Infection, Genetics and Evolution 11 (2011) 1577–1585

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Review

"Bird biting" mosquitoes and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology

Ary Farajollahi [a], Dina M. Fonseca [a], Laura D. Kramer [b,c], A. Marm Kilpatrick [d,*]

[a] Rutgers University, Center for Vector Biology, New Brunswick, NJ, USA
[b] Wadsworth Center, New York State Department of Health, Slingerlands, NY, USA
[c] State University of New York at Albany, Albany, NY, USA
[d] Dept. Ecology and Evolutionary Biology, University of California, Santa Cruz, CA, USA

ARTICLE INFO

Article history:
Received 6 April 2011
Received in revised form 15 August 2011
Accepted 16 August 2011
Available online 22 August 2011

Keywords:
Vector borne disease
Invasive species
West Nile virus
Arbovirus
Bridge vector
Vector competence

ABSTRACT

The transmission of vector-borne pathogens is greatly influenced by the ecology of their vector, which is in turn shaped by genetic ancestry, the environment, and the hosts that are fed on. One group of vectors, the mosquitoes in the *Culex pipiens* complex, play key roles in the transmission of a range of pathogens including several viruses such as West Nile and St. Louis encephalitis viruses, avian malaria (*Plasmodium* spp.), and filarial worms. The *Cx. pipiens* complex includes *Culex pipiens pipiens* with two forms, pipiens and molestus, *Culex pipiens pallens*, *Culex quinquefasciatus*, *Culex australicus*, and *Culex globocoxitus.* While several members of the complex have limited geographic distributions, *Cx. pipiens pipiens* and *Cx. quinquefasciatus* are found in all known urban and sub-urban temperate and tropical regions, respectively, across the world, where they are often principal disease vectors. In addition, hybrids are common in areas of overlap. Although gaps in our knowledge still remain, the advent of genetic tools has greatly enhanced our understanding of the history of speciation, domestication, dispersal, and hybridization. We review the taxonomy, genetics, evolution, behavior, and ecology of members of the *Cx. pipiens* complex and their role in the transmission of medically important pathogens. The adaptation of *Cx. pipiens* complex mosquitoes to human-altered environments led to their global distribution through dispersal via humans and, combined with their mixed feeding patterns on birds and mammals (including humans), increased the transmission of several avian pathogens to humans. We highlight several unanswered questions that will increase our ability to control diseases transmitted by these mosquitoes.

Contents

---

* Corresponding author. Address: EE Biology/EMS, Univ. of California, Santa Cruz, CA 95064, USA. Tel.: +1 831 459 5070.
E-mail address: akilpatr@ucsc.edu (A. Marm Kilpatrick).

1567-1348/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2011.08.013

1. Introduction

Vector-borne diseases such as malaria, plague, yellow fever, lymphatic filariasis have shaped our genetic make-up (Aubry, 2008; Tarantola et al., 2009), driven the rise and fall of civilizations (Vazeille et al., 2008), and the outcome of wars (Delatte et al., 2008). These and other vector-borne diseases such as dengue, Lyme disease and West Nile encephalitis, affect our ability to enjoy the outdoors (Coffinet et al., 2007; Kiehn et al., 2008) and, by separating humans from nature, potentially affect how we value biodiversity. Vector-borne pathogens include a wide range of organisms that are transmitted by a diverse set of species, including arthropods such as fleas, sandflies, ticks, and mosquitoes (Anosike et al., 2007). It follows that the specific life-history demands, abilities, and limitations of the vectors must have an enormous impact on transmission and thus the severity of disease outbreaks.

Determining the principal vectors for pathogens and what influences their transmission rates is a critical step in understanding patterns of transmission in space and time and in developing effective control interventions. Frequently an initial strategy for prevention of human diseases is to target the vectors most likely to bite humans. For pathogens where humans are an infectious host, a vector that bites humans exclusively with no or few "lost" bites to incompetent hosts such as pets, livestock, or wildlife, would generate the highest transmission rates (Kilpatrick et al., 2007; Townson and Nathan, 2008). This is the case for the dengue viruses, the filarial worms that cause lymphatic filariasis (*Wuchereria bancrofti*), and *Plasmodium falciparum*, the protozoan that causes human malaria. For these pathogens, humans are infectious hosts and the pathogens are primarily (but not exclusively) transmitted by mosquitoes that feed extensively on humans (Chandler et al., 1975; Siriyasatien et al., 2010).

Many human vector-borne diseases, however, are zoonoses that have amplification cycles involving species other than humans. These include Lyme disease, rickettsia, plague, and arboviral diseases such as yellow fever, West Nile, St. Louis, and eastern equine encephalitis, which have primates, small mammals, or birds as reservoirs. For many avian arboviruses, humans are dead-end hosts, because viremia (the concentration of virus in the blood) in humans for these viruses is too low to result in infection in biting vectors. This sometimes creates an apparent paradox because the principal vector of a human disease may be one that feeds primarily on non-human hosts and only a small fraction of its bloodmeals are derived from humans. This paradox is particularly well illustrated by *Culex pipiens* complex mosquitoes and the transmission of West Nile virus (WNV) in North America, as discussed in detail below.

In this review we examine in detail the taxonomy, phylogeny, ecology, population genetics, behavior, and vector competence of the *Cx. pipiens* complex, a group of morphologically and evolutionarily closely related mosquitoes with a long history of association with humans (Vinogradova, 2000). We discuss the role of these mosquitoes in the transmission of arboviruses including a review of host feeding patterns from blood meal analyses. We also discuss patterns of increased association between humans and these mosquitoes and the epidemiological consequences. Our aim is to highlight the role of vector ecology in transmission and its influence on the evolution of vector-borne pathogens, and integrate both these factors in determining the best approaches for control.

2. *Cx. pipiens* complex mosquitoes

2.1. Taxonomy of the *Cx. pipiens* complex

The current taxonomy in the Catalog of the Mosquitoes of the World (Knight, 1978) maintained by the Walter Reed Biosystematics Unit at the Smithsonian Institution (http://www.wrbu.si.edu), recognizes the following species as members of the *Cx. pipiens* complex: *Cx. pipiens*, *Culex quinquefasciatus*, *Culex australicus*, and *Culex globocoxitus* (Fig. 1). A species complex is usually defined as a group of evolutionarily closely related species that consequently are often difficult to separate morphologically (Collins and Paskewitz, 1996). This taxonomy is still controversial because of the historical dependence of taxonomy on morphological differences, the lack of such differences among many of the members of the *Cx. pipiens* complex, and the presence of hybrids (Harbach et al., 1985; Mattingly, 1965; Mattingly et al., 1951; Vinogradova, 2000; Zhao and Lu, 1999). Their close evolutionary association has been repeatedly supported by genetic analyses (Kent et al., 2007; Miller et al., 1996) as well as by the relative transferability of genetic markers across species (Smith et al., 2005). Although all species in the *Cx. pipiens* complex are identifiable by the shape of the male genitalia (Barr, 1957; Dobrotworsky, 1967), this trait cannot be used to identify females, the primary target of

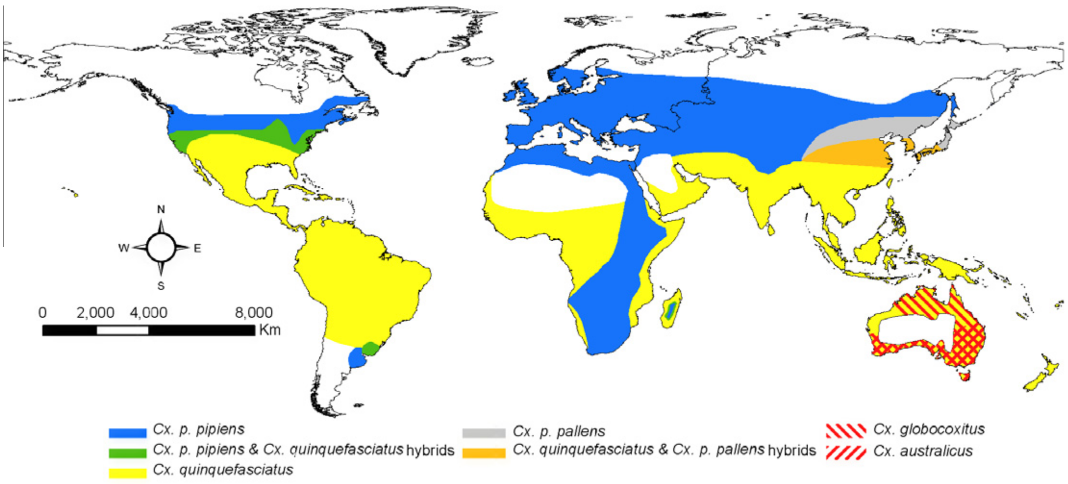

Fig. 1. Global distribution of the *Cx. pipiens* complex mosquitoes. Geographic range for *Cx. p. pipiens* may include both forms (pipiens and molestus) and in temperate Asia and Australia although *Cx. p. pipiens* form molestus can be found in urban environments we omitted it for clarity. Note that *Cx. australicus* and *Cx. globocoxitus* are restricted to Australia. (Adapted from (Mattingly, 1965; Smith and Fonseca, 2004; Vinogradova, 2000).

