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THE CONTRIBUTION OF *CULEX PIPIENS* COMPLEX MOSQUITOES TO TRANSMISSION AND PERSISTENCE OF WEST NILE VIRUS IN NORTH AMERICA

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ABSTRACT. Mosquitoes within the *Culex pipiens* complex have been implicated as major vectors of West Nile virus (WNV) in North America due to their seasonal abundance, vector competence and high field infection rates. However, the role of *Cx. p. pipiens* complex mosquitoes in enzootic amplification of WNV among avian hosts and epidemic transmission to humans varies throughout its geographical distribution. In the northeastern United States, *Cx. p. pipiens* is recognized as the primary enzootic vector responsible for amplification of virus among wild bird populations. However, because this mosquito is strongly ornithophilic, its role in transmission to humans appears to be more limited in this region. In the north central and Mid-Atlantic States by contrast, *Cx. p. pipiens* shows an increased affinity for human hosts and has been incriminated as a key bridge vector. In southern regions of the United States, *Culex p. quinquefasciatus* are more opportunistic feeders, and are thought to be principal enzootic and epidemic vectors. In western regions of the United States where *Culex tarsalis* predominates, especially in rural areas, *Cx. p. pipiens* and *Cx. p. quinquefasciatus* play roles that are more limited and are recognized as secondary vectors. In the southwestern United States *Cx. p. quinquefasciatus* also appears to be the predominant vector in urban habitats, but only a secondary vector in more rural environs. The direct involvement of *Cx. p. pipiens* form molestus in WNV transmission is largely unknown, but human-biting *Cx. p. pipiens* are more likely to have a probability of genetic ancestry with *Cx. p. pipiens* form molestus. The detection of WNV from overwintering populations of diapausing *Cx. p. pipiens* and non-diapausing *Cx. p. quinquefaciatus* and their role in local overwintering of WNV are addressed.

KEY WORDS *Culex pipiens*, *Culex quinquefasciatus*, West Nile virus, transmission, overwintering

INTRODUCTION

The detection and presumed introduction of West Nile virus (WNV) into the United States during the summer of 1999 (Anderson et al. 1999, Lanciotti et al. 1999) was a seminal event in realizing the potential threat of introduction and subsequent establishment of an exotic vector-borne disease in the western hemisphere. Within four years after its initial detection in New York City, this exotic virus, which was thought to have been introduced from the Middle East (Lanciotti et al. 1999), rapidly swept across the continental United States, moved north into Canada and southward into the Caribbean Islands and Central America to become the preeminent arboviral disease in North America. To date, WNV has caused over 30,000 cases of human disease and more than 1,000 fatalities in the United States alone (CDC 2010a, 2010b, 2011), and has clearly become a permanent part of the North American landscape causing seasonal epidemics. This unprecedented expansion and establishment of WNV in North America has been attributed to a number of factors including: (1) the emergence of a virus strain with greater virulence (Brault et al. 2004, 2007), transmission efficiency (Moudy et al. 2007) and epidemic potential (Davis et al. 2005); (2) the long-range movements of migratory birds (Peterson et al. 2003) and infected mosquito vectors (Venkatesan and Rasgon 2010); and (3) broad variety and widespread distribution of reservoir competent avian hosts (Komar 2003, Kilpatrick et al. 2007) and mosquito vectors (Turell et al. 2005). However, it is also quite likely that WNV may have never become established in North America where it not for the vectorial capacity and intimate involvement of urban *Culex* mosquito vectors within the pipiens complex.

Mosquitoes within the *Culex pipiens* complex are recognized as major vectors of WNV in North America due to their vector competence, high field infection rates, local abundance, and close association in time and space with virus foci and human cases. However, the role *Cx. pipiens* complex mosquitoes play in enzootic amplification of WNV among avian hosts and epidemic transmission to humans appears to vary widely throughout its geographical distribution. This review will examine regional differences in the role of *Culex p. pipiens* L., *Cx. p. quinquefaciatus* Say and their hybrids in transmission, local overwintering and long-term persistence of WNV in the United States based on national surveillance data compiled by the CDC ArboNet since 1999 and our current knowledge of their population biology and feeding behavior. The contribution of underground populations of *Cx.*

pipiens form molestus Forskal to the epidemiology of WNV in urban settings will also be explored.

REGIONAL ANALYSIS OF MOSQUITOES

Northeastern United States

A summary of mosquito pools that have tested positive for WNV from different regions of the US from 1999 to 2010 (CDC ArboNet) is shown in Table 1. Within the northeastern United States, where the first isolations were made in 1999 from *Culex p. pipiens* and *Aedes vexans* (Meigen) (Anderson et al. 1999, Nasci et al. 2001b), WNV has been identified from 33 species of mosquitoes representing eight different genera. However, over 96% of the positive pools have been obtained from *Culex* mosquitoes, among which 66% were from *Cx. p. pipiens,* 27.8% from *Culex restuans* Theobald, 6.1% from *Culex salinarius* Coquillett, 0.3% from *Culex erraticus* (Dyar and Knab), and < 0.1% from *Culex territans* Walker (Fig. 1). The preponderance of WNV positive pools obtained from field-collected *Cx. p. pipiens* and to a lesser degree, *Cx. restuans* clearly incriminate these two species as the most important vectors of WNV in the northeast. This conclusion is supported by their local abundance in virus foci and high minimum field infection rates revealed from mosquito surveillance conducted in Connecticut (Andreadis et al. 2001, 2004, Anderson et al. 2004, 2006, Andreadis and Armstrong 2007), Delaware (Gingrich et al. 2010), New York City (Kulasekera 2001) and New York State (Bernard et al. 2001, White et al. 2001, Ebel et al. 2005, Lukacik et al. 2006). Local regional populations of both species also have been shown to be moderately efficient vectors of WNV in the laboratory (Turell et al. 2000, 2001, 2005; Sardelis et al. 2001; Ebel et al. 2005).

