chen et al. 2004), and *An. albopictus* (Skuse) (Black et al. 1989). The distribution of *Ae. vexans* haplotypes is probably because of a unique history for haplotypes that results in local extinction of some geographically widespread lineages and persistence of others (Avisé et al. 1992). Within the majority of the sampled Kansas populations, we detected three or more haplotypes. Each individual population site may experience fluctuations in climate or precipitation. Multiple colonization events with haplotype loss or gains may occur during seasonal changes. Areas from which populations of *Ae. vexans* are lost during harsh winters may be recolonized by surviving individuals from neighboring areas, thereby contributing to increased variation within populations and regions. This hypothesis is supported by previous studies on the dispersal capability of this species. Adults of this species are known to fly great distances, commonly as far as 24 km (Stage et al. 1937), and have been reported to migrate >22 km in 24 h (Clarke 1943).

This first study on the genetic structure of *Ae. vexans* provides evidence that there is a large amount of genetic variation within populations and gene flow across broad geographical areas in this species. These conclusions are based on a single mitochondrial DNA marker by using populations over a broad geographical area. Future genetic analyses using other mitochondrial and nuclear genetic markers as well as analysis of gene flow among populations from a small geographical area are required to increase our understanding of genetic variation in this species. Elements that shape genetic variation in vector species also may influence variation in vectorial capacity and vector competence (Tabachnick and Black 1996). Knowledge of genetic variation within medically important insect species is important for understanding vector transmission, disease epidemiology and disease control (Tabachnick and Black 1995). A better understanding of these factors in *Ae. vexans* is important because of its status as a vector of, SLE, EEE, and West Nile virus.

### Acknowledgments

We thank Kevin Koblinsky for collecting the Kansas samples, and Mike Catangui, Dawn Wesson, and Jim Olson for providing additional samples. This research was supported in part by a University of Arkansas, Arkansas Agricultural Experiment Station research initiation grant.

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*Received 7 February 2005; accepted 2 September 2005.*

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