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## “BIRD BITING” MOSQUITOES AND HUMAN DISEASE: A REVIEW OF THE ROLE OF *CULEX PIFIENS* COMPLEX MOSQUITOES IN EPIDEMIOLOGY

Ary Farajollahi<sup>1</sup>, Dina M. Fonseca<sup>1</sup>, Laura D. Kramer<sup>2</sup>, and A. Marm Kilpatrick<sup>3</sup>

<sup>1</sup>Rutgers University, Center for Vector Biology, New Brunswick, New Jersey

<sup>2</sup>Wadsworth Center, New York State Department of Health, Slingerlands, New York, and State University of New York at Albany, Albany, New York

<sup>3</sup>Dept. Ecology and Evolutionary Biology, University of California, Santa Cruz, California

### 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 it feeds 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 *Cx. pipiens pipiens* with two forms, *pipiens* and *molestus*, *Cx. pipiens pallens*, *Cx. quinquefasciatus*, *Cx. australicus*, and *Cx. 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.

### Keywords

Vector borne disease; invasive; West Nile virus; arbovirus; bridge vector; vector competence; *Plasmodium*

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\*To whom correspondence should be addressed: A. Marm Kilpatrick, EE Biology/EMS, Univ. of California, Santa Cruz, CA 95064, akilpatr@ucsc.edu, (831) 459-5070.

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## 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 protozoon 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 *Cx. pipiens complex* mosquitoes and the transmission of 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

(wrbu.si.edu), recognizes the following species as members of the *Cx. pipiens* complex: *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. australicus*, and *Cx. globocoxitus* (Figure 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 especially the presence of hybrids (Harbach et al., 1985; Mattingly, 1965; Mattingly et al., 1951; Vinogradova, 2000; Zhao and Lu, 1999). 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 surveillance efforts. Nonetheless 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). 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* (possibly 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).

*Culex 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; Urbanelli et al., 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 there, only *Cx. quinquefasciatus* is infected with *Wolbachia pipientis*, a rickettsian intracellular parasite that can limit reproduction between insect populations (Dobson 2003). 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, *Culex 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 anaautogenous 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. 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 species whose females are often indistinguishable from those in the *Cx. pipiens* complex (especially if damaged during collection), but which are not members of the complex because they are genetically distinct. These include *Culex restuans*, *Cx. 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 hybridization between the two forms of *Cx. 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. pipiens* was hypothesized to make *Cx. 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. pipiens* mosquitoes with higher genetic ancestry from *Cx. 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. pipiens* with a strong *molestus* ancestry (>80%) fed on birds only 60% of the time (Kilpatrick et al., 2007) (*Cx. 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. *Culex pipiens* mosquitoes and humans

Ancestral *Cx. 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 16<sup>th</sup> 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 1950's (Mattingly 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, *Culex* 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 *Culex 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 entering 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 human (Michael and Gambhir, 2010).

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

*Cx. pipiens* complex mosquitoes play important roles in the transmission of several pathogens that infect humans including WNV, *St. Louis encephalitis virus*, and filarial worms (Bogh et al., 1998; Reisen et al., 1992; Turell et al., 2002) as well as wildlife pathogens such as bird malaria (*Plasmodium* spp, Lapointe et al 2005). This results partly from the wide variety of hosts on which they feed and also their very high abundances in developed areas. Their exact role and importance in different aspects of transmission (e.g. among avian hosts vs. 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 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 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., ; 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., ; 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, Kilpatrick et al. 2007, Hamer et al. 2008, 2009)(Kilpatrick et al., 2006b). *Culex 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, *Cx. 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 (Figure 2; Supplemental Online Table 1). All 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* (Figure 2; all three 95% confidence intervals overlap; ANOVA on arc-sin

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 (Figure 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 Figure 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 vs. 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., 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 30% of blood meals) by *Cx. pipiens* on humans (Figure 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 (Kilpatrick et al., 2006b; Kilpatrick et al., 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. filariasis, 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 coincidentally 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 1–3km, 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 *Culex pipiens* and *Culex 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 *St. Louis encephalitis virus* 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).