The ornithophilic feeding behavior of populations of *Cx. p. pipiens* from the northeastern United States is well-established (Crans 1964, Means 1968, Spielman 1971, Tempelis 1975, Magnarelli 1977, Apperson et al. 2002, 2004, Molaei et al. 2006) and clearly support a major role for this mosquito in transmission of WNV to birds throughout the region (Table 2). *Culex p. pipiens* involvement in both early and late season enzootic transmission is largely based upon the detection of WNV in July when populations of *Cx. p. pipiens* are typically increasing and the preponderance of virus positive pools found in August and September, when virus activity and *Cx. p. pipiens* populations are at their height (Andreadis et al. 2001, 2004, Anderson et al. 2004, 2006, Andreadis and Armstrong 2007). Additional support for early season initiation of enzootic transmission by *Cx. p. pipiens,* comes from the detection of WNV from overwintering females collected from hibernacula in New York

Table 1. Generic summary of West Nile virus positive mosquito pools reported to CDC ArboNet from different regions of the United States, 1999–2010.

	Northeast[1]			Southeast[2]			Midwest[3]			West[4]			Southwest[5]		
Mosquito	No. species	No. WNV pools	%	No. species	No. WNV pools	%	No. species	No. WNV pools	%	No. species	No. WNV pools	%	No. species	No. WNV pools	%
Culex	5	15,683	96.1	8	14,697	96.2	6	27,028	97.9	8	13,902	98.9	10	7,582	97.3
Aedes	14	363	2.2	12	386	2.5	14	435	1.6	6	79	0.6	9	177	2.3
Anopheles	5	32	0.2	4	59	0.4	4	83	0.3	4	17	0.1	2	9	0.1
Coquillettidia	1	35	0.2	1	25	0.2	1	30	0.1	1	1	<0.1	-	-	-
Culiseta	3	185	1.1	2	60	0.4	2	7	<0.1	4	50	0.3	-	-	-
Deinocerites	-	-	-	1	2	<0.1	-	-	-	-	-	-	-	-	-
Mansonia	-	-	-	1	4	<0.1	-	-	-	-	-	-	-	-	-
Orthopodomyia	1	2	<0.1	1	1	<0.1	1	4	<0.1	-	-	-	-	-	-
Psorophora	3	12	0.1	3	43	0.3	2	10	<0.1	1	2	<0.1	3	25	0.3
Uranotaenia	1	6	<0.1	1	2	<0.1	1	6	<0.1	-	-	-	-	-	-

[1] CT, DC, DE, MA, MD, ME, NJ, NY, PA, RI, VT (1999–2010).
[2] AL, AR, FL, GA, KY, LA, MS, NC, SC, TN, VA, WV (2001–2010).
[3] IA, IL, IN, KS, MI, MN, MO, ND, NE, OH, SD, WI (2001–2010).
[4] CA, CO, ID, MT, NV, OR, UT, WA, WY (2002–2010).
[5] AZ, NM, OK, TX (2002–2010).

