Genetic Variation Associated with Mammalian Feeding in *Culex pipiens* from a West Nile Virus Epidemic Region in Chicago, Illinois

Shaoming Huang,1 Gabriel L. Hamer,2 Goudarz Molaei,1 Edward D. Walker,3 Tony L. Goldberg,4 Uriel D. Kitron,5 and Theodore G. Andreadis1

Abstract

Mosquitoes of the *Culex pipiens* complex are important vectors of West Nile virus in the United States. We examined the genetic variations of *Cx. pipiens* mosquitoes from Chicago, Illinois that were determined to be principally ornithophilic but exhibited a relatively higher inclination for mammalian hosts including humans. Microsatellite analysis of 10 polymorphic markers was performed on 346 engorged *Cx. pipiens* specimens with identified avian or mammalian blood meals. Our results indicated that there were no significant differences in allelic richness, the pattern of conformity to Hardy-Weinberg equilibrium, and linkage disequilibrium, nor was there overall genetic differentiation between specimens with avian- and mammalian-derived blood meals. However, *Cx. pipiens* form *pipiens* with mammalian- (including human-) derived blood meals had significantly higher ancestry (*p* < 0.001) and proportion of hybrids (*p* < 0.01) from the *Cx. pipiens* form *molestus* (population from New York City) than did those with avian-derived blood meals. By contrast, there were no significant differences in the ancestry (*p* > 0.05) and the proportion of hybrids (*p* > 0.05) from *Cx. quinquefasciatus* (population from Harris Country, Texas). No temporal genetic variation was detected in accordance with the observation that there was no shift in blood feeding from birds to mammals. The results of this study in conjunction with regional host-feeding behavior suggest that the probability of genetic ancestry from *Cx. pipiens* f. *molestus* may predispose mosquitoes to feed more readily on mammals; however, the genetic mechanisms are unknown.

Key Words: Microsatellite analysis—Hybrid ancestry—Host-feeding behavior—*Cx. pipiens* form *pipiens*—*Cx. pipiens* form *molestus*.

Introduction

**West Nile virus** (WNV) continues to be the major mosquito-borne arbovirus in the United States since its discovery in New York City in 1999 (Lanciotti et al. 1999). The Chicago suburban area (Cook County, IL) has experienced high levels of virus activity with accompanying human cases, ranking Illinois the first and second in the United States in 2002 and 2005, respectively (Illinois Department of Public Health: http://www.idph.state.il.us/envhealth/wnv.htm). The mosquito *Cx. pipiens* (L.) has been implicated as the principal enzootic and epidemic vector in this region based upon incriminating evidence including: local abundance (Lampman et al. 2006), high prevalence of infection, diverse feeding on avian and mammalian hosts including humans (Hamer et al. 2008a), and vector competence (Sardelis et al. 2001, Turell et al. 2001, 2005).

Host-feeding pattern studies indicate that populations of *Cx. pipiens* from the northeastern United States are principally ornithophilic (Apperson et al. 2002, Molaei et al. 2006), whereas populations from the southeast (Apperson et al. 2004, Savage et al. 2007), mid-Atlantic (Kilpatrick et al. 2006), and upper midwest (Hamer et al. 2008a) are more mammalophilic in nature. The underlying bases for these apparent variations are poorly understood. One hypothesis suggests that varying degrees of introgression between aboveground *Cx. pipiens* form *pipiens* and the underground *Cx. pipiens* form *molestus*, which is reported to be highly

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mammalophilic and aggressive human biter (Harbach et al. 1984), is an important contributing factor (Spielman 2001, Fonseca et al. 2004). Indeed, microsatellite analysis has demonstrated that the U.S. Cx. pipiens populations contain a relatively large number of hybrids with signatures of European forms of pipiens and molestus (Fonseca et al. 2004). However, in an examination of populations from the northeastern United States, the number of hybrids was shown to be considerably lower when a U.S. population of Cx. pipiens f. molestus from New York City was used to identify hybrids (Huang et al. 2008).