surveillance efforts. Further, *Cx. pipiens* has two recognized subspecies, *Cx. pipiens pipiens*, an Old World taxa originally distributed from Northern Europe to the highlands of South Africa (Harbach et al., 1985), and *Cx. p. pallens*, distributed east of the Urals across temperate Asia (Fonseca et al., 2009). *Culex p. pipiens* also has two recognized forms "pipiens" and "molestus", which differ dramatically in ecology (see more details under "Behavior and physiology" below). *Culex p. pipiens* (including both forms or their hybrids, (Fonseca et al., 2004)) have been accidentally introduced to temperate zones in North America and South America, while only *Cx. p. pipiens* form molestus has been introduced to cities in Japan, Republic of South Korea, and Australia (Vinogradova, 2000).

Cx. quinquefasciatus thrives in tropical and sub-tropical regions, including the African lowlands, Americas, Asia, and Australia (Fonseca et al., 2006). Together *Cx. pipiens* and *Cx. quinquefasciatus* occur across most inhabited areas globally and are often closely associated with humans, earning them the names of northern and southern house mosquitoes, respectively. Where their ranges overlap, *Cx. pipiens* (both subspecies) and *Cx. quinquefasciatus* can hybridize extensively as repeatedly shown by genitalia analysis, allozyme polymorphisms and more recently, microsatellite (nuclear DNA) analysis. There is extensive introgression between populations of *Cx. pipiens* and *Cx. quinquefasciatus* in North America, Argentina, Madagascar, Japan and Republic of South Korea (Barr, 1957; Cornel et al., 2003; Fonseca et al., 2009; Humeres et al., 1998; Kothera et al., 2009; Urbanelli et al., 1997, 1995; Wang et al., 2000).

This introgression is in stark contrast to the sympatric, but non-hybridizing populations of *Cx. pipiens* and *Cx. quinquefasciatus* in South Africa (Cornel et al., 2003). The lack of hybridization in South Africa between *Cx. pipiens* and *Cx. quinquefasciatus* is supported by the fact that in that location, only *Cx. quinquefasciatus* is infected with *Wolbachia pipientis*, a *rickettsian* intracellular parasite that can limit reproduction between insect populations. Of further interest, throughout most of North America *Cx. pipiens* is considered the primary vector of WNV (Kilpatrick et al., 2005; Kramer et al., 2008; Turell et al., 2002), while in Africa it is not (McIntosh et al., 1967).

2.2. Behavior and physiology across the complex

The *Cx. pipiens* complex includes populations with distinct behaviors and physiologies that greatly influence their vectorial capacity, or the efficiency of pathogen transmission. In addition to their preferred larval habitat (underground hypogeous versus above-ground epigeous, rural versus urban) and geographic range distribution, members of the *Cx. pipiens* complex also exhibit wide urban variations in host feeding patterns, gonotrophic development (autogeny versus anautogeny), and means or presence of adult female hibernation (quiescence versus diapause). Hibernation of *Cx. pipiens* complex mosquitoes involves the harmonization of many behavioral, biochemical, and physiological pathways within the mosquito and is often initiated by environmental signals (e.g. photoperiod, temperature, nutrient availability, and moisture) that result in significant physiological or behavioral changes.

Under the influence of a short photoperiod, *Cx. p. pipiens* form pipiens and *Cx. p. pallens* females will mate but will not seek a blood meal (Eldridge, 1987). Indeed, their ability to digest blood under short photoperiods is severely hampered by the downregulation of lipases, enzymes that digest blood (Robich and Denlinger, 2005). Instead, females raised under short photoperiod accumulate fat by feeding on nectar and other carbohydrate rich sources, a task aided by the simultaneous upregulation of proteins involved in carbohydrate digestion (Robich and Denlinger, 2005). Mated but not blood fed females retreat to cold and moist secluded/safe areas usually partly underground, such as basements and caves (Eldridge, 1987). Inside these hibernaculae, they survive freezing winters in partial torpor or diapause. In contrast, *Cx. quinquefasciatus*, *Cx. globocoxitus*, *Cx. australicus*, and *Cx. p. pipiens* form molestus do not diapause and will develop continuous cohorts across the seasons, although lower temperatures will slow down development (Dobrotworsky, 1967; Eldridge, 1987). For example, *Cx. australicus* in more temperate southern regions in Australia will retreat to protected areas but does not exhibit gonotrophic dissociation (also called ovarian arrest) and therefore does not undergo true diapause (Dobrotworsky, 1967). The propensity to enter diapause appears to be relatively consistent within taxa in the complex, although this may be a circular argument as that ability is often used to differentiate the taxa. One exception are populations of *Cx. p. pipiens* from South Africa that appear to be incapable of true diapause (Jupp, 1987) a pattern that deserves to be further explored. If *Cx. pipiens* mosquitoes enter diapause in late fall and cease blood feeding, this ends their contribution to transmission, whereas in areas with similar climate but where *Cx. quinquefasciatus* is present, the transmission season might be extended.

A second trait that varies across the *Cx. pipiens* complex is the expression of autogeny, or the ability to lay eggs without first obtaining vertebrate blood. Autogeny can increase mosquito abundance, especially if hosts for blood meals are limiting, but it could also decrease transmission of pathogens since mosquitoes would not need to feed to lay their first batch of eggs. Autogenous oviposition behavior may be influenced by larval overcrowding or diet: evidence suggests that genetically anautogenous mosquitoes cannot become autogenous by superabundant larval feeding, but autogenous development can be suppressed by the starving or overcrowding of genetically autogenous larvae (Spielman, 1971). Autogeny is a trait associated with *Cx. p. pipiens* form molestus, which in cold climates survives in underground sites such as sewage or subway systems in cities (Fonseca et al., 2004; Spielman, 2001), but autogeny can also be common in aboveground populations of *Cx. p. pipiens* form molestus in mild climates such as those in southern Europe (Gomes et al., 2009), northern Africa (Knight and Malek, 1951), and parts of northern California (Iltis, 1966).

A third trait that varies substantially within the complex is the propensity to feed on avian or mammalian blood for egg production. This is discussed in more detail in Section 4, below.

2.3. Accurate identification of Culex species

The accurate identification of mosquitoes is critical for vector surveillance and control because the abundance and infection of different vectors frequently indicates different levels of risk of transmission. Accurate speciation of *Cx. pipiens* complex mosquitoes relies on a wide variety of methods for precise identification. Quantitative differences in the shape of the male genitalia (DV/D ratio), and quantitative characters in wing venation (cross vein index ratio) have been the gold standard to separate *Cx. pipiens* from *Cx. quinquefasciatus* (Barr, 1957). However, hybrids often show intermediate phenotypic and genotypic manifestations of the parent population, thus making reliance on some of the above mentioned morphological characters unreliable (Aspen et al., 2003; Aspen and Savage, 2003; Cornel et al., 2003; Sanogo et al., 2008; Urbanelli et al., 1997). Also, there are no known morphological differences between the two forms of *Cx. p. pipiens* (Harbach et al., 1984) and therefore their identification in temperate latitudes has been traditionally associated with differences in egg development (autogeny as frequently observed in *Cx. p. pipiens* form molestus) and/or preferred larval habitat - (underground in areas of difficult access for *Cx. p. pipiens* form molestus or aboveground for *Cx. p. pipiens* form pipiens).

There are also several other species of *Culex* mosquitoes whose females are often indistinguishable from those in the *Cx. pipiens*

Table 1
Summary of the available molecular assays to identify *taxa* within the *Cx. pipiens* complex and morphologically related species.

