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Genetic population structure of West Nile Virus vector Mosquitoes

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Abstract

Culex mosquitoes are considered as one of the most important vectors of West Nile virus (WNV) and other arboviruses detected in at least 34 species of mosquitoes in the United States. This review paper summarizes previous studies on the genetic diversity of West Nile Virus vectors and focuses on population structure. In addition, it also attempt to review significant information about molecular markers used in investigating the geographical and temporal patterns of genetic diversity in *Culex* mosquitoes. Genetically independent markers are the best strategies for the correct identification of population demes, gene flow and species relationships when working with *Culex* mosquitoes. The apparently low or restricted gene flow of mosquito vectors may be due to the large geographic distance or isolation by distance and physical barriers to dispersal may explain the spatial pattern of current genetic diversity in some *Culex* species. On the other hand, other studies where gene flow is evident, the recognition of the existence of gene flow between populations provides useful information on their potential, and possibly of the infectious agent they transmit. The genetic structure observed in this study may lead to the best understanding of their genetic variations for the development of effective strategies for vector control.

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Introduction

The mosquito, *Culex pipiens* Linnaeus (Diptera: Culicidae), is a ubiquitous species that colonizes a large variety of biotopes throughout temperate and tropical countries. It is usually considered as a complex including: *C. p. pipiens* Linnaeus, *C. p. quinquefasciatus* Say, C. p. *pallens* Coquillett and *C. p. molestus* Forska (Knight, 1978). These are morphologically, physiologically, ecologically and behaviourally different. *Culex p. pipiens* (temperate type) and *C. p. quinquefasciatus* (tropical type) are the most widely found members and are both closely associated with human activity. They are major vectors of *Wuchereria bancrofti* and West Nile virus (Anderson *et al.,* 1999).

Mosquitoes of the *Culex pipiens* complex are distributed all over the world and are of great fundamental, medical and veterinary importance as active bloodsuckers and vectors (Vinogradova, 2000). They are anautogenous, eurygamous and diapausing during wintertime. After diapause, females lay egg batches of 150-240 eggs on the water surface where the larvae hatch within one or two days. Depending on climate conditions larval development takes one week up to several weeks with several generations per year (Becker *et al.,* 2010). The larvae of *Cx. pipiens* can be found in nearly every natural, artificial, permanent or semi-permanent water body as well as in rural or urban areas (Weitzel *et al.,* 2009).

Studies indicated that *Culex quinquefasciatus* originated in Southeast Asia and then established in the New World through slave ships and colonized Africa (Fonseca *et al.,* 2006). Gravid *Cx. quinquefasciatus* females lay a single egg raft averaging 155 eggs during each gonotrophic cycle; the number of eggs depends on mosquito age, blood source and blood volume (Subra, 1981). Egg rafts are laid on the surface of a suitable water body selected, using the chemical cues derived from the conspecific egg rafts (Laurence and Pickett, 1985). Larval to adult development is dependent on temperature, nutrition and population density and can be as short as at 7 days under optimal conditions (30°C) (Rueda, 1990). Females mate within 2-6 days of emergence and may begin to seek hosts within 48 hours of emergence. The duration of larval stages was 118 hours for males and 135 hours for females. Females of *Cx. quinquefasciatus* emerge in large number than males in the regions where the seasons are more distinct. Since this mosquito must require blood meal for reproduction and does not undergo a reproductive diapause, hence this species is active and reproduces year round (Bhattacharya and Basu, 2016).

Recent analyses have shown a high degree of hybridization between these forms in North American populations relative to European *Cx. pipiens f. pipiens* populations (Fonseca *et al.,* 2004). Hybridization between these forms has been shown to negatively impact host specificity and increase vector capacity to transmit WNV to humans (Ciota *et al.,* 2013). These hybrids can therefore act as bridge vectors transmitting zoonotic agents between birds and mammalian hosts, particularly humans (Huang *et al.,* 2009).