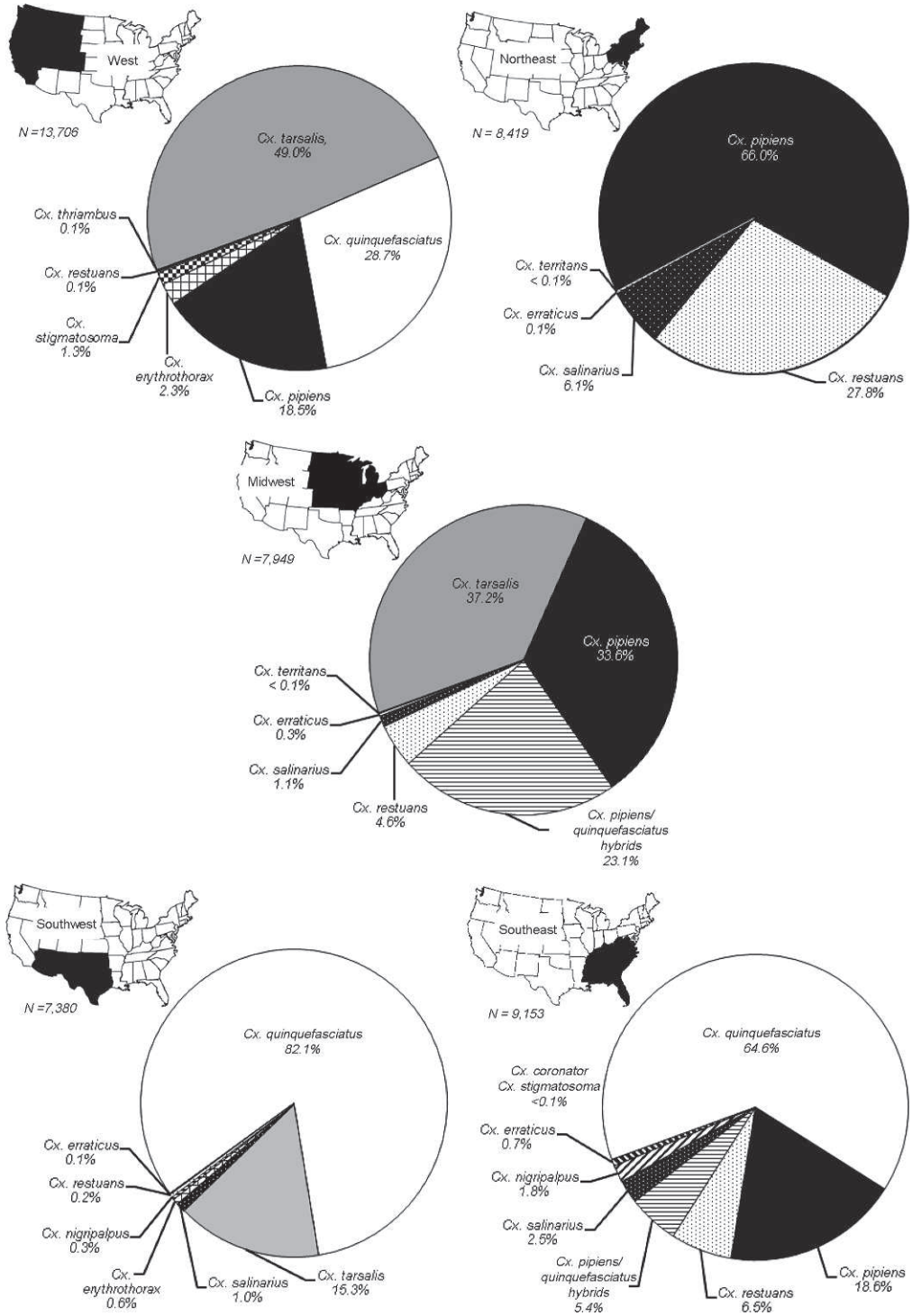

Fig. 1. Comparative proportion of West Nile virus positive mosquito pools obtained from field-collected *Culex* species reported to CDC ArboNet from different regions of the United States, 1999–2010.

Table 2. Role of *Culex p. pipiens* complex mosquitoes in enzootic and epidemic transmission of WNV in different geographic regions of the US based on the prevalence of virus detection among *Culex* mosquitoes and host feeding behavior.

Region	% WNV+ pools among *Culex*	Feeding behavior		Vectorial capacity for transmission	
		Bird	Mammal	Enzootic	Epidemic
Cx. p. pipiens					
Northeast	66.0	++++	+	high	low
Southeast	18.6	+++	++	moderate	low
Midwest	33.6	+++	++	moderate	moderate
West	18.5	++++	+	moderate	low
Cx. p. quinquefasciatus					
Southeast	64.6	+++	++	high	moderate
West	28.7	+++	++	moderate	moderate
Southwest	82.1	+++	++	high	high

City, New Jersey and Pennsylvania (Nasci et al. 2001a, Farajollahi et al 2005, Bugbee and Forte 2004, Andreadis et al. 2010), and documentation of vertical transmission of the virus by resident populations in the laboratory (Dohm et al. 2002, Anderson et al. 2008). The important role of *Cx. p. pipiens* in amplification of WNV in wild bird populations is further supported by the identification of avian communal roosts as amplification foci in urban centers in the Northeast during the transmission season (Diuk-Wasser at al. 2010), and the detection of significantly greater numbers of WNV-infected *Cx. pipiens* in traps placed in the tree canopy when compared to similar traps placed on the ground (Anderson et al. 2004, Andreadis and Armstrong 2007).

While the role that *Cx. pipiens* plays in enzootic transmission of WNV among wild bird populations is unequivocal, its involvement in epidemic transmission to humans in the northeastern United States appears to vary widely depending on the location. The majority of investigations on feeding patterns of this species clearly indicate that populations from Connecticut (Magnarelli 1977, Molaei et al. 2006), Massachusetts (Spielman 1971), New Jersey (Crans 1964) and New York (Means 1968, Tempelis 1975, Apperson et al. 2002, 2004), predominately feed on birds and rarely feed on humans. However, Apperson et al. (2004) identified mammalian-derived blood meals in 38% of blooded *Cx. p. pipiens*, 11% of which were human-derived, collected from natural and man-made resting sites in suburban areas of New Jersey, while Gingrich and Williams (2005) reported a high percentage (69%) of mammalian-derived blood meals (albeit no human) from populations in Delaware. Although apparently rare, human derived blood meals have been occasionally identified ($< 1\%$) in *Cx. p. pipiens* populations from urban sites in Connecticut as well (Molaei et al. 2006). Using a risk-assessment model that combined data on mosquito abundance, infection prevalence, vector competence, and biting behavior, Kilpatrick et al. (2005) estimated that local populations of *Cx. p. pipiens* and *Cx. restuans* from New Jersey and New York might be responsible for up to 80% of human infections in that region. In an investigation of populations from the mid-Atlantic region (Maryland and Washington DC), Kilpatrick et al. (2006) similarly implicated *Cx. p. pipiens* as a major epidemic vector based on a late-summer shift in feeding behavior from avian to mammalian hosts. The involvement of *Cx. p. pipiens* as an epidemic as well as epizootic vector thus appears probable, especially in densely populated urban areas where this species predominates. However, its contribution to epidemic transmission varies greatly, depending on regional differences in host feeding patterns (Table 2).

In addition to *Cx. p. pipiens*, it is appropriate to comment on the role of two other notable species of *Culex* mosquitoes that also have been incriminated as vectors of WNV in the Northeast, *Cx. restuans* and *Cx. salinarius*. The abundance of *Cx. restuans* in June and July, early season detection of WNV (Andreadis et al. 2001, 2004, Andreadis and Armstrong 2007) and high vectorial capacity (Ebel et al. 2005), support the supposition that this mosquito plays an important role as an enzootic vector involved in early amplification of WNV among wild birds in the northeastern US. In addition to being the most abundant *Culex* species at this time of the year, it is widely distributed throughout the region and it occurs in both urban and rural environs (Ebel et al. 2005). This conclusion is fully consistent with its well-documented ornithophilic feeding preferences (Means 1968, Magnarelli 1977, Apperson et al. 2002, 2004, Molaei et al. 2006). However, there are several reports in the literature (Hayes 1961, Murphey et al. 1967, Means 1968, 1987) indicating that although *Cx. restuans* prefers feeding on birds, females from this region will bite humans on occasion, and a human-derived blood meal has been identified from

blooded females collected in suburban New Jersey (Apperson et al. 2004). These findings taken in concert with the multiple isolations of WNV obtained from this species in August and September (Andreadis et al. 2001, 2004, Anderson et al. 2004, Andreadis and Armstrong 2007) do not preclude its involvement as a bridge vector to humans. However, because of its generally lower abundance at this time of the year, virus transmission to humans is likely to be relatively rare.