Microsatellite genotyping and blood meal analyses of engorged mosquitoes from Washington DC and Maryland, suggest that the probability of ancestry from Cx. pipiens f. molestus on humans (Kilpatrick et al. 2007). Such studies on the genetic predisposition and its relevance to the host-feeding behavior of Cx. pipiens from the metropolitan Chicago area have not been conducted. The current research initiative was undertaken to examine genetic variations among a relatively large number of blood-fed mosquitoes acquiring blood meals from avian and/or mammalian hosts in order to examine the potential influence of introgressions in the host-feeding behavior of Cx. pipiens in this region for the first time.

Materials and Methods

Details on mosquito collection at study sites in metropolitan Chicago and blood-meal identification are described elsewhere (Hamer et al. 2008b). The sample included 346 Cx. pipiens f. pipiens mosquitoes, identified to species by polymerase chain reaction (Hamer et al. 2008b) including 254 that pipiens f. pipiens mosquitoes, identified to species by polymerase chain reaction (Hamer et al. 2008b) including 254 that

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele no.</th>
<th>HE</th>
<th>HO</th>
<th>FIS</th>
<th>Allele No.</th>
<th>HE</th>
<th>HO</th>
<th>FIS</th>
</tr>
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<tbody>
<tr>
<td>CxqTri4 F/R</td>
<td>5 (4.9)</td>
<td>0.57</td>
<td>0.53</td>
<td>0.06</td>
<td>5 (5.0)</td>
<td>0.54</td>
<td>0.55</td>
<td>-0.02</td>
</tr>
<tr>
<td>CxqGT6b F/R</td>
<td>7 (5.3)</td>
<td>0.71</td>
<td>0.67</td>
<td>0.05</td>
<td>6 (5.0)</td>
<td>0.70</td>
<td>0.62</td>
<td>0.10</td>
</tr>
<tr>
<td>CxqGT4 F3/R</td>
<td>8 (5.9)</td>
<td>0.25</td>
<td>0.17</td>
<td>0.31</td>
<td>5 (4.0)</td>
<td>0.24</td>
<td>0.21</td>
<td>0.14</td>
</tr>
<tr>
<td>CQ11 F2/R3</td>
<td>13 (10.5)</td>
<td>0.73</td>
<td>0.25</td>
<td>0.63</td>
<td>10 (10.0)</td>
<td>0.75</td>
<td>0.30</td>
<td>0.57</td>
</tr>
<tr>
<td>CxqGT12 F2/R2</td>
<td>9 (8.6)</td>
<td>0.69</td>
<td>0.58</td>
<td>0.14</td>
<td>9 (10.0)</td>
<td>0.65</td>
<td>0.47</td>
<td>0.28</td>
</tr>
<tr>
<td>CxqGT4 F/R</td>
<td>9 (10.7)</td>
<td>0.74</td>
<td>0.69</td>
<td>0.08</td>
<td>8 (12.9)</td>
<td>0.73</td>
<td>0.67</td>
<td>0.08</td>
</tr>
<tr>
<td>CxqGT9 F2/R</td>
<td>15 (15.6)</td>
<td>0.85</td>
<td>0.81</td>
<td>0.05</td>
<td>12 (13.0)</td>
<td>0.89</td>
<td>0.77</td>
<td>0.11</td>
</tr>
<tr>
<td>CxqGT40 F/R</td>
<td>24 (24)</td>
<td>0.72</td>
<td>0.60</td>
<td>0.16</td>
<td>18 (23.6)</td>
<td>0.77</td>
<td>0.67</td>
<td>0.12</td>
</tr>
<tr>
<td>CxqGT46 F/R</td>
<td>20 (20.7)</td>
<td>0.88</td>
<td>0.71</td>
<td>0.09</td>
<td>17 (17.9)</td>
<td>0.87</td>
<td>0.61</td>
<td>0.26</td>
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<tr>
<td>CxqGT51 F/R</td>
<td>28 (29.2)</td>
<td>0.89</td>
<td>0.89</td>
<td>-0.02</td>
<td>22 (24.0)</td>
<td>0.89</td>
<td>0.90</td>
<td>-0.02</td>
</tr>
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Average 13.8 (13.5) 0.70 0.59 0.16 11.2 (12.5) 0.70 0.58 0.16