Birds are the main reservoir hosts of WNV, and mosquitoes are the main vector for the virus transmission from birds to humans, horses, and other birds. WNV epidemics mainly occur in summer and autumn in temperate, subtropical, and tropical areas. Because WNV is highly influenced by regular, seasonal climate, and environmental changes, it is particularly amenable to spatial and temporal analysis (Epp, 2009). During the mosquito season, mosquitoes become infected with the West Nile Virus primarily through bird-blood meals and then retransmit the virus to any one of multiple bird species, a cycle which amplifies the virus. Governed by environmental conditions and host behaviors, infected mosquitoes can spread WNV to other incidental hosts, such as humans and horses. Endemic to Africa, Asia, Europe, Australia, and now the Caribbean and the Americas, the origin of the original strain of WNV is the Middle East, but the mode of introduction is unknown (Rainham, 2004).

In the United States, WNV spread from east to west, but since 2001, the Southern states have usually been

affected earlier in the season than the North because of the warmer weather (Nolan, 2013). The recent unusually mild winters, early springs, and early summers have aided transmission of the virus in Texas in the summer of 2012. After seeing the worst toll from West Nile, Texas declared a state of emergency, which has reached 270 cases and 11 deaths in Dallas County alone. The increasing occurrences of warmer and wetter weather patterns in the northern United States have increased the mosquito life span leading to increased incidence of WNV infections (Ghosh *et al.*, 2010). The southward dissemination of WNV into the Caribbean and Central and South America is attributed to migratory birds (Jacob *et al.,* 2009)

There has been much research on *Culex mosquitoes* throughout the world on various subjects, such as population genetic structure. This review paper summarizes previous studies on the genetic diversity of West Nile Virus vectors and focuses on population structure. In addition, it also attempt to review significant information about molecular markers used in investigating the geographical and temporal patterns of genetic diversity in *Culex* mosquitoes. Understanding the distribution of these vectors can help improve viral surveillance activities and mosquito control efforts. Therefore, a better knowledge of the genetic structure of these insect populations is required for the development of effective strategies for vector control.

The Population Structure of Mosquito Vectors

In predicting the dynamics of disease epidemics, it is significant to understand key factors linked to transmission through the vector such as the biological diversity involved, the population dynamics of these species, and the spatial extent of their population. Population genetic studies can provide insight on many of these aspects. For example: Population structure and transmission at a lower taxonomic level, the subdivision of the vector into discrete populations (population structure) will also affect the scale at which transmission occurs. Indeed, different population structures can alter disease transmission parameters and modify predictions of disease dynamics (Wonham *et al.,* 2006). As in the case for cryptic vector species, this isolation can lead to inter population differences in competence, virulence, resistance, etc. Gene flow can provide us with estimates of the distance, direction and rate of gene flow between discrete populations.

If gene flow is high, the vector may have a high probability of colonizing new areas or recolonizing sites where local control programmes were successful at eradication. Patterns of gene flow across populations can also provide indications of dispersal mechanisms. For example, if population divergence gradually increases with geographic distance (*i.e.*, isolation by distance), natural dispersal of the vectors between neighbouring populations may be occurring. Depending on the biology of the organism, alternative patterns may suggest that other dispersal mechanisms are operating, including anthropogenic sources (*e.g.*, vector migration via human transportation systems).

Tools for Measuring Population Structure

Some of the more useful measures of population subdivision are the F-statistics developed by Wright (1965). F-statistics can be thought of as a measure of the correlation of alleles within individuals and are related to inbreeding coefficients. F_{IS} and F_{ST} which describe how genetic variation is distributed in a subdivided population (Wright, 1951; Weir & Cockerham, 1984).

F_{IS} is the inbreeding coefficient, which measures the departure from random mating within subpopulations, and can take values from strongly negative (total outbreeding) and (total inbreeding). It results from the sampled population being genetically further subdivided (Wahlund, 1928), or more rarely, from active mate choice favoring relatives. F_{IS} represents the combination of both genetic viscosity and mating between relatives as we cannot, based on the available data, discriminate between the two. F_{ST} is a measure for genetic differentiation among subpopulations, and can take values from 0 to 1.