Name	Locus	Taxa it targets	Reference
"Crabtree"	Ribosomal	*Cx. pipiens* sl *Cx. restuans* *Cx. salinarius*	Crabtree et al. (1995)
Subtractive hybridization	Nuclear	*Cx. p. pipiens* *Cx. p. quinquefasciatus*	Crabtree et al., (1997)
Aspen et al.	Ribosomal	*Cx. pipiens* sl, *Cx. nigripalpus*	Aspen et al., (2003)
Smith & Fonseca	Nuclear (Ace2)	*Cx. p. pipiens* *Cx. quinquefasciatus* *Cx. p. pallens* *Cx. torrentium* *Cx. australicus* *Cx. pervigilans*	Smith and Fonseca, (2004)
Ace.2	Nuclear (Ace2)	*Cx. pipiens* *Cx. quinquefasciatus*	Aspen and Savage, (2003)
HotAce	Nuclear (Ace2)	*Cx. pipiens* *Cx. quinquefasciatus*	Savage et al., (2007)
Kasai et al.	Nuclear (Ace2)	*Cx. p. pallens* *Cx. p. pipiens* f. molestus	Kasai et al., (2008)
Bahnck & Fonseca	Nuclear (CQ11)	*Cx. p. pipiens* f. pipiens *Cx. p. pipiens* f. molestus	Bahnck and Fonseca, (2006)

complex (especially if damaged during collection), but which are not members of the complex because they are genetically distinct. These include *Culex restuans*, *Culex nigripalpus*, and *Culex salinarius* in North America, *Culex torrentium* in northern Europe, *Culex pervigilans* in New Zealand, and *Culex vagans* in central and eastern Asia. To facilitate mosquito identification, several polymerase chain reaction-based assays that use species-specific primers targeting 12S-ribosomal (Crabtree et al., 1995), the acetylcholinesterase 2 locus (Aspen and Savage, 2003; Smith and Fonseca, 2004), or other nuclear sequences (Bahnck and Fonseca, 2006) have been developed (Table 1).

2.4. The two Cx. p. pipiens forms: recent developments

Recent work has shown that hybrizidation between the two forms of *Cx. p. pipiens* may have important implications for pathogen transmission. Genetic isolation exists between northern European populations of the two forms of *Cx. p. pipiens*, whereas extensive hybridization is present in the United States (Bahnck and Fonseca, 2006; Fonseca et al., 2004). Hybridization between bird biting and more mammalian biting forms of *Cx. p. pipiens* was hypothesized to make *Cx. p. pipiens* a superior bridge vector of WNV to humans. This is because mosquitoes would be frequently infected from feeding on birds, but could also transmit the virus to humans (Fonseca et al., 2004). Subsequently, two studies showed that North American *Cx. p. pipiens* mosquitoes with higher genetic ancestry from *Cx. p. pipiens* form molestus were in fact more likely to feed on humans (Kilpatrick et al., 2007) and mammals (Huang et al., 2009). This indicates that high "molestus" ancestry in a population may have led to increased transmission of WNV to humans. These results were somewhat surprising since recombination associated with hybridization would be expected to rapidly disassociate behavioral traits from the combination of neutral microsatellite markers that indicates species ancestry. Indeed, recombination may explain why US *Cx. p. pipiens* with a strong molestus ancestry (>80%) fed on birds only 60% of the time (Kilpatrick et al., 2007) (*Cx. p. pipiens* with little (<10%) molestus ancestry fed on birds >90% of the time). The strong association between behavioral and neutral markers may indicate an influx of genes from pure *Cx. p. pipiens* form molestus populations into the aboveground populations, possibly during the summer.

3. *Cx. pipiens* mosquitoes and humans

Ancestral *Cx. p. pipiens* may have been an African species that colonized temperate northern European regions as well as the highlands of Africa after the last glaciations. More recently, possibly as early as the 16th century, it arrived in the New World and is now found in cities and suburbs in all temperate climates (Vinogradova, 2000). In contrast, the ancestral distribution of *Cx. quinquefasciatus* was indubitably tropical, possibly in south-east Asia (Fonseca et al., 2006), although further population genetic studies including extensive sampling in East Africa and Asia are necessary. The presence of *Cx. quinquefasciatus* in Western Africa is likely recent, as suggested by the early ecological observations of the species in the 1950s (Mattingly et al., 1951) and by more recent genetic analysis (Fonseca et al., 2006). Thus, *Cx. quinquefasciatus* was likely not introduced into the New World with the slave trade as previously proposed (Vinogradova, 2000) and instead may have reached western Africa in boats returning from the Americas.

The success of the *Cx. pipiens* complex mosquitoes is partly due to their ability to exploit the large amounts of "food" found in standing water generated by humans and livestock. Unlike most other species of mosquitoes, *Cx. pipiens* complex species commonly thrive in aquatic habitats with a high organic content (Bockarie et al., 2009, Vinogradova, 2000). Many researchers have also attributed the worldwide distribution and abundance of *Cx. p. pipiens* and *Cx. quinquefasciatus* to their ability to exploit several modes of human transportation (Barr, 1957; Kilpatrick et al., 2004). Filthy bilges of large ships may have provided habitat for juvenile mosquitoes, and the abundant human and animal occupants may have provided a suitable blood source for mosquitoes to undergo several generations, particularly during long voyages. In addition, these journeys may have selected for mosquitoes ability to mate in confined spaces and survival on ships likely required feeding on mammals.

The traits of the types of mosquitoes that have spread across the world is demonstrated by recent worldwide population genetic analysis of the yellow fever mosquito, *Aedes aegypti* (Brown et al., 2011), a species that currently exhibits a pantropical distribution. All populations of *Ae. aegypti*, outside Africa appear to derive from a single African population, and potentially a single domestication event from which they spread across the world through human commerce of slaves and goods. Little evidence of secondary expansion of *Ae. aegypti* from Africa was found, underscoring the stringent requirements of life associated with humans and the rarity of such events.

In the *Cx. pipiens* complex, however, there were two separate domestication events. The advent of agriculture in North Africa may have led to *Cx. p. pipiens* form molestus (Fonseca et al., 2004) whereas the advent of organized agriculture and high density civilizations in southeast Asia likely resulted in the domestic forms of *Cx. quinquefasciatus* (Fonseca et al., 2006; Kenoyer, 1998)

Cx. p. pipiens form molestus fits the stereotype of the "domestic" mosquito: it thrives in highly polluted sewers, mates in confined spaces, often enters houses, and feeds readily on mammals, especially humans as evidenced by their role as principal vectors of lymphatic filariasis in Egypt (Abdel-Hamid et al., 2011). Likewise, the existence of domestic populations of *Cx. quinquefasciatus* is supported by the critical role of this species in the transmission of lymphatic filariasis in China and Southeast Asia (Liu et al., 1991; Sudomo et al., 2010). Without a highly specialized vector this parasite may not have become exclusively transmitted among human (Michael and Gambhir, 2010).

4. Pathogens transmitted by *Cx. pipiens* complex mosquitoes

Culex pipiens complex mosquitoes play important roles in the transmission of several pathogens that infect humans including WNV, *St. Louis encephalitis virus* (SLEV), and filarial worms (Bogh et al., 1998; Reisen et al., 1992; Turell et al., 2002) as well as wildlife pathogens such as avian malaria (*Plasmodium* spp, Kimura et al., 2011). This results partly from the wide variety of hosts on which they feed and from their high abundances in developed areas. Their exact role and importance in different aspects of transmission (e.g. among avian hosts versus between avian hosts and humans or other mammals such as horses) has sometimes been debated, but is becoming increasingly clear.

Variation in feeding between the different mosquito species and different populations within a species plays an important role in the pathogens they transmit. For example, in southeast Asia *Cx. quinquefasciatus* feeds predominantly on humans and is the principal vector of human lymphatic filariasis whereas in Hawaii *Cx. quinquefasciatus* likely feeds predominantly on birds because it is the most efficient vector of the local species of avian malaria (*Plasmodium relictum*) and avian pox among the endemic endangered birds (Fonseca et al., 1998; Van Riper et al., 1986).