Numbers in bold indicate significant heterozygote deficiency after sequential Bonferroni correction (p < 0.05). Allele numbers in parentheses represent those adjusted to account for uneven sample size according to the rarefaction method. N, numbers of specimen genotyped; HE, expected heterozygosity; HO, observed heterozygosity; FIS, inbreeding coefficient; S. E., standard error.
Results

Microsatellite analyses of 346 Cx. pipiens using 10 polymorphic markers revealed that there were no significant differences in allelic richness between Cx. pipiens with avian- and mammalian-derived blood meals both before and after adjustment for uneven sample size (Table 1). Deviations from Hardy-Weinberg equilibrium were observed in six markers (Table 1); however, the patterns of conformity were not different between Cx. pipiens with avian- and mammalian-derived blood meals. No significant tests for linkage disequilibrium were observed in all the locus pairs after sequential Bonferroni correction. Pairwise $F_{ST}$ and $R_{ST}$ values indicated that there was no overall genetic differentiation between the Cx. pipiens f. pipiens that had fed on birds and mammals (Table 2).

Results of Bayesian clustering indicated that Cx. pipiens f. pipiens, Cx. pipiens f. molestus, and Cx. quinquefasciatus were

<table>
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<tr>
<th></th>
<th>Cx. quinquefasciatus</th>
<th>Cx. pipiens fed on birds</th>
<th>Cx. pipiens fed on mammals</th>
<th>Cx. pipiens f. molestus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cx. quinquefasciatus</td>
<td>0.3453</td>
<td>0.2726</td>
<td>0.2874</td>
<td>0.4940</td>
</tr>
<tr>
<td>Cx. pipiens f. pipiens fed on birds</td>
<td>0.3522</td>
<td>0.0012</td>
<td>0.1897</td>
<td>0.1843</td>
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<tr>
<td>Cx. pipiens f. pipiens fed on mammals</td>
<td>0.4970</td>
<td>0.1951</td>
<td>0.1797</td>
<td>0.0001</td>
</tr>
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</table>

Numbers above the diagonal are $F_{ST}$ values and those below the diagonal are $R_{ST}$ values. Numbers in bold are significant ($p < 0.001$) after sequential Bonferroni correction.

FIG. 1. Bayesian clustering analysis of Culex pipiens f. pipiens collected from Chicago suburban area. The yellow, green, and red colors represent Cx. quinquefasciatus, Cx. pipiens f. pipiens, and Cx. pipiens f. molestus cluster, respectively. Each mosquito is represented by a thin vertical line partitioned into three colored segments corresponding to the individual’s estimated ancestry in the three clusters. Mosquito populations and groups are separated by vertical black lines. (A) Genetic comparison between Cx. pipiens mosquitoes fed on avian and mammalian hosts. (B) Analysis of seasonal shift in genetic composition in engorged Cx. pipiens mosquitoes. Culex pipiens f. pipiens mosquitoes collected in each year are arranged in temporal order from June to October.
genetically distinct entities (Fig. 1A), as indicated by the $F_{ST}$ and $R_{ST}$ values as well (Table 2). Furthermore, a pattern of asymmetric gene flow was evident from $C.\text{pipiens f. molestus}$ and $C.\text{quinquefasciatus}$ populations to $C.\text{pipiens f. pipiens}$ population (Fig. 1A). This apparent unidirectional introgression from $C.\text{pipiens f. molestus}$ resulted in significant ancestry variation in the $C.\text{pipiens f. pipiens}$ population. On average, $Culex\text{pipiens f.}\text{pipiens}$ mosquitoes that had fed on mammals had significantly higher ancestry ($p < 0.001$), and there was a greater proportion of hybrids ($p < 0.01$) from $C.\text{pipiens f. molestus}$ than those that fed on birds (Figs. 1A, 2). Three specimens of $C.\text{pipiens f.}\text{pipiens}$ with mammalian-derived blood and none with avian-derived blood had molestus ancestry greater than 0.5, a value theoretically denoting half proportion of the genome is derived from the parental population.