Use of Genetic Markers in Assessing the Genetic Structure

Describing the genetic population structure of a particular species over a specific geographic region requires genetic studies using some form of genetic/molecular markers. Each molecular marker provides information on the historical demography of viral vectors at different points in time. Mutation rate(s) for mitochondrial genes are usually higher than for nuclear genes, but slower than for microsatellite loci. Also, the recombination rate, mode of inheritance and genome location must all be considered for adequate resolution of different evolutionary processes (Wandeler *et al.,* 2007; Dixit *et al.,* 2011). Agreement between different markers may provide a robust perspective on population structure, especially if they operate under different evolutionary constraints (Linton *et al.,* 2003; Reidenbach *et al.,* 2009). On the other hand, discrepancies can emerge when using the total-evidence approach (i.e., concatenating multiple loci), as different genes may depict different molecular signals indicative of natural selection, introgression or lack of marker resolution (Sallum *et al.,* 2007).

Frequently used types of markers include mitochondrial DNA, microsatellites, allozymes, and single nucleotide polymorphisms (SNPs) (Estoup and Angers, 1998; Morin *et al.*, 2004). It is important to stress that direct genetic analyses are necessary for conclusions regarding the genetic population structure.

Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been widely used to answer questions about molecular taxonomy, phylogenetic relationships and population structure mosquitoes (Table 1). High copy numbers and the availability of conserved primers and PCR protocols make mtDNA an ideal starting point to investigate genetic diversity (Moreno *et al.,* 2010). To allow a good comparison of the studies based on mtDNA, the sequences available from data banks should be of similar size and from the same region of the gene(s). However, in species with such complicated demographic histories, the use of mtDNA markers may not be the most relevant choice.

Indeed, variation at mtDNA only reflect the demography in terms of the maternal line; it could be also considered as an unique locus, so the accuracy of the information given is thus more sensitive to stochastic events such as population bottlenecks. In addition, it could be affected by either direct selection on mitochondrial genes or by linkage disequilibrium with any other selected cytoplasm component such as symbiotic or parasitic bacteria (Hurst and Jiggins, 2005).Mitochondrial DNA is better to detect large-scale geographical differences due to maternal inheritance, Ne (effective population size) for mtDNA is a quarter of that of nuclear markers, and genetic drift may produce a strong signal of spatial population processse (i.e., migration)

Table 1. Summary of Studies on the genetic diversity of West Nile Virus Vectors, Culex mosquitoes.

Species	Markers	Genetic Population structure	References
Cx. pipiens s.l	Microsatellite	Lack of latitudinal influence on the population structure; *Little to Moderate Temporal variation	Edillo F. *et al.* 2009. Effects of latitude and longitude on the population structure of Culex pipiens s.l., vectors of West Nile virus in North America
Cx. p. quinquefasciatus	Microsatellite	Low genetic variability among geographic samples	Nayar J. *2003. Temporal and geographic genetic variation in Culex pipiens quinquefasciatus (Diptera: Culicidae) from Florida.*
Cx. pipiens	Allozyme	Moderate genotypic variation	Cui F. *et al.* 2007 Genetic differentiation of Culex pipiens (Diptera: Culicidae) in China. Bull. Entomol. Res. 2007, 97, 291-297.
Cx. pipiens & Cx. torrentium	Mitochondrial DNA	Moderate genetic differentiation	Werblow *et al.* 2014. Population structure and distribution patterns of the sibling mosquito species Culex pipiens and Culex torrentium (Diptera: Culicidae) reveal different evolutionary paths.