In contrast, for human zoonotic pathogens with avian hosts, it is the mixed feeding patterns of species in the *Cx. pipiens* complex that result in them playing key roles. For example, in the northeastern and north central US, the predominant vector of WNV is *Cx. pipiens* (Andreadis et al., 2004; Hamer et al., 2008; Kilpatrick et al., 2005; Turell et al., 2002), which transmits virus among a variety of avian hosts, and also is important in transmission of virus to humans (Hamer et al., 2008; Kilpatrick et al., 2005), especially later in the transmission season (Kilpatrick et al., 2006b). Evidence for the importance of *Cx. pipiens* mosquitoes in the transmission of WNV comes from the large number of virus isolations from field collected individuals (Andreadis et al., 2004; Lukacik et al., 2006), their moderately efficient vector competence for WNV (Sardelis et al., 2001; Tiawsirisup et al., 2005; Turell et al., 2005), their abundance in urban environments (Andreadis et al., 2004; Kilpatrick et al., 2005; Lukacik et al., 2006; Ruiz et al., 2010; Savage et al., 2006), their mixed host feeding behavior (Apperson et al., 2004; Hamer et al., 2008; Kilpatrick et al., 2006b), their ability to vertically pass the virus from an infected female to her offspring (Dohm et al., 2002), and their capacity to serve as an overwintering reservoir of WNV (Farajollahi et al., 2005; Nasci et al., 2001). In addition, their higher abundance in urban environments has been hypothesized as a key factor in increasing WNV transmission rates in urbanized areas (Bowden et al., 2011; Brown et al., 2008; Gomez et al., 2008).

5. Host feeding

Many questions still remain on the exact roles of different mosquito vectors in arbovirus transmission cycles. This has partly stemmed from recent research that has challenged previous characterizations of the feeding patterns exhibited by *Culex* mosquitoes, and the level of transmission risk to humans associated with these vectors (Fonseca et al., 2004; Kilpatrick et al., 2005, 2007; Hamer et al., 2008, 2009; Kilpatrick et al., 2006b). *Cx. pipiens* mosquitoes are known to be enzootic vectors for several arboviruses, and historically had been classified as ornithophilic mosquitoes. However, they are increasingly recognized as important bridge vectors based on comprehensive integrated studies that examine host preferences, vector/host abundance, virus infection rates, and vector competence. Here we review variation in feeding patterns of *Cx. pipiens* and *Cx. quinquefasciatus* mosquitoes in the context of arbovirus transmission. Further, we attempt to provide a broader perspective by comparing them to two other *Culex* species from North America, *Culex tarsalis* and *Cx. restuans*, which are also important in arboviral transmission.

We found seven studies of the feeding patterns of *Cx. pipiens* mosquitoes in North America, nine studies of *Cx. quinquefasciatus* (six from North America, two from Australia, and one from Mexico), seven studies of feeding patterns of *Cx. restuans*, and ten studies of *Cx. tarsalis*, all from North America (Fig. 2; Supplemental Online Table 1). All of these studies determined the fraction of blood meals derived from mammals and birds (and usually from other vertebrate classes), and all determined the fraction that had fed on humans. This enables an examination of each species' role and efficiency in the transmission of avian pathogens to humans, as well as their efficiency in transmitting both human and non-human mammal pathogens.

Somewhat surprisingly, across all populations studied, there was no significant difference in the fraction of feedings taken from birds or humans between *Cx. pipiens* and *Cx. quinquefasciatus* (Fig. 2; all three 95% confidence intervals overlap; ANOVA on arcsin square root transformed data to normalize residuals: all p's > 0.1) Interestingly, populations of *Cx. quinquefasciatus* from Australia, while genetically different from North American populations, did not show widely disparate feeding patterns. Perhaps even more surprising, there were no significant differences between the mammal, human, or avian fraction of feedings among any of the four *Culex* species (Fig. 2; ANOVAs on transformed data, all p's > 0.07).

These surprising results stem, in part, from substantial spatial variability in feeding patterns among populations as is clear from Fig. 2. The causes of this variability are not well known, but likely reflect variation in the abundance of different hosts, and variation in genetic predisposition of the mosquitoes at different sites that influence feeding patterns. Although several recent studies simultaneously examined feeding patterns and estimated the local abundance of at least part of the host community (usually birds) (Hamer et al., 2009; Hassan et al., 2003; Kent et al., 2009; Kilpatrick et al., 2006a), we are unaware of any studies that have estimated the abundance of all avian and mammalian hosts simultaneously with data on mosquito feeding patterns. This would be necessary to determine the influence of host abundance on mammal versus bird feeding. Further, no study has estimated the abundance of amphibian or reptile hosts which sometimes appear to make up a non-trivial fraction of *Cx. pipiens* feedings (Apperson et al.,

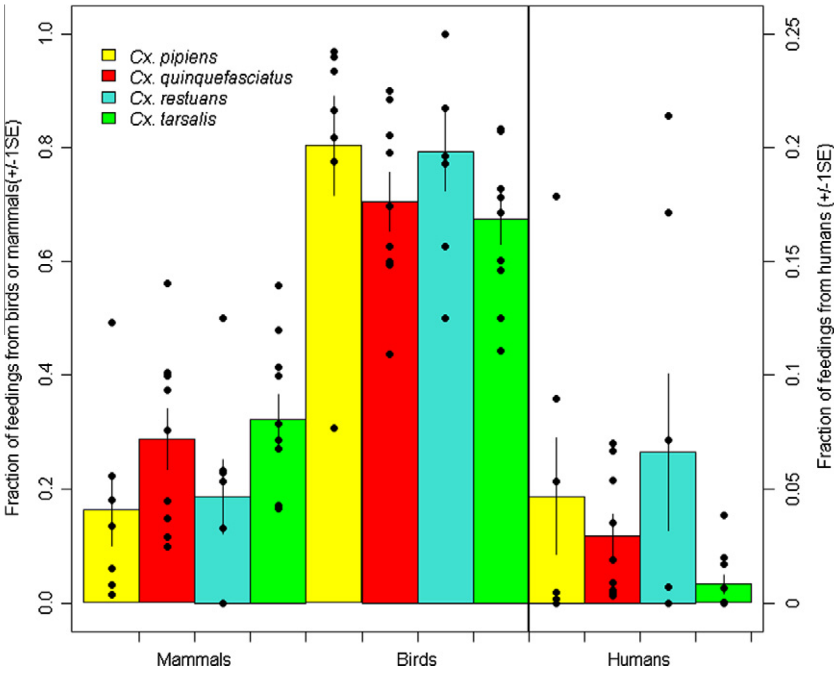

Fig. 2. Feeding patterns of four species of *Culex* mosquitoes. Left axis shows the fraction of feedings from birds and mammals (first eight columns) represented by the mean (column height), 95% confidence interval (whiskers), and raw data (points). Right axis and last four columns show the fraction of feedings from humans (a subset of the mammals) on a different scale. See Supplemental Online Table 1 for sources and raw data.

2004). Finally, some studies have estimated the densities of domestic mammals and birds (usually chickens, cows, pigs, etc.) while examining vector feeding, but these studies provide limited information for pathogens that circulate in wild animals.

The consequences of these feeding patterns for transmission of avian and mammalian pathogens to humans are profound. As noted earlier, the appreciable feeding (up to 18% of blood meals) by *Cx. pipiens* on humans (Fig. 2) makes them likely to be the most important bridge vector of the primarily avian pathogen, WNV, to humans in several regions of North America (Hamer et al., 2008; Kilpatrick et al., 2005). It is worth noting that increased feeding on humans by any of these vectors decreases the enzootic intensity of transmission for WNV and SLEV, but simultaneously increases the "force-of-infection" experienced by humans, at least initially (Kilpatrick et al., 2006b, 2007). The feeding pattern that would, in the worst case, maximize human incidence is a complex function of the other factors that influence transmission, including vector abundance, survival, other hosts fed on, etc. However, it can be stated that transmission of avian pathogens to humans will initially increase with increasing feedings on humans until the fraction of feedings on humans (which are dead-end hosts for WNV) is so large that transmission is inefficient. In contrast, if humans can serve as an amplifying host for a pathogen (e.g. filiariasis, dengue virus), increasing feeding on humans will both increase enzootic transmission and the force-of-infection experienced by humans in a monotonic fashion. As a result, for these human-amplified pathogens, any control strategies that decrease feeding on humans, without increasing other factors (e.g. vector abundance) should reduce transmission. This is the logic behind zooprophylaxis, placing non-human animal hosts near humans to divert vector feeding. As has been noted before, this technique is likely to be most effective when the placement of animal hosts near humans decreases human feeding but does not increase vector density, which is a likely scenario only if larval habitats are limiting.