No significant differences either in ancestry (Fig. 1A; average ancestry difference $= 0.003 \pm 0.002$, $p > 0.05$) or in proportion of hybrids (proportion test: proportion difference $= 0.06 \pm 0.03$, $p > 0.05$) from $C.\text{quinquefasciatus}$ were observed between mosquitoes that fed on birds and mammals. Additionally, the Bayesian clustering analysis showed no seasonal changes in proportion of $C.\text{pipiens f.}\text{molestus}$ and $C.\text{quinquefasciatus}$ ancestries nor in hybrid percentages (Fig. 1B).

**Discussion**

The present microsatellite analysis of genetic variation in a relatively large sample of blood fed $C.\text{pipiens}$, the principal vector of WNV in metropolitan Chicago (Hamer et al. 2008a, 2008b, 2009), suggests the importance of population substructure to bird or mammal host selection. Specifically, our results document a significant association between molestus hybrid ancestry in individuals and the presence of mammalian blood, reinforcing an earlier proposition that host selection by $C.\text{pipiens}$ mosquitoes is influenced by genetic predisposition (Kilpatrick et al. 2007).

Considerable geographic variation in host selection patterns of $C.\text{pipiens}$ populations from various regions in the United States has been reported (Apperson et al. 2002, 2004, Molaei et al. 2006, Savage et al. 2007, Hamer et al. 2008a). In the northeastern United States, most (>94%) blood meals were from birds (Apperson et al. 2002, Molaei et al. 2006). However, recent studies in more southerly and midwestern regions showed that a substantial number of individuals had fed on mammals, including humans: Washington DC and Maryland (13%) (Kilpatrick et al. 2006), Chicago (22.4%) (Hamer et al. 2008a), Tennessee (24%) (Apperson et al. 2004), and New Jersey (38%) (Apperson et al. 2004). Although no significant differences in genetic structure and degrees of hybridization were found among populations predominantly feeding on birds in the northeast (Huang et al. 2008), higher fractions of molestus ancestry were detected in the populations with a substantially greater percentage of mammalian-derived blood meals, particularly human, from Washington DC and Maryland (Kilpatrick et al. 2007). In our study, $C.\text{pipiens}$ specimens with mammalian-derived blood meals had a significantly higher proportion of molestus ancestry, suggesting an underlying genetic basis for mammalian versus avian host selection. Although the genetic mechanism is unknown, it would be of value to determine if it is a selectable phenotype, albeit likely a complex one.

FIG. 2. Analysis of ancestry and hybrid proportion in engorged $Culex\text{pipiens f.}\text{pipiens}$ mosquitoes with avian- and mammalian-derived blood from $C.\text{pipiens f. molestus}$. Average ancestry and hybrid proportion were plotted on the Y-axes to the left and right, respectively.
Because Cx. pipiens has been indicted as the sole (i.e., enzootic, epizootic, and bridge) vector of West Nile virus in the Chicago region (Hamer et al. 2008a), an analysis of the mechanisms influencing feeding on birds (virus amplifying hosts) and mammals (especially, humans) is warranted. Bridging transmission by this species would require flexibility in the phenotype, such that an earlier feeding on a viremic bird was followed by a later feeding on a human. Field evidence for a virus-infected Cx. pipiens feeding on a human has recently been published (Hamer et al. 2008a). Our data suggest that such a link is not accompanied by a seasonal shift to Cx. pipiens with marked molestus ancestry (Fig. 1B). Earlier reports on the blood-feeding behavior of several mosquito species indicate a temporal shift from avian to mammalian hosts, or from a number of avian species to different birds (Tempelis et al. 1965, 1967, Edman and Taylor 1968). A recent study by Kilpatrick et al. (2007) also made note of genetic determinants for a seasonal change in host-feeding of Cx. pipiens from avian to mammalian hosts (particularly humans) as determined by microsatellite analysis of engorged females collected from Washington DC and Maryland. However, the genetic composition showed no correlated seasonal trend. A seasonal shift from mostly American robin, a major host species to other birds, has also been reported in Connecticut (Molaei et al. 2006), but analyses of unengorged populations from the region showed no temporal population differentiation nor changes in molestus ancestry or hybrid percentages (Huang et al. 2008). Cx. pipiens from Chicago was shown to exhibit a similar pattern of decline in feeding on American robin followed by an increase in feeding on other bird species, particularly house sparrow, though no seasonal shift from bird-to-mammal feeding was noticed (Hamer et al. 2009). Our genetic analyses on the same populations did not reveal temporal changes in ancestries or hybrid percentages from Cx. pipiens f. molestus and Cx. quinquefasciatus. Host availability and abundance could potentially play a significant role in seasonal variations in host-feeding behavior, but further investigations are required to more precisely evaluate the contributions of these as well as other (e.g., genetic) factors.