Species	Markers	Genetic Population structure	References
Cx. quinquefasciatus	Microsatellite	Moderate genetic structuring	Wilke A. *et al.* 2014. Population genetics of neotropical Culex quinquefasciatus (Diptera:Culicidae). Parasites & Vectors.
Cx. pipiens & *Cx. restuans*	Microsatellite	Little genetic differentiation between geographical distance; *Little genetic differentiation between years	Kilpatrick A *et al.* 2010. Spatial and temporal variation in vector competence of *Culex pipiens* and *Cx. restuans* mosquitoes for West Nile virus.
Cx. pipiens	Microsatellite	Little genetic differentiation between rural and urban areas	Huang S *et al.* 2008. Genetic insights into the population structure of *Culex pipiens* (Diptera: Culicidae) in the northeastern United States by using microsatellite analysis
Cx. pipiens	Microsatellite	*No temporal genetic changes/variation	Huang S, Molaei G, Andreadis TG, 2008. Genetic insights into the population structure of *Culex pipiens* (Diptera: Culicidae) in the northeastern United States by using microsatellite analysis. *Am J Trop Med Hyg 79:* 518 - 527
Cx. pipiens	Microsatellite	*No temporal genetic changes/variation	Huang S. 2009. Genetic variation associated with mammalian feeding in Culex pipiens from a West Nile virus epidemic region in Chicago, Illinois.
Cx. p. quinquefasciatus	Allozyme	Little genetic differentiation between geographical distance;*Minimal population substructuring	Nayar J. *et al. 2003. Temporal and geographic genetic variation in Culex pipiens quinquefasciatus (Diptera: Culicidae) from Florida.*
Cx. quinquefasciatus & Cx. tarsalis	mitochondrial DNA	Little genetic differentiation	Rainham D. 2004. Ecological complexity and West Nile virus: Perspectives on improving public health response. Canadian Journal Public Health
Cx. pipiens & Cx. torrentium	Allozyme	Little genetic differentiation; lack of migration barriers	Weitzel T. 2009. Genetic differentiation of populations within the Culex pipiens complex and phylogeny of related species.
Culex tritaeniorhynchus	Allozyme	Little genetic differentiation	Kanojia PC *et al.* 2010. Morphometric and allozyme variation in Culex tritaeniorhynchus mosquito populations from India
Cx. pipiens & Cx. torrentium	mitochondrial DNA	Moderate to very great between populations	Werblow A *et al.* 2014. Population Structure and Distribution Patterns of the Sibling Mosquito Species *Culex pipiens* and *Culex torrentium* (Diptera: Culicidae) Reveal Different Evolutionary Paths.
Culex quinquefasciatus	mitochondrial DNA	Little genetic differentiation	Morais S *et al.* 2012. Low genetic diversity in Wolbachia-Infected *Culex quinque* fasciatus (Diptera: Culicidae) from Brazil and Argentina.
Culex quinquefasciatus	mitochondrial DNA	Low genetic differentition	Fonseca D. 2006. Pathways of expansion and multiple introductions illustrated by large genetic differentiation among worldwide populations of the southern house mosquito
Culex quinquefasciatus	mitochondrial DNA	Low genetic diversity	Low V. 2014. Mitochondrial DNA analyses reveal low genetic diversity in Culex quinquefasciatus from residential areas in Malaysia.
Cx. quinquefasciatus	*mitochondrial DNA and microsatellite*	Great/Large genetic differences	Pfeiler E *et al.* 2013. Genetic diversity and population genetics of mosquitoes (Diptera: Culicidae: Culex spp.) from the Sonoran Desert of North America.
Cx. quinquefasciatus	*mitochondrial DNA and microsatellite*	Moderate genetic differentiation	Fonseca *et al.* 1998. Microsatellite primers for *Culex pipiens* quinquefasciatus, the vector of avianmalaria in Hawaii

Microsatellite loci

Microsatellite loci have been widely developed and used primarily to estimate patterns and rates of gene flow of Culex mosquitoes (Table 1). Contemporary levels of gene flow among Culex and other mosquito populations are generally assessed using microsatellites because, due to their high mutation rate, they can detect differentiation even in weakly structured species (Temu *et al.,* 2004; Huang *et al.,* 2008; Kilpatrick *et al.,* 2010; Wilke, 2014) In contrast, large-scale geographical differences are better detected using mtDNA polymorphisms because, due to maternal inheritance, Ne for mtDNA is a quarter of that of nuclear markers, and genetic drift may produce a strong signal of spatial population processes (i.e., migration) (Chen *et al.,* 2004). Nonetheless, the mutation rate for mtDNA is slower than for microsatellites, thus erroneous estimates of patterns and rates of contemporary gene flow could be obtained under the phylogeographic framework, as mtDNA may portray signals of more ancient demographic processes (Foley and Torres 2006; Reiff *et al.,* 2007). Microsatellites can also assess levels of gene flow among *Culex* populations due to their high mutation rate that can detect differentiation even in weakly structured species.

Allozyme

The allozyme technique for species diagnostic allele distribution can be applied to most kinds of organisms including *Cx. quinquefasciatus* (Table 1). The reliability of marker systems is obvious by their banding pattern, and is guided by the use of reference samples. The inheritance of cytoplasmic enzyme-genes follows Mendelian rules. The allele combinations (genotypes) are usually readable in the banding pattern. The technique of allozyme eletrophoresis has been successfully and widely used to assist in resolving taxonomic problems and to infer on genetic relationships (Thorpe & Solé-Cava, 1994).