One of the next frontiers in determining the role of vectors in the transmission of zoonotic pathogens that infect multiple vertebrate classes (e.g. mammals and birds) will be assessing the under- or over-utilization (a smaller or larger fraction, respectively, of feedings coming from a species than expected from the fraction of the host community a species represents) of avian and mammalian hosts through simultaneous studies of local host abundance of both mammals and birds coincidently with feeding patterns. It is worth emphasizing that data on host abundance should be collected at the same locations where engorged mosquitoes are collected. Host abundances can vary by at least an order of magnitude between sites separated by only 1–3 km, making "semi-local" host abundance data of limited utility in understanding mosquito feeding patterns. Finally, there has been relatively little work done in the last decade on the mechanistic causes of over- or under-utilization of host species. Over-utilization of a species can arise from a preference of biting vectors for that species, an overlap between mosquito microclimate selection and host roosting behavior (especially for nocturnal or crepuscular feeding vectors), or relatively lower host defensive behavior against biting vectors. The fact that any of these mechanisms can cause over-utilization makes the use of the term "preference" to describe raw feeding patterns is somewhat misleading. Thus, it is critical that the mechanisms underlying feeding patterns are distinguished to maximize the understanding gained and for implementing interventions such as alteration of host or mosquito microhabitats.

6. Variability in vector competence

Across members of the *Cx. pipiens* complex, there is evidence of genetic (heritable) control underlying feeding behavior and vector competence, although the identification of the actual genes that determine those traits is in its infancy (Bartholomay et al., 2010). For example, based on the analysis of neutral genomic DNA loci (microsatellites), *Cx. pipiens* collected from distant locations in New York State were more genetically distinct and differed in vector competence for WNV more than mosquitoes collected from a single location (Kilpatrick et al., 2010). In addition, although temporal variation was evident in all locations, genetic ancestry was associated with differences in vector competence, with form pipiens mosquitoes more likely to become infected with WNV in one of two populations studied (Kilpatrick et al., 2010). This pattern was also replicated in recent studies of vector competence conducted with laboratory hybrids of colonized *Cx. pipiens* form pipiens, form molestus, and *Cx. quinquefasciatus* that indicated significant differences in vector competence for WNV (Kramer, Kilpatrick and Fonseca, personal communication). Thus it appears that genetic variation of *Cx. pipiens* complex mosquitoes can affect the ability of the mosquito to become infected, allow virus to disseminate, and/or transmit virus.

There is also evidence that the genetics of the virus influences the ecological cycle of WNV through dynamic interactions with *Cx. pipiens* and *Cx. tarsalis* mosquitoes. An evolved genotype of WNV that was first detected in 2001 (termed WN02) completely displaced the introduced 1999 genotype (termed NY99) throughout the United States by 2004 (Davis et al., 2005; Ebel et al., 2004). Subsequent research showed that the viral strains in the evolved genotype, WN02, increased vector competence (the fraction of mosquitoes transmitting the virus) in both *Cx. pipiens* and *Cx. tarsalis* mosquitoes (Ebel et al., 2004; Moudy et al., 2007), and the difference was especially pronounced at higher temperatures (Kilpatrick et al., 2008). This occurred despite only three consistent nucleotide differences between the NY99 clade of WNV and the strains in the WN02 clade, and only one of these differences leads to an amino acid change, a valine to alanine at position 159 (Davis et al., 2005; Ebel et al., 2004). Interestingly, there were no consistent differences in vector competence between the 1999 and WN02 isolates with *Cx. quinquefasciatus* (Vanlandingham et al., 2004).

Mosquitoes may also shape the viral transmission cycle through their effect on the virus itself. Like all RNA viruses, WNV has a high mutation rate and replicates to high titers rapidly in competent hosts. Studies on field-collected *Cx. pipiens* indicated WNV isolated from mosquito pools demonstrated twice as much heterogeneity in nucleotide sequence as virus isolated from dead infected American crows from the same locations (Bertolotti et al., 2008; Jerzak et al., 2005). Experimental passage studies with both WNV and SLEV confirm that *Cx. pipiens* mosquitoes serve as a source for significant intrahost genetic diversity (Ciota et al., 2009; Jerzak et al., 2007). Despite this, the capacity to maintain such viral diversity in mosquitoes over time may be limited by species-specific differences in seasonal maintenance, vector competence, and/or within-host bottlenecks (Ciota and Kramer, 2010).

7. Conclusions and perspectives

We have provided an overview of the diverse *Cx. pipiens* complex of mosquitoes. The diversity in ecology, physiology, and behavior is somewhat surprising given the relatively close genetic relationships among members of the complex, but is partly explained by the intraspecific diversity in genetics, behavior, and vector competence that results in steep spatial and temporal discontinuities in disease transmission. This diversity, especially in feeding patterns, results in these mosquitoes being key vectors for pathogens ranging from avian malaria to strictly human filariasis.

Despite the substantial recent work many outstanding issues require further study. These include, but are not limited to: (1) the factors influencing hybridization and genetic introgression between *Cx. pipiens* and *Cx. quinquefasciatus*, as well as between the two forms of *Cx. pipiens*, form pipiens and form molestus; (2) the causes of variation in feeding patterns for all mosquitoes in the complex, including the role of mosquito attraction, host defense, and overlap in microhabitats of host-seeking mosquitoes and hosts, as well as availability-driven selection; and (3) the causes of variation in competence of *Cx. pipiens* complex mosquitoes for various pathogens, including the relative importance of genetic and environmental influences. The results of these studies will enable better mapping of the risk of infection in space and time, more efficient control and mosquito population management efforts, and insight into the evolutionary relationships underlying host-pathogen interactions.

Acknowledgements

We thank Eric Williges for GIS assistance in creating the global distribution map. Funding was provided by CDC grant CCU220532, NIH grant 1RO1AI090159-01, NIH/NIAID Contract N01A125490, and NSF grants DEB-1115069 and EF-0914866.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.meegid.2011.08.013.

References

Abdel-Hamid, Y.M., Soliman, M.I., Kenawy, M.A., 2011. Geographical distribution and relative abundance of culicine mosquitoes in relation to transmission of lymphatic filariasis in El Menoufia Governorate. Egypt. J. Egypt. Soc. Parasitol. 41, 109–118.

Andreadis, T.G., Anderson, J.F., Vossbrinck, C.R., Main, A.J., 2004. Epidemiology of West Nile virus in Connecticut: a five-year analysis of mosquito data 1999–2003. Vector-Borne Zoonotic Dis. 4, 360–378.

Anosike, J.C., Nwoke, B.E., Okere, A.N., Oku, E.E., Asor, J.E., Emmy-Egbe, I.O., Adimike, D.A., 2007. Epidemiology of tree-hole breeding mosquitoes in the tropical rainforest of Imo State, south-east Nigeria. Ann. Agric. Environ. Med. 14, 31–38.

Apperson, C.S., Hassan, H.K., Harrison, B.A., Savage, H.M., Aspen, S.E., Farajollahi, A., Crans, W., Daniels, T.J., Falco, R.C., Benedict, M., Anderson, M., McMillen, L., Unnasch, T.R., 2004. Host feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. Vector-Borne Zoonotic Dis. 4, 71–82.

Aspen, S., Crabtree, M.B., Savage, H.M., 2003. Polymerase chain reaction assay identifies *Culex nigripalpus*: part of an assay for molecular identification of the common *Culex* (*Culex*) mosquitoes of the eastern United States. J. Am. Mosq. Control Assoc. 19, 115–120.

Aspen, S., Savage, H.M., 2003. Polymerase chain reaction assay identifies North American members of the *Culex pipiens* complex based on nucleotide sequence differences in the acetylcholinesterase gene Ace.2. J. Am. Mosq. Control Assoc. 19, 323–328.

Aubry, P., 2008. Dengue outbreaks in the French West-Indies in a context of arbovirosis emergence and reemergence. Bull. Acad. Natl. Med. 192, 781–793.

Bahnck, C.M., Fonseca, D.M., 2006. Rapid assay to identify the two genetic forms of *Culex* (*Culex*) *pipiens* L. (Diptera: culicidae) and hybrid populations. Am. J. Trop. Med. Hyg. 75, 251–255.

Barr, A.R., 1957. The distribution of *Culex p. pipiens* and *Culex p. quinquefasciatus* in North America. Am. J. Trop. Med. Hyg. 6, 153–165.