Attempts to associate the pronounced mammalian blood-feeding behavior of Cx. pipiens with genetic composition are based on the occurrence of possible hybridization between the ornithophilic Cx. pipiens f. pipiens and the mammalophilic Cx. pipiens f. molestus. The latter form has been shown to be an aggressive human biter in Europe (Harbach et al. 1984). However, the feeding preference and geographic distribution of molestus populations in the United States have not been thoroughly investigated. The feeding behavior of hybrid populations has only been studied in a mixed population containing both forms in Boston, Massachusetts (Spielman 1964, 2001), where 6 of 353 mosquitoes (1.7%) were reportedly heterozygotes for autogeny (Spielman 1964), consistent with low degrees of hybridization demonstrated in other Cx. pipiens populations in northeastern United States (Huang et al. 2008). However, 3 of 9 and 6 of 13 human-biting mosquitoes were identified as heterozygotes in the aforementioned studies (Spielman 1964, 2001). Our results further indicate a positive correlation between genetic ancestry and mammalian blood-feeding behavior in Cx. pipiens. Collectively, the limited extent of hybridization between Cx. pipiens f. pipiens and Cx. pipiens f. molestus can be considered a contributing genetic factor that may influence host-feeding behavior.

We did not identify an association between Cx. quinquefasciatus ancestry and mammalian blood-feeding by Cx. pipiens f. pipiens. This was most likely due to the locations of our collection sites (41°-42° N) that were well beyond the recognized sympatric zone (36° N and 39° N latitude), where Cx. pipiens f. pipiens and Cx. quinquefasciatus extensively hybridize (Barr 1957, Urbanelli et al. 1997). A recent study on mosquitoes collected from Champaign, Illinois (40° 05’ N), which is approximately 160 km south of our sampling sites, detected 7.5% hybrids by DV/D ratio (that is the relative positions of the dorsal [D] and ventral [V] arms of male phallosome), suggesting that the hybridization zone may be wider than previously thought (Sanogo et al. 2008). Our microsatellite analysis detected approximately 5.5% hybrids, which may represent a gradient penetration of Cx. quinquefasciatus alleles into the northern edge along the north-south axis. Blood-meal analysis of Cx. pipiens complex mosquitoes from Memphis, Tennessee did not reveal greater mammalian blood-feeding among hybrids of Cx. pipiens and Cx. quinquefasciatus (Savage et al. 2007), and the impact of hybridization was not assessed.

In conclusion, our microsatellite analyses indicate the presence of heterogeneity in the Cx. pipiens population. Our results also suggest that the probability of genetic ancestry from Cx. pipiens f. molestus may predispose mosquitoes to feed more readily on mammals, although the genetic mechanisms are not known. The impact of extensive gene flow on the population structure of this species and its implications for blood-feeding behavior and vectorial capacity merit further investigation. These studies will help clarify the role that Cx. pipiens plays in both enzootic and epidemic transmission of arboviruses including WNV.

Acknowledgments

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No competing financial interests exist.

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Apperson, CS, Harrison, BA, Unnasch, TR, Hassan, HK, et al. Host-feeding habits of Culex and other mosquitoes (Diptera: Culicidae) in the Borough of Queens in New York City, with