## 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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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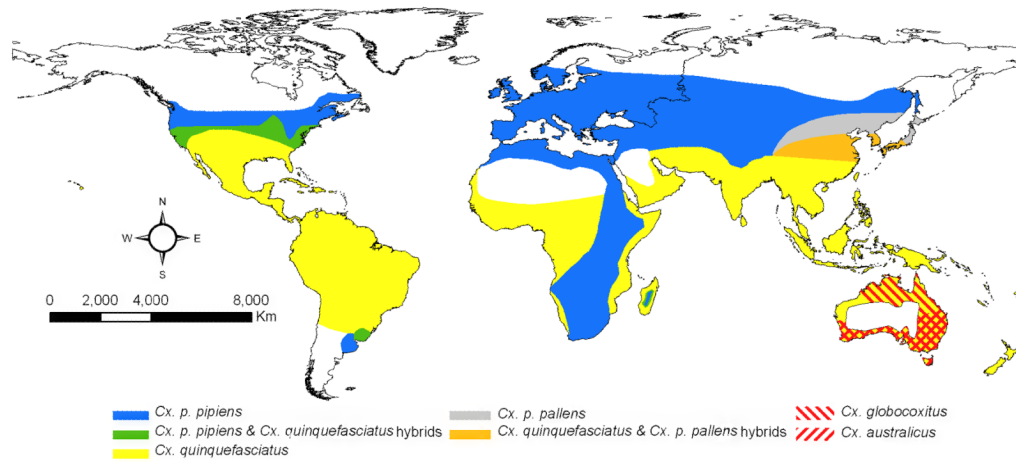
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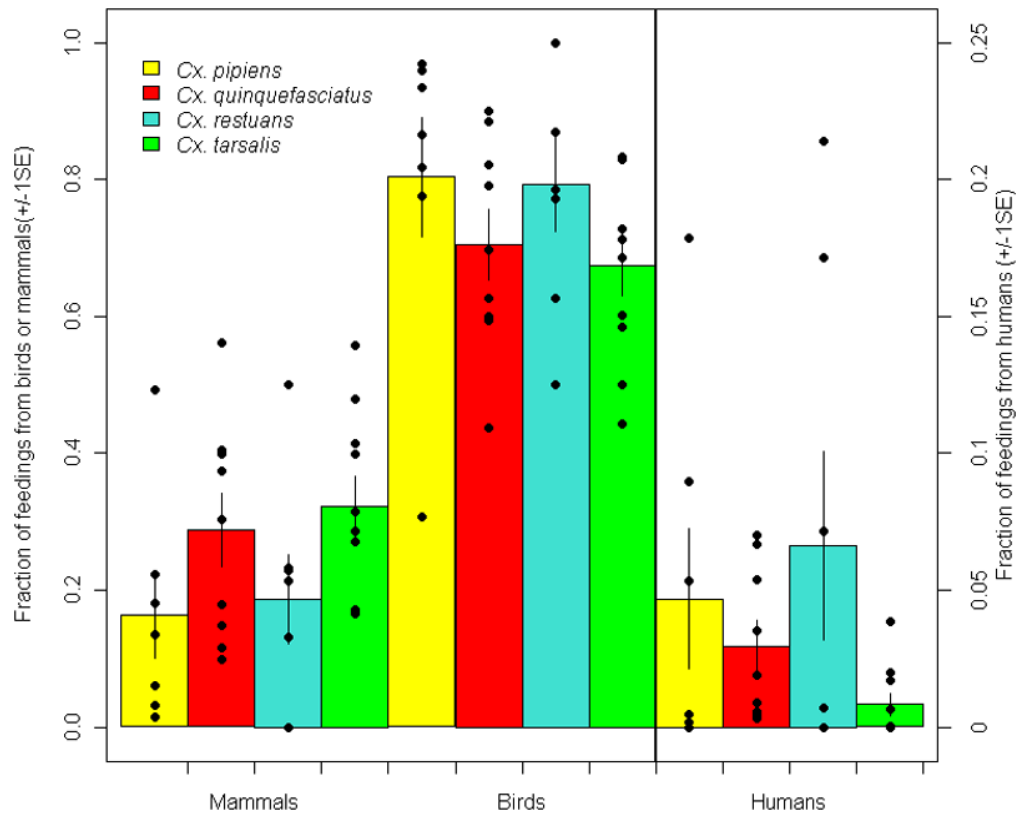
### Highlights

- 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, avian malaria (*Plasmodium* spp.) and filarial worms that cause elephantiasis.
- The advent of genetic tools has greatly enhanced our understanding of the history of speciation, domestication, dispersal and hybridization.
- The adaptation of *Cx. pipiens* complex mosquitoes to human-altered environments combined with their mixed feeding patterns on birds and mammals (including humans) greatly increase the transmission of several avian pathogens to humans.



**Figure 1.**

Global distribution of the *Cx. pipiens complex* mosquitoes. Geographic range for *Cx. 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).



**Figure 2.** Feeding patterns of four species of *Culex* mosquitoes. Left axis shows the fraction of feedings from mammals and birds (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.

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