Most empirical population genetic studies have been based on genetic markers assumed to primarily reflect selectively neutral DNA variation (most allozymes, microsatellites and mtDNA markers). Recent molecular developments now provide increased opportunities for studying markers known to be located within functional genes of potential importance for fitness (Leukart *et al.,* 2003). Neutral markers are expected to reflect the evolutionary processes of mutation, random genetic drift, and gene flow that act on the whole genome, making these markers particularly suitable when studying genetic structure and reproductive relationships. In the other hand, studying loci that are under selection may provide locus-specific information of relevance for the understanding of local adaptation and natural selection on the genes assayed. It is important to realize, however, that a genetic pattern observed at a locus under selection may be valid only for that particular locus. Selective forces acting on other loci may result in disparate differentiation pattern. Similarly, it may often be statistically demanding to demonstrate unambiguously that a particular genetic marker shows signs of selection.

Useful genetic markers are expected to have several key features: selective neutrality, ease of scoring in all specimens of the species and sufficient variability to allow for measures of genetic differentiation, and genetic clustering of individuals. They also should be robust to re-genotyping and/or allow comparison with genotypes from new samples (Lowe *et al.,* 2004). Depending on the purpose, the selected markers should be suitable for phylogeography analyses (such as mitochondrial DNA), allow interpretation about the breeding structure (such as codominant allozymes or microsatellites) or be sufficiently numerous in the genome to tease apart the effects of demographic history from those of natural selection (Lowe *et al.,* 2004; Stapley *et al.,* 2010).

Genetic Population Structure

Gene flow due to geographic distance, also known as isolation by distance (IBD), is an equilibrium model based on the hypothesis that gene flow is operating among neighboring popula tions, with the goal to identify the geographical distance that makes them genetically distinct. Under IBD, random mating is more likely to occur between mosquitoes in nearby

sites than those more distantly located (Wright, 1951; Jensen *et al.,* 2005).

A study conducted by Edillo *et al.* (2009) on the effects of latitude and longitude on the population structure of *Culex pipiens* s.l., in North America using microsatellite loci showed a strong population structure between *Cx. p. pipiens* and *Cx. p. quinquefasciatus* existed. Among *Cx. p. pipiens,* a 100-km increase in the latitudinal change resulted in an increased square root of F_{ST} by 0.002. A 100-km increase in the longitudinal change caused an increased square root of F_{ST} by 0.035. A lack of latitudinal influence on the structure between *Cx. p. pipiens* populations suggests a uniform signal using the 12 microsatellite markers, which might increase the risk of West Nile virus (WNV) transmission toward northern areas because of longer breeding season, extend hostseeking period, and larger population size.

Another study Geographic Genetic Variation in *Culex pipiens quinquefasciatus* (Diptera: Culicidae) from Florida by Nayar *et al.* (2003) at 12 enzymes (10 "neutral" gene enzymes with 11 putative loci and two "complex" gene enzymes) showed low genetic variability was also observed among geographic samples of *Cx. p. quinquefasciatus* collected from Florida. Similar results were reported by Cheng *et al.* (1982) on their analysis of 17 neutral gene loci from Texas, Louisiana, and Florida samples. Gene flow estimates based on F_{ST} =0.05, indicating low levels of gene flow among the geographic samples of *Cx. p. quinquefasciatus*.

Cui *et al.* (2007) on his study of the population genetic structure of the *C. pipiens* complex (*C. p. quinquefasciatus* and *C. p. pallens*) in China using polymorphic allozyme loci which was carried out at different scales over a south-north transect across the country to determine genetic differentiation and isolation by distance. The overall genotypic differentiation found across China was moderate (F_{ST} = 0.059) and highly significant. This genetic variation was partially explained by distance, as a significant increase of differentiation was found with geographic distance. The intra-province genotypic differentiation within each province was low but significant. Results showed that the overall genotypic differentiation across 20 Chinese *C. pipiens* populations was moderate (Fst = 0.059), despite the maximum distance between the populations being about 2000km and there being isolation by distance at this scale On a regional scale (intra-province), a low (F_{ST} = 0.007-0.016) and significant genetic differentiation was found, with no clear geographical pattern. On a wider scale (inter-province), the genetic differentiation was higher (F_{ST} = 0.059), and an isolation by distance emerged. The results are compared with previous population genetic surveys of this mosquito species in different geographic areas over the world. The overall pattern suggests that Culex pipiens requires considerable distance (500-1000 km) to show isolation by distance, irrespective of the subspecies (*C. p. pipiens*, *C. p. quinquefasciatus* and *C. p. pallens*) or the geographic location.