Bartholomay, L.C., Waterhouse, R.M., Mayhew, G.F., Campbell, C.L., Michel, K., Zou, Z., Ramirez, J.L., Das, S., Alvarez, K., Arensburger, P., Bryant, B., Chapman, S.B., Dong, Y.M., Erickson, S.M., Karunaratne, S., Kokoza, V., Kodira, C.D., Pignatelli, P., Shin, S.W., Vanlandingham, D.L., Atkinson, P.W., Birren, B., Christophides, G.K., Clem, R.J., Hemingway, J., Higgs, S., Megy, K., Ranson, H., Zdobnov, E.M., Raikhel, A.S., Christensen, B.M., Dimopoulos, G., Muskavitch, M.A.T., 2010. Pathogenomics of *Culex quinquefasciatus* and meta-analysis of infection responses to diverse pathogens. Science 330, 88–90.

Bertolotti, L., Kitron, U.D., Walker, E.D., Ruiz, M.O., Brawn, J.D., Loss, S.R., Hamer, G.L., Goldberg, T.L., 2008. Fine-scale genetic variation and evolution of West Nile Virus in a transmission "hot spot" in suburban Chicago, USA. Virology 374, 381–389.

Bockarie, M.J., Pedersen, E.M., White, G.B., Michael, E., 2009. Role of vector control in the global program to eliminate lymphatic filariasis. Annu. Rev. Entomol. 54, 469–487.

Bogh, C., Pedersen, E.M., Mukoko, D.A., Ouma, J.H., 1998. Permethrin-impregnated bednet effects on resting and feeding behaviour of lymphatic filariasis vector mosquitoes in Kenya. Med. Vet. Entomol. 12, 52–59.

Bowden, S.E., Magori, K., Drake, J.M., 2011. Regional differences in the association between land cover and West Nile virus disease incidence in humans in the United States. Am. J. Trop. Med. Hyg. 84, 234–238.

Brown, H.E., Childs, J.E., Diuk-Wasser, M.A., Fish, D., 2008. Ecological factors associated with west nile virus transmission, northeastern United States. Emerg. Infect. Dis. 14, 1539–1545.

Brown, J.E., McBride, C.S., Johnson, P., Ritchie, S., Paupy, C., Bossin, H., Lutomiah, J., Fernandez-Salas, I., Ponlawat, A., Cornel, A.J., Black, W.C.t., Gorrochotegui-Escalante, N., Urdaneta-Marquez, L., Sylla, M., Slotman, M., Murray, K.O., Walker, C., Powell, J.R., 2011. Worldwide patterns of genetic differentiation imply multiple 'domestications' of *Aedes aegypti*, a major vector of human diseases. Proceedings. Biological sciences/The Royal Society.

Chandler, J.A., Boreham, P.F., Highton, R.B., Hill, M.N., 1975. A study of the host selection patterns of the mosquitoes of the Kisumu area of Kenya. Trans. R. Soc. Trop. Med. Hyg. 69, 415–425.

Ciota, A.T., Jia, Y.Q., Payne, A.F., Jerzak, G., Davis, L.J., Young, D.S., Ehrbar, D., Kramer, L.D., 2009. Experimental passage of St. Louis encephalitis virus in vivo in mosquitoes and chickens reveals evolutionarily significant virus characteristics. PLoS ONE 4.

Ciota, A.T., Kramer, L.D., 2010. Insights into arbovirus evolution and adaptation from experimental studies. Viruses-Basel 2, 2594–2617.

Coffinet, T., Mourou, J.R., Pradines, B., Toto, J.C., Jarjaval, F., Amalvict, R., Kombila, M., Carnevale, P., Pages, F., 2007. First record of *Aedes albopictus* in Gabon. J. Am. Mosq. Control Assoc. 23, 471–472.

Collins, F.H., Paskewitz, S.M., 1996. A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic *Anopheles* species. Insect Mol. Biol. 5, 1–9.

Cornel, A.J., Mcabee, R.D., Rasgon, J., Stanich, M.A., Scott, T.W., Coetzee, M., 2003. Differences in extent of genetic introgression between sympatric *Culex pipiens* and *Culex quinquefasciatus* (Diptera: Culicidae) in California and South Africa. J. Med. Entomol. 40, 36–51.

Crabtree, M.B., Savage, H.M., Miller, B.R., 1995. Development of a species-diagnostic polymerase chain reaction assay for the identification of *Culex* vectors of St. Louis encephalitis virus based on interspecies sequence variation in ribosomal DNA spacers. Am. J. Trop. Med. Hyg.s 53, 105–109.

Crabtree, M.B., Savage, H.M., Miller, B.R., 1997. Development of a polymerase chain reaction assay for differentitaion between *Culex pipiens pipiens* and *Cx. p. quinquefasciatus* (Diptera: Culicidae) in North America based on genomic differences identified by subtractive hybridization. J. Med. Entomol. 34, 532–537.

Davis, C.T., Ebel, G.D., Lanciotti, R.S., Brault, A.C., Guzman, H., Siirin, M., Lambert, A., Parsons, R.E., Beasley, D.W.C., Novak, R.J., Elizondo-Quiroga, D., Green, E.N., Young, D.S., Stark, L.M., Drebot, M.A., Artsob, H., Tesh, R.B., Kramer, L.D., Barrett, A.D.T., 2005. Phylogenetic analysis of North American West Nile virus isolates, 2001–2004: evidence for the emergence of a dominant genotype. Virology 342, 252–265.

Delatte, H., Paupy, C., Dehecq, J.S., Thiria, J., Failloux, A.B., Fontenille, D., 2008. *Aedes albopictus*, vector of chikungunya and dengue viruses in Reunion Island: biology and control. Parasite 15, 3–13.

Dobrotworsky, N.V., 1967. The problem of the *Culex pipiens* complex in the South Pacific (including Australia). Bull. World Health Organ. 37, 251–255.

Dohm, D.J., Sardelis, M.R., Turell, M.J., 2002. Experimental vertical transmission of West Nile virus by *Culex pipiens* (Diptera: Culicidae). J. Med. Entomol. 39, 640–644.

Ebel, G.D., Carricaburu, J., Young, D., Bernard, K.A., Kramer, L.D., 2004. Genetic and phenotypic variation of West Nile virus in New York, 2000–2003. Am. J. Trop. Med. Hyg. 71, 493–500.

Eldridge, B.F., 1987. Diapause and related phenomena in *Culex* mosquitoes: their relation to arbovirus disease ecology. In: Harris, K.F. (Ed.), Curr. Top. Vector Res.. Springer-Verlag, New York.

Farajollahi, A., Crans, W.J., Bryant, P., Wolf, B., Burkhalter, K.L., Godsey, M.S., Aspen, S.E., Nasci, R.S., 2005. Detection of West Nile viral RNA from an overwintering pool of *Culex pipens* pipiens (Diptera: Culicidae) in New Jersey, 2003. J. Med. Entomol. 42, 490–494.

Fonseca, D.M., Atkinson, C.T., Fleischer, R.C., 1998. Microsatellite primers for *Culex pipiens quinquefasciatus*, the vector of avian malaria in Hawaii. Mol. Ecol. 7, 1617–1619.

Fonseca, D.M., Keyghobadi, N., Malcolm, C.A., Mehmet, C., Schaffner, F., Mogi, M., Fleischer, R.C., Wilkerson, R.C., 2004. Emerging vectors in the *Culex pipiens* complex. Science 303, 1535–1538.

Fonseca, D.M., Smith, J.L., Kim, H.C., Mogi, M., 2009. Population genetics of the mosquito *Culex pipiens* pallens reveals sex-linked asymmetric introgression by *Culex quinquefasciatus*. Infect. Genet. Evol. 9, 1197–1203.

Fonseca, D.M., Smith, J.L., Wilkerson, R.C., Fleischer, R.C., 2006. Pathways of expansion and multiple introductions illustrated by large genetic differentiation among worldwide populations of the southern house mosquito. Am. J. Trop. Med. Hyg. 74, 284–289.

Gomes, B., Sousa, C.A., Novo, M.T., Freitas, F.B., Alves, R., Corte-Real, A.R., Salgueiro, P., Donnelly, M.J., Almeida, A.P., Pinto, J., 2009. Asymmetric introgression between sympatric molestus and pipiens forms of *Culex pipiens* (Diptera: Culicidae) in the Comporta region, Portugal. BMC Evol. Biol. 9, 262.