Culex salinarius is among the most frequently captured *Culex* species in coastal regions of the northeastern United States where a large majority of human cases occur, and is locally abundant in August and September when virus activity is at its height (Andreadis et al. 2001, 2004; Kulasekera et al. 2001; Anderson et al. 2004; Gingrich and Casillas 2004; Andreadis and Armstrong 2007; Rochlin et al. 2008). In contrast to *Cx. p. pipiens* and *Cx. restuans, Cx. salinarius* is a well-recognized generalist that feeds indiscriminately on both birds and mammals and readily bites humans (Crans 1964, Murphey et al. 1967, Means 1987). Studies by Apperson et al. (2002, 2004) with local populations from New Jersey and New York reaffirmed the wide-ranging feeding habits reported in these prior investigations; with identification of mammal to bird feeding ratios of 4:1 in blooded females collected from a WNV focus in Queens, New York in 2000, and 3:1 in blooded females collected from WNV endemic peri-urban areas in New Jersey in 2001 with 8.6% of the mammalian blood meals identified as human-derived. In an analysis of local populations from Connecticut, Molaei et al. (2006) similarly found that *Cx. salinarius* readily feeds on both birds (36%) and mammals (53%) including humans, and further detected mixed blood meals in 11% of these females, a necessary condition for transmission to humans. The frequent isolations of WNV from this species in late August and September (Andreadis et al. 2001, Bernard et al. 2001, Kulasekera et al. 2001, White et al. 2001, Anderson et al. 2004) when the majority of human cases were reported, in union with its abundance at this time of the year, broad feeding habits, and demonstrated vector competence (Sardelis et al. 2001) which equals that of *Cx. p. pipiens* (Anderson et al. 2012), make *Cx. salinarius* a likely bridge vector to humans, horses and other mammals in northeastern United States.

Southeastern United States

The first WNV positive mosquito pools reported from the southeastern United States were in 2001 (Blackmore et al. 2003, Rutledge et al. 2003, Godsey et al. 2005a), two years following the initial discovery in New York City. Since then, WNV has been detected in 34 species of mosquitoes in 10 genera (Table 1). However, as in the Northeast, the overwhelming majority of virus positive pools (96%) have been made from mosquitoes in the genus *Culex* (8 species). Among the *Culex* mosquitoes, members of the *Cx. p. pipiens* complex have accounted for over 88% of the WNV positive pools reported to ArboNet, and based on total numbers and infection rates, *Culex p. quinquefasciatus*, a common and moderately efficient vector (Sardelis et al. 2001, Turell et al. 2005, Richards et al. 2007), appears to be the predominant vector species (Rutledge et al. 2003; Godsey et al. 2005a, 2005b; Gibbs et al. 2006; Lindsey et al. 2008) (Fig. 1).

The role that members of the *Cx. p. pipiens* complex play in enzootic/epizootic transmission among birds and epidemic transmission to humans appears to vary throughout the Southeast as it does in the Northeast (Table 2). In a series of detailed studies in the southern portion of the *Cx. p. pipiens/quinquefaciatus* hybrid zone in Shelby County, Tennessee, Savage et al. (2006, 2007, 2008) found that members of the *Cx. pipiens* complex accounted for 97% of all WNV positive mosquitoes with no significant differences in infection rates among members within the complex. They also reported significantly higher infection rates in urban sites (Memphis) associated with larger populations of *Cx. p. pipiens* complex mosquitoes and human cases, reaffirming their importance in these foci. An analysis of feeding preferences again showed no differences among members of the complex, which fed predominantly upon avian hosts (73%), supporting their primary role as enzootic vectors. However, a substantial number of mammalian-derived blood meals (14%), including humans (1%) were also identified. Despite the comparatively low rate of human feeding, Savage et al. (2007) concluded that the very high rates of WNV infection in *Cx. p. pipiens* complex mosquitoes combined with the extremely high mosquito population levels in this region, supported a role for members within the complex in transmission to humans. The involvement of *Cx. restuans* as a principal enzootic vector to birds and occasional vector to humans was also noted.

Studies conducted in more rural areas in the Tennessee Valley by contrast, indicate a reduced role for *Cx. p. pipiens/Cx. quinquefasciatus*, where the most commonly infected species were *Culex erraticus* and *Cx. salinarius* (Cupp et al. 2007). Although the vector competence of *Cx. erraticus* for WNV has not been evaluated, the authors of this investigation felt its abundance, wide distribution, and strong ornithophilic feeding behavior (Hassan et al. 2003), make it a potentially important enzootic vector in this region of the southeastern United States. They also noted that *Cx. salinarius* likely played a role as an important

bridge vector as suspected in the northeastern United States.