Werblow *et al.* (2014) on the population structure and distribution patterns of the sibling mosquito species *Culex pipiens* and *Culex torrentium* (Diptera: Culicidae) using mitochondrial DNA showed indications of genetic differentiation between populations from central and eastern Germany within *Cx. pipiens* and also some indications for strong genetic differentiation between populations from the western parts of Germany and central and east-German populations of *Cx. torrentium*. It is also obvious that there is a moderate differentiation between western and eastern populations of *Cx. pipiens* in Germany. The reason for this might be that *Cx. pipiens* occurs in two bioforms (*Cx. pipiens* form *pipiens* and Cx. *pipiens* form molestus) and that these bioforms differ in their relative abundance in different parts of Germany. Isolation by distance was then using distance based redundancy analysis (dbRDA). However, only a very low proportion of the genetic variation could be significantly explained by geographical distance; the spatial coordinates only explained 2% of the genetic variability in *Cx. pipiens* and 5% of the genetic variation within *Cx. torrentium*.

Another study conducted by Bruno *et al.* (2014) on the population genetics of neotropical *Culex quinquefasciatus* polymorphic microsatellite markers showed an Fst mean value was 0.12 indicating moderate genetic structuring, pairwise Fst value comparisons between populations ranged from 0.08 to 0.29 and all of them were statistically significant ($P < 0.01$). Population structure comprised a clear North-south dichotomy in clustering. Such interpretation is in accordance to Morais *et al.* (2010) who first noted that this species varies geographically in the Neotropics using morphometric wing characteristics. The presence of correlation between genetic and geographic distances suggests that genetic isolation by distance might occur but considering that samples came from different biomes, ecological components are also an influential factor on population structure genetic characteristics.

Spatial and temporal variation in vector competence of *Culex pipiens* and *Cx. restuans* Mosquitoes using polymorphic microsatellite markers conducted by Kilpatrick *et al.* (2010) revealed some evidence for genetic differentiation between mosquitoes that became infected with WNV after feeding on infected blood (susceptible) and those that did not become infected (resistant) in Suffolk County, NY *Cx. pipiens* mosquitoes (Fst = 0.0203) and Staten Island, NY *Cx. pipiens* mosquitoes (Fst = 0.0135). However, there was little evidence of genetic differentiation between mosquitoes that had either disseminated infections or transmitted WNV (F_{ST} values for three county-year comparisons = -0.0483 to 0.0092), which was partly caused by the smaller sample size of disseminated and transmitting mosquitoes in these comparisons.

In their study, different temperatures and other environmental factors at the different sites may have affected the parental generation that laid the egg rafts collected for our experimental vector competence assays physiologically and by altering the genetics and phenotypes of mosquito populations. This possibility was supported by the significant genetic differentiation between counties, and these differences in turn might have influenced vector competence despite our rearing all larvae and maintaining all adults at one temperature (30°C). Although our collections of mosquito populations were not conducted frequently enough to enable determination of influences of temperature or other environmental influences on vector competence, suggestive evidence has been observed in previous studies with seasonal patterns of susceptibility to infection (Reisen *et al.,* 1996; Hardy *et al.,* 1990).

More broadly, their results suggest that vector competence is not a static intrinsic trait of a particular mosquito population, and spatial variation within a species can be larger than between species. Instead, their study suggests that vector competence of a mosquito population can vary over time and appears to be dependent on intrinsic and extrinsic influences, such as environmental and genetic factors, and possibly their interaction. This finding is interesting because temperature has received substantial attention as a determinant of the geographic distribution and transmission intensity of particular vector-borne diseases, and vector competence has been suggested as one possible contributing factor (Reisen *et al.,* 1991; Rogers and Randolph, 2006).