Gomez, A., Kilpatrick, A.M., Kramer, L.D., Dupuis, A.P., Jones, M.J., Goetz, S.J., Marra, P.P., Daszak, P., Aguirre, A.A., 2008. Land use and West Nile virus seroprevalence in wild mammals. Emerg. Infect. Dis. 14, 962–965.

Hamer, G.L., Kitron, U.D., Brawn, J.D., Loss, S.R., Ruiz, M.O., Goldberg, T.L., Walker, E.D., 2008. *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. J. Med. Entomol. 45, 125–128.

Hamer, G.L., Kitron, U.D., Goldberg, T.L., Brawn, J.D., Loss, S.R., Ruiz, M.O., Hayes, D.B., Walker, E.D., 2009. Host selection by *Culex pipiens* mosquitoes and West Nile Virus amplification. Am. J. Trop. Med. Hyg. 80, 268–278.

Harbach, R.E., Dahl, C., White, G.B., 1985. *Culex* (*Culex*) *pipiens* Linnaeus (Diptera: Culicidae): concepts, type designations, and description. Proc. Entomol. Soc. Wash. 87, 1–24.

Harbach, R.E., Harrison, B.A., Gad, A.M., 1984. *Culex* (*Culex*) *molestus* Forskal (Diptera: Culicidae): neotype designation, description, variation, and taxonomic status. Proc. Entomol. Soc. Wash. 86, 521–542.

Hassan, H.K., Cupp, E.W., Hill, G.E., Katholi, C.R., Klingler, K., Unnasch, T.R., 2003. Avian host preference by vectors of eastern equine encephalomyelitis virus. Am. J. Trop. Med. Hyg. 69, 641–647.

Huang, S.M., Hamer, G.L., Molaei, G., Walker, E.D., Goldberg, T.L., Kitron, U.D., Andreadis, T.G., 2009. Genetic variation associated with mammalian feeding in *Culex pipiens* from a West Nile virus epidemic region in Chicago, Illinois. Vector-Borne Zoonotic Dis. 9, 637–642.

Humeres, S.G., Almiron, W.R., Sabattini, M.S., Gardenal, C.N., 1998. Estimation of genetic divergence and gene flow between *Culex pipiens* and *Culex quinquefasciatus* (Diptera: Culicidae) in Argentina. Mem. Inst. Oswaldo Cruz 93, 57–62.

Iltis, W.G., 1966. Biosystematics of the *Culex pipiens* complex in northern California. University of California, Davis.

Jerzak, G., Bernard, K.A., Kramer, L.D., Ebel, G.D., 2005. Genetic variation in West Nile virus from naturally infected mosquitoes and birds suggests quasispecies structure and strong purifying selection. J. Gen. Virol. 86, 2175–2183.

Jerzak, G.V.S., Bernard, K., Kramer, L.D., Shi, P.Y., Ebel, G.D., 2007. The West Nile virus-mutant spectrum is host-dependant and a determinant of mortality in mice. Virology 360, 469–476.

Jupp, P.G., 1987. Comparative studies on morphology and laboratory biology of *Culex* (*Culex*) *pipiens* Linnaeus (Diptera: Culicidae) from South Africa and England. J. Entomol. Soc. South Africa 50, 455–461.

Kasai, S., Komagata, O., Tomita, T., Sawabe, K., Tsuda, Y., Kurahashi, H., Ishikawa, T., Motoki, M., Takahashi, T., Tanikawa, T., Yoshida, M., Shinjo, G., Hashimoto, T., Higa, Y., Kobayashi, M., 2008. PCR-based identification of *Culex pipiens* complex collected in Japan. Japanese J. Infectious Dis. 61, 184–191.

Kenoyer, J.M., 1998. Ancient Cities of the Indus Valley Civilization. Oxford University Press, Oxford.

Kent, R., Juliusson, L., Weissmann, M., Evans, S., Komar, N., 2009. Seasonal blood feeding behavior of *Culex tarsalis* (Diptera: Culicidae) in Weld County, Colorado, 2007. J. Med. Entomol. 46, 380–390.

Kent, R.J., Harrington, L.C., Norris, D.E., 2007. Genetic differences between *Culex pipiens* f. molestus and *Culex pipiens* f. pipiens (Diptera: Culicidae) in New York. J. Med. Entomol. 44, 50–59.

Kiehn, L., Murphy, K.E., Yudin, M.H., Loeb, M., 2008. Self-reported protective behaviour against West Nile Virus among pregnant women in Toronto. J. Obstet. Gynaecol. Can 30, 1103–1109.

Kilpatrick, A.M., Daszak, P., Jones, M.J., Marra, P.P., Kramer, L.D., 2006a. Host heterogeneity dominates West Nile virus transmission. Proc. Royal Soc. B: Biol. Sci. 273, 2327–2333.

Kilpatrick, A.M., Fonseca, D.M., Ebel, G.D., Reddy, M.R., Kramer, L.D., 2010. Spatial and temporal variation in vector competence of *Culex pipiens* and *Cx. restuans* mosquitoes for West Nile virus. Am. J. Trop. Med. Hyg. 77, 667–671.

Kilpatrick, A.M., Gluzberg, Y., Burgett, J., Daszak, P., 2004. A quantitative risk assessment of the pathways by which West Nile virus could reach Hawaii. EcoHealth 1, 205–209.

Kilpatrick, A.M., Kramer, L.D., Campbell, S., Alleyne, E.O., Dobson, A.P., Daszak, P., 2005. West Nile virus risk assessment and the bridge vector paradigm. Emerg. Infect. Dis. 11, 425–429.

Kilpatrick, A.M., Kramer, L.D., Jones, M.J., Marra, P.P., Daszak, P., 2006b. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biol. 4, 606–610.

Kilpatrick, A.M., Kramer, L.D., Jones, M.J., Marra, P.P., Daszak, P., Fonseca, D.M., 2007. Genetic influences on mosquito feeding behavior and the emergence of zoonotic pathogens. Am. J. Trop. Med. Hyg. 77, 667–671.

Kilpatrick, A.M., Meola, M.A., Moudy, R.M., Kramer, L.D., 2008. Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. PLoS Pathog. 4, e1000092.

Kimura, M., Darbro, J.M., Harrington, L.C., 2011. Avian malaria parasites share congeneric mosquito vectors. Journal of Parasitology 96, 144–151.

Knight, K.L., 1978. Supplement to a catalog of the mosquitoes of the World (Diptera: Culicidae). Thomas Say Foundation 6, 1–107.

Knight, K.L., Malek, A.A.A., 1951. A morphological and biological study of *Culex pipiens* in the Cairo area of Egypt. Bull. Soc. Fouad I Entomol. 35, 175–185.

Kothera, L., Zimmerman, E.M., Richards, C.M., Savage, H.M., 2009. Microsatellite characterization of subspecies and their hybrids in *Culex pipiens* complex (Diptera: Culicidae) mosquitoes along a north-south transect in the central United States. J. Med. Entomol. 46, 236–248.

Kramer, L.D., Styer, L.M., Ebel, G.D., 2008. A global perspective on the epidemiology of West Nile virus. Annu. Rev. Entomol. 53, 61–81.

Liu, J.Y., Liu, X.J., Chen, Z., Tu, Z.P., Zheng, G.B., Chen, Y.N., Zhang, Y.Z., Weng, S.P., Huang, X.H., Yang, F.Z., 1991. Filariasis and its control in Fujian, China. The Southeast Asian J. Trop. Med. Public Health 22, 147–154.

Lukacik, G., Anand, M., Shusas, E.J., Howard, J.J., Oliver, J., Chen, H., Backenson, P.B., Kauffman, E.B., Bernard, K.A., Kramer, L.D., White, D.J., 2006. West Nile virus surveillance in mosquitoes in New York state, 2000–2004. J. Am. Mosq. Control Assoc. 22, 264–271.

Mattingly, P.F., 1965. The systematics of the *Culex pipiens* complex. Bull. World Health Organ. 37, 257–261.

Mattingly, P.F., Rozeboom, L.E., Knight, K.L., Laven, H., Drummond, F.H., Christophers, S.R., Shute, P.G., 1951. The *Culex pipiens* complex. T. Royal Entomol. Soc. London 102, 331–382.