In surveillance studies in East Baton Rouge Parish, Louisiana, where a large number of human cases occurred from 2002 to 2004, over 87% of all WNV positive pools were obtained from *Cx. p. quinquefaciatus* (Godsey et al. 2005b, Palmisano et al. 2005, Gleiser et al. 2007, Mackay et al. 2008). Also underscored in those investigations was the abundance and widespread distribution of *Cx. p. quinquefaciatus* in urban areas and peak temporal association between the onset of human disease and mosquito WNV infection rates. These findings and the demonstrated feeding of local populations of *Cx. p. quinquefaciatus* on human hosts at a relatively high rate (7% to 15% of all blood meals) (Niebylski and Meek 1992, Mackay et al. 2010), clearly incriminate this species as the most important vector for both enzootic amplification and transmission of WNV to humans in southern Louisiana.

Among non-*Cx. pipiens* complex mosquitoes, *Culex nigripalpus* Theobald has been implicated as a potentially important vector in some regions of the southern United States where it is locally abundant (Godsey et al. 2005a). Although the overall number of WNV positive pools reported from *Cx. nigripalpus* have been comparatively few (1.8% of all *Culex*) (Fig. 1), this moderately competent vector species (Sardelis et al. 2001) is believed to be one of the more important vectors in Florida, where transmission patterns have been sporadic and largely focal with rare epidemics (Blackmore et al. 2003, Rutledge et al. 2003, Hribar et al. 2004). *Culex nigripalpus* is an opportunistic species (Edman 1974) that feeds on avian hosts during the winter and spring, and then purportedly shifts to mammalian hosts, including humans during the summer and fall (Edman and Taylor 1968). These factors, coupled with its abundance, suggest that *Cx. nigripalpus* likely serves as an enzootic as well as epidemic vector in this region. It has been further suggested that in south Florida drought brings *Cx. nigripalpus* and wild birds into close contact facilitating epizootic WNV amplification and generating infection rates necessary to support high levels of WNV transmission (Shaman et al. 2005).

Midwestern United States

The expansion of WNV into the midwestern United States in 2001 was followed by extensive epidemics of human disease throughout the region in 2002 and 2003 (7,067 human cases) (Hayes et al. 2005). Associated with this was the incrimination of *Culex tarsalis* Coquillett, a highly competent vector (Goddard et al. 2002; Turell et al. 2002, 2005) and opportunistic feeder that prefers avian hosts, but will readily attack humans (Hayes et al. 1973, Tempelis 1975, Reisen and Reeves 1990). This species has since become the most commonly reported WNV-positive mosquito in rural locations of the Midwest, making up more than one-third of all *Culex* pools reported to the CDC ArboNet through 2010 (Fig. 1). In certain regions of the midwest, such as Grand Forks, North Dakota, for example, it is regarded as the most important vector of WNV, serving as both the enzootic and bridge vector to humans and horses (Bell et al. 2005).

Members of the *Cx. p. pipiens* complex, by contrast, appear to be the predominant vectors in more densely populated urban and suburban environments in the Midwest (Gu et al. 2006, Hamer et al. 2008a, 2009, Harrison et al. 2009) accounting for over 56% of the WNV-positive pools reported through 2010 to the CDC ArboNet (Fig. 1). Their role as primary enzootic vectors involved in amplification of WNV among wild bird populations in these settings is well recognized (Hamer et al. 2008b, 2009) and widely acknowledged throughout the region (Table 2). The most compelling evidence supporting their role in transmission of WNV to humans comes from a series of investigations conducted in an endemic transmission area in metropolitan Chicago, Illinois, where Hamer et al. (2008a, 2009) documented an unusually high rate of human feeding by *Cx. pipiens* (16% of total blood meals examined). Based on the: 1.) relatively high rate of feeding on humans, 2.) high prevalence of WNV infection in local *Cx. p. pipiens* populations (12 per 1,000), 3.) identification of a WNV-positive female *Cx. p. pipiens* with a human blood meal, and 4.) low rate of WNV infection in non-*Culex* mosquitoes (1 per 1000), the authors concluded that *Cx. p. pipiens* likely serves as both the enzootic and epidemic vector in this metropolitan area. Microsatellite analysis of populations of *Cx. p. pipiens* from Chicago suggested that the probability of genetic ancestry from *Cx. p. pipiens* form molestus may have predisposed these mosquitoes to readily feed on mammals, although the genetic mechanisms are not known (Huang et al. 2009).

The degree to which *Cx. p. pipiens* contribute to human transmission in other metropolitan districts in the Midwest is less well known and may be markedly reduced. Mosquito and arbovirus surveys conducted in semi-urban regions of southeastern Kansas in 2007 (Harrison et al. 2009) detected high WNV infection rates in *Cx. p. pipiens* (26 per 1,000) that would be typically associated with an elevated risk of human infection. However, only a single human case was subsequently documented.

Western United States

Mosquito and arbovirus surveillance conducted in the western United States since 2002 have

resulted in the detection of WNV from eight species of *Culex* (Table 1). The most frequently reported WNV-positive species has been *Cx. tarsalis*, which made up nearly one-half (49.0%) of the positive pools, followed by *Cx. p. quinquefasciatus* (28.7%), *Cx. p. pipiens* (18.5%), *Culex erythrothorax* Dyar (2.3%), *Culex stigmatosoma* Dyar (1.3%), *Cx. restuans* (0.1%), *Culex thriambus* Dyar (0.1%), and *Cx. territans* (< 0.1%) (Fig. 1).