It remains to be determined whether temporal variability in vector competence and not just susceptibility to infection can be consistently linked to environmental factors in a predictive manner. Temperature is already known to have an impact on survivorship, feeding frequency, immature developmental rates, and vector competence directly, 31, 60, 61 all of which affect vectorial capacity (Delatte *et al.,* 2009; Rueda *et al.,* 1990).

Badillo *et al.* (2011) on the distribution of potential West Nile virus vectors, *Culex pipiens pipiens* and *Culex pipiens* quinquefasciatus (Diptera: Culicidae), in Mexico City using microsatellite markers revealed that *Culex pipiens quinquefasciatus* hybrid mosquitoes were found in all six collection sites. Furthermore, hybrid mosquito densities were qualitatively higher during the rainy season. Hybrids were not observed throughout the year.

Notably they were not detected in the dry months (January to May). Two peaks in abundance of hybrid mosquitoes were observed : the first in June and the second in August. Temperatures at the collection sites fluctuated from 8°C to 23°C. Hybrids were found at temperatures from 12.5°C to 17°C.

Nayar *et al.* (2003) conducted a study on the temporal and geographic genetic variation in *Culex pipiens quinquefasciatus* (Diptera: Culicidae) from Florida at 12 enzymes (10 "neutral" gene enzymes with 11 putative loci and two "complex" gene enzymes) showed low value of *F*st of 0.058 indicated minimum population substructuring among the temporal samples.

Huang *et al.* (2009) on the genetic variation associated with mammalian feeding in *Culex pipiens* from a West Nile Virus epidemic region in Chicago, Illinois using microsatellite markers revealed 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.

Physical barriers to dispersal may impede gene flow between mosquito populations like *Culex* complex, but only for those species that lack the capacity to traverse or circumnavigate them. Barriers to gene flow in *Cx. p. pipiens* populations may include both by geographical distance and topography, especially in species with low vagility. Mountainous terrain and river are particularly important barriers (Failloux *et al.,* 1997; Julvez *et al.,* 1990). This ability will not only depend on the species ecology (i.e., flight range and the type of breeding sites), but also on the geographic extent and the shape of such barriers. Physical barriers may act in conjunction with other climatic or biological barriers and their effects might vary over time, thus making it difficult to assess their real input to genetic exchange (Fairley *et al.,* 2002; Muturi *et al.,* 2010). Mountain ranges (cordilleras), oceans, rivers and forests can hamper gene flow for mosquitoes according to different molecular markers (Table 1). Ocean barriers might have also been the initial driving force causing diversification between mosquito species (Coetzee *et al.,* 1999).

The population structure of *Cx. pipiens* might also be explained by other reasons such as a recent bottleneck. Both of these events could be the result of genetic drift within certain populations and could lead, as seen in the case of *Cx. pipiens*, to a reduced overall genetic variability.

Conclusion

Since dispersal and demographic changes are difficult to track in small organisms such as *Culex* mosquito vectors, population genetics analyses play very significant role to inform researchers and the general public about the various epidemiologically important characteristics. This compilation of various of researches illuminates the key features of the population genetics structure of *Culex* mosquitoes. Genetically independent markers are the best strategies for the correct identification of population demes, gene flow and species relationships when working with *Culex* mosquitoes.

The presumably low or restricted gene flow may be due to the large geographic distance or isolation by distance and physical barriers to dispersal may explain the spatial pattern of current genetic diversity in some *Culex* species. On the other hand, in other studies where gene flow is apparent, the recognition of the existence of gene flow between populations provides useful information on their potential, and possibly of the infectious agent they transmit.

The genetic structure observed in this study may lead to the best understanding of Culex demographic diversity as well as their genetic variations patterns. Moreover, understanding the distribution of these vectors can help improve viral surveillance activities and mosquito control efforts.

Analysis of the available data stresses that future population genetics studies in *Culex* mosquitoes should include a more exhaustive worldwide sampling in order to infer genetic structure. It is important, in particular, to know whether restricted gene flow really exists between tropical non-diapausing and temperate-diapausing populations in the native area, and if phenotypically similar populations share a genetic kinship.

Particularly, the photoperiodical diapause, which has a demonstrated genetic basis. The current availability of more informative markers and large collections of samples including different time periods would, however, make possible the investigation and modeling of invasion routes (Cristescu, 2015), and the comprehensive study of the genetic structuring of such vector.

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