McIntosh, B.M., Jupp, P.G., Dickinson, D.B., McGillivray, G.M., Sweetnam, J., 1967. Ecological studies on Sindbis and West Nile viruses in South Africa. I. Viral activity as revealed by infection of mosquitoes and sentinel fowls. South African J. Med. Sci. 32, 1–14.

Michael, E., Gambhir, M., 2010. Vector transmission heterogeneity and the population dynamics and control of lymphatic filariasis. Adv. Exp. Med. Biol. 673, 13–31.

Miller, B.R., Crabtree, M.B., Savage, H.M., 1996. Phylogeny of fourteen *Culex* mosquito species, including the *Culex pipiens* complex, inferred from the internal transcribed spacers of ribosomal DNA. Insect Mol. Biol. 5, 93–107.

Moudy, R.M., Meola, M.A., Morin, L.L., Ebel, G.D., Kramer, L.D., 2007. A newly emergent genotype of West Nile virus is transmitted earlier and more efficiently by *Culex* mosquitoes. Am. J. Trop. Med. Hyg. 77, 365–370.

Nasci, R.S., Savage, H.M., White, D.J., Miller, J.R., Cropp, B.C., Godsey, M.S., Kerst, A.J., Bennett, P., Gottfried, K., Lanciotti, R.S., 2001. West Nile virus in overwintering *Culex* mosquitoes, New York City, 2000. Emerg. Infect. Dis. 7, 742–744.

Reisen, W.K., Milby, M.M., Presser, S.B., Hardy, J.L., 1992. Ecology of mosquitos and St. Louis encephalitis virus in the Los Angeles basin of California, 1987–1990. J. Med. Entomol. 29, 582–598.

Robich, R.M., Denlinger, D.L., 2005. Diapause in the mosquito *Culex pipiens* evokes a metabolic switch from blood feeding to sugar gluttony. Proc. Natl. Acad. Sci. USA 102, 15912–15917.

Ruiz, M.O., Chaves, L.F., Hamer, G.L., Sun, T., Brown, W.M., Walker, E.D., Haramis, L., Goldberg, T.L., Kitron, U.D., 2010. Local impact of temperature and precipitation on West Nile virus infection in *Culex* species mosquitoes in northeast Illinois, USA. Parasites & Vectors 3, 19.

Sanogo, Y.O., Kim, C.H., Lampman, R., Halvorsen, J.G., Gad, A.M., Novak, R.J., 2008. Identification of male specimens of the *Culex pipiens* complex (Diptera: Culicidae) in the hybrid zone using morphology and molecular techniques. J. Med. Entomol. 45, 203–209.

Sardelis, M.R., Turell, M.J., Dohm, D.J., O'Guinn, M.L., 2001. Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. Emerg. Infect. Dis. 7, 1018–1022.

Savage, H.M., Aggarwal, D., Apperson, C.S., Katholi, C.R., Gordon, E., Hassan, H.K., Anderson, M., Charnetzky, D., McMillen, L., Unnasch, E.A., Unnasch, T.R., 2007. Host choice and West Nile virus infection rates in blood fed mosquitoes, including members of the *Culex pipiens* complex, from Memphis and Shelby County, Tennessee 2002–2003. Vector-Borne Zoonotic Dis. 7, 365–386.

Savage, H.M., Anderson, M., Gordon, E., McMillen, L., Colton, L., Charnetzky, D., Delorey, M., Aspen, S., Burkhalter, K., Biggerstaff, B.J., Godsey, M., 2006. Oviposition activity patterns and West Nile virus infection rates for members of the *Culex pipiens* complex at different habitat types within the hybrid zone, Shelby County, TN, 2002 (Diptera: Culicidae). J. Med. Entomol. 43, 1227–1238.

Siriyasatien, P., Pengsakul, T., Kittichai, V., Phumee, A., Kaewsaitiam, S., Thavara, U., Tawatsin, A., Asavadachanukorn, P., Mulla, M.S., 2010. Identification of blood meal of field caught *Aedes aegypti* (L.) by multiplex PCR. Southeast Asian J. Trop. Med. Public Health 41, 43–47.

Smith, J.L., Fonseca, D.M., 2004. Rapid assays for identification of members of the *Culex* (*Culex*) *pipiens* complex, their hybrids, and other sibling species (Diptera: culicidae). Am. J. Trop. Med. Hyg. 70, 339–345.

Smith, J.L., Keyghobadi, N., Matrone, M.A., Escher, R., Fonseca, D.M., 2005. Cross-species comparison of microsatellite loci in the *Culex pipiens* complex and beyond. Mol. Ecol. Notes 5, 697–700.

Spielman, A., 1971. Bionomics of autogenous mosquitoes. Annu. Rev. Entomol. 16, 231–248.

Spielman, A., 2001. Structure and seasonality of nearctic *Culex pipiens* populations. Ann. N. Y. Acad. Sci. 951, 220–234.

Sudomo, M., Chayabejara, S., Duong, S., Hernandez, L., Wu, W.P., Bergquist, R., 2010. Elimination of lymphatic filariasis in Southeast Asia. Adv. Parasit. 72, 205–233.

Tarantola, A., Quatresous, I., Ledrans, M., Lassel, L., Krastinova, E., Cordel, H., Lapidus, N., Debruyne, M., Poveda, J.D., Boude-Chevalier, M., Schuffenecker, I., Zeller, H., Grandadam, M., Tolou, H., Paquet, C., 2009. Imported cases of dengue fever diagnosed in metropolitan France, from January 2001 to December 2006. Med. Mal. Infect. 39, 41–47.

Tiawsirisup, S., Platt, K.B., Evans, R.B., Rowley, W.A., 2005. A comparision of West Nile virus transmission by *Ochlerotatus trivittatus* (COQ.), *Culex pipiens* (L.), and *Aedes albopictus* (Skuse). Vector-Borne Zoonotic Dis. 5, 40–47.

Townson, H., Nathan, M.B., 2008. Resurgence of chikungunya. Trans. R Soc. Trop. Med. Hyg. 102, 308–309.

Turell, M.J., Dohm, D.J., Sardelis, M.R., O Guinn, M.L., Andreadis, T.G., Blow, J.A., 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. J. Med. Entomol. 42, 57–62.

Turell, M.J., Sardelis, M.R., O'Guinn, M.L., Dohm, D.J., 2002. Potential vectors of West Nile virus in North America, in: Mackenzie, J., Barrett, A., Deubel, V. (Eds.), Japanese Encephalitis and West Nile Viruses, vol. 267, Current Topics in Microbiology and Immunology. Springer-Verlag, Berlin, pp. 241–252.

Urbanelli, S., Silvestrini, F., Reisen, W.K., De Vito, E., Bullini, L., 1997. Californian hybrid zone between *Culex pipiens pipiens* and *Cx. P. quinquefasciatus* revisited (Diptera:Culicidae). J. Med. Entomol. 34, 116–127.

Urbanelli, S., Silvestrini, F., Sabatinelli, G., Raveloarifera, F., Petrarca, V., Bullini, L., 1995. Characterization of the *Culex pipiens* complex (Diptera: Culicidae) in Madagascar. J. Med. Entomol. 32, 778–786.

Van Riper, C., III, Van., Riper, S.G., Goff, M.L., Laird, M., 1986. The epizootiology and ecological significance of malaria in Hawaiian (USA) land birds. Ecol. Monogr. 56, 327–344.

Vanlandingham, D.L., Schneider, B.S., Klingler, K., Fair, J., Beasley, D., Huang, J., Hamilton, P., Higgs, S., 2004. Real-time reverse transcriptase-polymerase chain reaction quantification of West Nile virus transmitted by *Culex pipiens quinquefasciatus*. Am. J. Trop. Med. Hyg. 71, 120–123.

Vazeille, M., Moutailler, S., Pages, F., Jarjaval, F., Failloux, A.B., 2008. Introduction of *Aedes albopictus* in Gabon: what consequences for dengue and chikungunya transmission? Trop. Med. Int. Health 13, 1176–1179.

Vinogradova, E.B., 2000. *Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Pensoft, Sofia.

Wang, E., Ni, H., Xu, R., Barrett, A.D., Watowich, S.J., Gubler, D.J., Weaver, S.C., 2000. Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. J. Virol. 74, 3227–3234.

Zhao, T.-Y., Lu, B.-L., 1999. The clasdics of *Culex pipiens* complex. Acta Zootaxonomica Sinica 24, 206–210.