Much attention has focused on *Cx. tarsalis,* which is considered the primary enzootic and epidemic vector of WNV throughout much of the western region, especially in rural areas. This is largely due to its high vector efficiency (Goddard et al. 2002, Turell et al. 2002, Reisen et al. 2005, Anderson et al. 2012), widespread abundance (Bolling et al. 2009, Reisen and Reeves 1990, Winters et al. 2008), high natural infection rate (Bolling et al. 2007) and propensity of local populations to feed on birds and mammals, including humans (Kent et al. 2009, Thiemann et al. 2011). Strong support for this view comes from studies in northeastern Colorado conducted from 2003 to 2007, where the abundance of *Cx. tarsalis* and vector index for WNV-infected females were strongly associated with the large number of human disease cases that occurred during that period (Bolling et al. 2007, 2009). Similarly, Gujral et al. (2007) reported higher vector indices for WNV transmission among local populations of *Cx. tarsalis* than *Cx. p. pipiens* in two adjacent cites in northern Colorado (Loveland and Fort Collins) that had severe outbreaks of human disease in 2003. Bowden et al. (2011) further demonstrated that the incidence of human WNV disease in the northwest was positively associated with agricultural land covers (grassland, crops, herbaceous wetland) and not urban land covers as observed in other regions of the country where *Cx. pipiens* complex mosquitoes predominate. This analysis is consistent with the preferred breeding sites for *Cx. tarsalis*, which include natural ground pools and ditches with emergent vegetation, open grassland and freshwater pools associated with agricultural sources (Reisen and Reeves 1990).

A more prominent role for *Cx. p. pipiens* complex mosquitoes in the ecology and epizootiology/epidemiology of WNV transmission in the western United States comes from studies in California where WNV was first isolated from a pool of *Cx. tarsalis* collected from Imperial County near the Mexican border during July 2003 (Reisen et al. 2004). In urban/suburban areas of Sacramento and Yolo counties located in the north central regions of the state, *Cx. p. pipiens* appears to function primarily as a WNV-amplifying enzootic vector (Montgomery et al. 2011), but has also been incriminated as an epidemic vector as well. Support for the latter comes from investigations during a severe human outbreak that occurred in these counties during 2005 (Elnaimen et al. 2008). *Culex p. pipiens* was the most abundant urban vector collected in CO_2-baited traps placed in residential areas where the epidemic occurred, accounting for 66.8% of all *Culex* mosquitoes. *Culex p. pipiens* also made up 68.3% of the WNV-infected pools followed by *Cx. tarsalis* (28.8%), and had an infection rate that was more than double that detected in *Cx. tarsalis*. These findings led the authors to conclude that *Cx. p. pipiens* was the primary vector likely involved in human transmission. However, the supposition that *Cx. p. pipiens* functions as a bridge vector in these residential settings could not be corroborated in a subsequent analysis of blood meals from field-caught *Cx. p. pipiens* collected in 2007 and 2008 from urban/suburban centers in the same two counties, where > 99% of the blood meals were determined to be of avian origin and not a single incident of human feeding was detected (Montgomery et al. 2011).

In the rural lower Coachella Valley of southern California, *Cx. tarsalis* is viewed as the primary enzootic vector responsible for maintenance and amplification of WNV (Reisen et al. 2004, 2008b, Lothrop et al. 2008). This is based on the frequent detection of virus in this species and its overall abundance, which generally mirrors the temporal and spatial distribution of enzootic transmission throughout the region. *Culex p. quinquefasciatus*, by contrast, is considered the primary enzootic and bridge vector to humans in the more urbanized Upper Valley (Reisen et al. 2004, 2008b, Lothrop et al. 2008), due to its abundance in peridomestic habitats and diverse feeding habits that include humans (Reisen et al. 1990, Reisen and Reeves 1990). It is also thought to be involved in most tangential transmission of WNV to humans in peridomestic environs in Kern County, where rapid spring amplification was associated with early season increases in WNV infection incidence in *Cx. p. quinquefasiatus* (Reisen at al. 2009).

Host-feeding patterns and WNV infection rates in mosquitoes collected from urbanized centers in neighboring Orange, Riverside and San Bernardino Counties, equally implicate *Cx. p. quinquefasciatus* as the primary vector of WNV in this region of southern California as well (Molaei et al. 2010). This mosquito was among the most commonly trapped species and the main source of WNV over a two-year period (2006–2008), representing nearly 80% of all WNV-positive mosquito pools, and blood meal analysis revealed opportunistic feeding on a diversity of competent avian (88.4%) and mammalian (11.6%) hosts including humans (1.9%), further indicating its involvement in enzootic as well as epidemic transmission (Table 2).

Mosquito and arbovirus surveillance conducted in Los Angeles County, California, from 2003 to 2008 that included major human epidemics, similarly identified *Cx. p. quinquefasciatus* as the most abundant species, and on the basis of infection incidence, the species most frequently involved in enzootic and epidemic transmission in urban Los Angeles (Kwan et al. 2010). Also identified were *Cx. tarsalis* and *Cx. stigmatosoma*, the latter a highly competent vector for WNV (Goddard et al. 2002, Reisen et al. 2008a) that feeds almost exclusively on birds (Reisen et al. 1990, Molaei et al. 2010). However, both species were significantly less abundant than *Cx. p. quinquefasciatus* (4.1% of total *Culex* collection), were not consistently found infected throughout the 8-year period, and accounted for only 7.2% of the WNV positive pools identified in 2004 when the greatest number of human cases (n = 168) were recorded.

Other species of *Culex* found naturally infected with WNV in southern California include *Cx. erythrothorax,* a widely distributed species that develops in permanent and semipermanent marshes supporting dense tule and cattail stands (Reisen and Reeves 1990). *Culex erythrothorax* is a competent vector for WNV (Goddard et al. 2002) and opportunistic feeder (Reisen and Reeves 1990, Molaei et al. 2010) that may serve as an occasional bridge vector to humans. However, this mosquito feeds rather infrequently on competent avian hosts (Molaei et al. 2010) and exhibits substantially lower WNV infection rates in comparison to all other *Culex* vectors (Kwan et al. 2010, Molaei et al. 2010) indicating that it is not likely to be a significant vector of WNV in this region.

Southwestern United States

Within the southwestern United States, WNV has been detected in 10 different species of *Culex* mosquitoes (Table 1), but the overwhelming majority of virus positive pools reported to CDC ArboNet have been from *Cx. p. quinquefasciatus* (82.1% of all *Culex*) (Fig. 1). In Harris County, Texas, which includes the Houston metropolitan area where WNV was first detected in June 2002, *Cx. p. quinquefasciatus* is the dominant *Culex* species, and based on its abundance, feeding habits, and high WNV infection rate, is considered the principal vector of WNV in the area (Lillibridge et al. 2004, Dennett et al. 2007, Molaei et al. 2007). During the 2002 epidemic, when 105 human cases were reported throughout the metropolitan area, it was the only species to test positive for the virus, with nearly 14% of 69,490 pools WNV-positive and a minimum field infection rate of 3.3 per 1,000 (Lillibridge et al. 2004). Surveillance activities conducted in the same area in subsequent years (2003–2006) further demonstrated a strong positive correlation between WNV-positive *Cx. quinquefasciatus* pools, WNV-positive blue jays and the incidence of monthly human cases (Dennett et al. 2007). The role of this mosquito in both enzootic and epidemic transmission was explicitly revealed in two host-feeding studies (Dennett et al. 2007, Molaei et al. 2007) which showed local populations were very opportunistic, exhibiting considerable variation in blood-feeding behavior that included: 1.) A variety of competent avian hosts (42% and 39% of total blood meals), 2.) Several mammals (58% and 53% of total blood meals), and 3.) Humans (0.7% and 23% of mammalian blood meals) (Table 2). Molaei et al. (2007) further identified mixed avian and mammalian blood in 8% of the blood meals from *Cx. p. quinquefasciatus* reaffirming its potential role as a likely bridge vector.

Culex p. quinquefasciatus also appears to be the predominant vector in urban habitats in Denton and surrounding counties in the Dallas-Ft. Worth metropolitan area located in north central Texas (Bolling et al. 2005), while in more rural Lubbock County in northwestern Texas, *Cx. tarsalis* constitutes the great majority of WNV positive mosquitoes (Bradford et al. 2005).

Studies in the Rio Grande Valley of New Mexico, which include the Albuquerque metropolitan area, provide evidence to suggest separate enzootic and epidemic cycles of WNV transmission that involve different species of *Culex* mosquitoes. *Culex tarsalis* appears to be involved in early season amplification of WNV in wild avian hosts, especially in rural areas of the Valley, whereas *Cx. salinarius* and *Cx. p. quinquefasciatus* are the two species most likely involved in epidemic transmission to humans in more urban locales. According to this scenario, the virus builds through multiple amplification cycles involving avian hosts and *Cx. tarsalis* and eventually spreads throughout the metropolitan area to populations of *Cx. salinarius* and *Cx. p. quinquefasiatus*, which are locally abundant and exhibit comparable WNV infection rates (DiMenna et al. 2006, 2007).

In more semiarid areas of Doña Ana County, New Mexico, *Cx. tarsalis* is reported to be the primary vector of WNV based on the frequency and preponderance of WNV-positive pools identified from this species (Pitzer et al. 2009). However, because it is most abundant in sparsely populated riparian and agricultural areas, its involvement in transmission to humans appears minimal. *Culex p. quinquefasciatus*, on the other hand, is reportedly a secondary vector, based on the detection of substantially fewer WNV-positive pools from this species, but may be responsible for most WNV transmission in urbanized areas where 82% of the positive pools for this species were collected.

Table 3. Detection of West Nile virus in overwintering populations of *Culex p. pipiens* in the US.

Location	Year	No. mosquitoes	No. virus + pools		Reference
			Isolation	PCR	
New York	2000	2,360	1	2	Nasci et al. 2001a
New Jersey	2001–03	1,324	-	1	Farajollahi et al. 2005
Pennsylvania	2003	501	-	1	Bugbee & Forte 2004
Colorado	2003–04	8,017	-	-	Bolling et al. 2007
New York	2006–09	3,240	1	-	Andreadis et al. 2010

CONTRIBUTION OF CULEX PIPIENS COMPLEX MOSQUITOES TO OVERWINTERING AND PERSISTENCE OF WEST NILE VIRUS

The role that *Cx. p. pipiens* complex mosquitoes play in overwintering and long-term persistence of WNV has been the subject of several investigations and has been recently reviewed (Kramer and Ebel 2003, Reisen and Brault 2007, Kramer et al. 2008). In cool temperate regions where transmission ceases during the winter months, WNV has been detected in hibernating *Cx. p. pipiens* on several occasions (Nasci et al. 2001a, Bugbee and Forte 2004, Farajollahi et al. 2005, Andreadis et al. 2010), and this species is thought to serve as a natural overwintering host responsible for amplifying transmission of the virus in the spring. This view is consisitent with epidemiological data documenting the annual reemergence of WNV from the same geographic locales (Andreadis et al. 2004; Reisen et al. 2006, 2008b, 2009; Bolling et al. 2007), and molecular evidence of year-to-year persistence of similar viral subclades from foci in the northeastern (Armstrong et al. 2011) and midwestern United States (Amore et al. 2010). However, the prevalence of viral infection in the overwintering population of *Cx. p. pipiens* appears to be quite low (Table 3), and the manner in which prehibernating females become infected with WNV in the fall before entering hibernacula is not entirely clear.

It is widely acknowledged that above ground populations of *Culex p. pipiens* overwinter in natural and man-made shelters as non-blood fed, nulliparous, inseminated females (Service 1969, Hayes 1973, Slaff and Crans 1977, Sulaiman and Service 1983, Jaenson 1987, Onyeka and Boreham 1987, Vinogradova 2000). Since the majority of females that enter diapause do not blood feed (Eldridge 1987, Mitchell 1988), infection of these females must occur through vertical transmission of the virus. Vertical transmission of WNV by *Cx. p. pipiens* has been demonstrated in the laboratory but appears to be relatively inefficient (Dohm et al. 2002, Goddard et al. 2003, Anderson et al. 2008). According to Rosen (1987), flaviviruses seem to enter the fully formed egg through the micropyle at the time of fertilization rather than infecting developing eggs in the ovary, a more efficient mechanism observed with bunyaviruses. Nevertheless, WNV has also been isolated from field-collected males, nulliparous females, and adults reared from field-collected larvae and WNV-infected females (Anderson and Main 2006, Anderson et al. 2006, Reisen et al. 2006, McAbbe et al. 2008) reaffirming its occurrence in natural populations. Unequivocal evidence that vertically infected female *Cx. p. pipiens* that enter diapause in the fall are able to initiate infection the following spring comes from an investigation by Anderson and Main (2006), who documented horizontal transmission of WNV by a vertically infected female that had been in diapause for more than 5½ months. Based on an estimated infection rate of ~0.05 infected females/1000, these authors concluded that in temperate climates, transgenerational transmission of WNV by *Cx. p. pipiens* is an important means of enabling the virus to persist during the winter and amplify in the spring.

An alternative mechanism wherein older prehibernating females that had previously acquired an infectious blood meal enter hibernacula in the fall and survive the winter to initiate infection in the spring, albeit rare, remains plausible. Parous female *Cx. p. pipiens* from the northeastern United States are known to enter hibernacula in the fall, and despite significant mortality during the winter months, some individuals survive to emerge in the spring (Jumars et al. 1969, Andreadis et al. 2010). However, it is unknown whether the parous state of these females is due to blood feeding and oviposition prior to entering the hibernaculum or autogenous egg production. It is generally presumed that diapausing populations of *Cx. p. pipiens* from northern latitudes are anautogenous and must acquire a blood meal to produce eggs. Autogenous populations have been identified in North America (Richards 1941, Wray 1946, Rozeboom 1951, Spielman 1964, 1971, Kent et al. 2007, Huang et al. 2008, Mutebi and Savage 2009) but only among non-diapausing *Cx. p. pipiens* form molestus that are confined to enclosed spaces in urban subterranean habitats such as sewer systems and flooded basements. Populations of these two physiological biotypes are for the most part reproductively isolated due

to differences in their breeding sites (Rozeboom and Gilford 1954, Spielman 1964, 2001) and based on comparative microsatellite analyses are genetically distinct entities (Kent et al. 2007, Huang et al. 2008). However, evidence of molestus genetic ancestry among a small portion of the aboveground population (Fonseca et al. 2004;Kent et al. 2007; Kilpatrick et al. 2007; Huang et al. 2008, 2009), and the documentation of occasional episodes of interbreeding where sympatric populations coexist in the northeastern United States (Spielman 1971, 2001), suggest some level of gene flow and possible hybridization between the two biotypes (Kent et al. 2007). The degree to which hybridization occurs where populations of these two biotypes are sympatric in nature and whether specific genes for autogeny are expressed in above ground populations are intriguing questions that remain to be explored. Autogeny has been reported in above ground populations of *Cx. p. pipiens* from southern Europe (Gomes et al. 2009) and the Middle East (Nudelman et al. 1988) but not from North America. The role of *Cx. p. pipiens* form molestus in seasonal transmission and persistence of WNV in North America is entirely unknown. It has also been suggested that *Cx. salinarius* may have a role in maintaining WNV in the northeastern United States due to its ability to vertically and horizontally transmit WNV similarly to *Cx. p. pipiens* (Anderson et al. 2012).

In southern regions of the United States where low levels of WNV activity in birds and mosquitoes have been detected during the winter months (Tesh et al. 2004, Reisen et al. 2006), it has been suggested that the virus may persist through continued transmission involving *Cx. p. quinquefasciatus* (Reisen and Brault 2007, Richards et al. 2007). Unlike *Cx. p. pipiens*, *Cx. p. quinquefasciatus* does not enter a true diapause but rather overwinters in quiescence (Reisen et al. 1986). Adults become inactive during cold periods, resting under buildings and in storm drains and sewers (Tesh et al. 2004), but during warm periods becomes active again and renew blood feeding. According to Tesh et al. (2004), this intermittent host-seeking activity throughout the winter likely accounts for continued low-level WNV transmission among resident avian populations in the western Gulf region of Louisiana and Texas and is the principal mechanism by which the virus overwinters. Continued WNV transmission was similarly detected in southern California by the recovery of viral RNA from dead American crows. This observation in concert with the isolation of WNV from male and adults emerging from field-collected immature *Cx. p. quinquefasciatus,* indicated that vertical transmission may be possible during mild winter conditions in southern California (Reisen et al. 2006). West Nile virus has also been detected in *Cx. p. quinquefasciatus* mosquitoes collected as larvae from southern Louisiana (Unlu et al. 2010).

It is also noteworthy that WNV-positive pools have been identified from winter-resting and early season *Cx. erraticus* females in Alabama, well before significant numbers of this species became active, adding further evidence that overwintering mosquitoes in this region maintain virus between transmission seasons (Cupp et al. 2007). WNV has also been detected in overwintering larvae of *Cx. erythrothorax* collected in late October from Utah demonstrating vertical transmission in this mosquito species, and suggesting that vertical transmission may similarly contribute to WNV overwintering in this region (Philips and Christensen 2006).

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