*Am. J. Trop. Med. Hyg.*, 77(4), 2007, pp. 667–671


# Genetic Influences on Mosquito Feeding Behavior and the Emergence of Zoonotic Pathogens

A. Marm Kilpatrick,* Laura D. Kramer, Matthew J. Jones, Peter P. Marra, Peter Daszak, and Dina M. Fonseca*

*Consortium for Conservation Medicine, New York, New York; Wadsworth Center, New York State Department of Health, Slingerlands, New York; Department of Biomedical Sciences, State University of New York at Albany; Smithsonian Environmental Research Center, Edgewater, Maryland; Smithsonian Migratory Bird Center, National Zoological Park, Washington, District of Columbia; Rutgers University, Center for Vector Biology, New Brunswick, New Jersey*

*Abstract.* The feeding behavior of vectors influences the likelihood of pathogen invasion and the exposure of humans to vector-borne zoonotic pathogens. We used multilocus microsatellite genetic typing of an introduced mosquito vector and DNA sequencing of mosquito blood meals to determine the impact of hybrid ancestry on feeding behavior and the emergence of West Nile virus (WNV). The probability of ancestry of *Culex pipiens* mosquitoes from two bionomically divergent forms, form molestus and form pipiens, influenced the probability that they fed on humans but did not explain a late summer feeding shift from birds to humans. We used a simple model to show that the occurrence of pure form molestus mosquitoes would have decreased the likelihood of WNV invasion ($R_0$ in bird populations) 3- to 8-fold, whereas the occurrence of pure forms pipiens mosquitoes would have halved human exposure compared with the hybrids that are present. Data and modeling suggest that feeding preferences may be influenced by genetic ancestry and contribute to the emergence of vector-borne pathogens transmitted by introduced species, including malaria, and dengue, Chikungunya, yellow fever, and West Nile viruses.

## INTRODUCTION

Exposure of humans to multi-host zoonotic vector-borne pathogens is determined by the feeding patterns of the vectors.[1] Different vector species are well known to have different host feeding patterns,[2,3] and genetically distinct populations of a single species sometimes show varying levels of attraction to humans.[4–6] Strong links between genetic ancestry and feeding preferences may have important implications for the transmission of pathogens vectored by introduced species, which often show different genetic backgrounds compared with the populations of origin in their native range.[7,8] In turn, the feeding behavior of introduced vector populations may facilitate or inhibit the transmission of pathogens to humans, depending on the competence of humans and other hosts for the pathogen and the probability of feeding on each host. A number of significant pathogens, including malaria, and yellow fever virus, dengue virus, and West Nile virus (WNV) have been introduced outside their endemic range where they are transmitted by introduced vectors.[9,10]

*Culex pipiens* is a principal vector of WNV in the northeastern and northcentral United States[11,12] and Europe[13] and has been introduced throughout the world inadvertently by human transport.[14] It has two distinct forms in Northern Europe: form pipiens and form molestus.[15] Although conclusive evidence has been lacking, the two forms are thought to have different host preferences[16] and therefore different vectorial capacities. Form pipiens is thought to feed mainly on birds and form molestus mainly on mammals, especially humans.[17] The two forms have been shown to be genetically isolated in Northern Europe, but there is clear evidence of hybridization in North America.[14] In addition, *Culex quinquefasciatus*, a tropical to sub-tropical species that is ubiquitous in southern North America, hybridizes extensively with both forms of *Cx. pipiens* across a broad latitudinal zone creating an extraordinarily complex genetic background.[14,18] *Cx. quinquefasciatus* is known to bite both mammals and birds in significant proportions.[19,20]

It has been proposed that the mixed mammal and avian feeding habits of North American *Cx. pipiens*[16,21–23] result from the mixed ancestry of temperate populations of this species.[14] Subsequently, it was shown that *Cx. pipiens* in the mid-Atlantic exhibit a feeding shift from feeding primarily on birds in early summer to feeding on humans nearly a third of the time in fall.[23] These authors proposed that the feeding shift most likely resulted from changes in the availability of *Cx. pipiens*' preferred avian host, American robins (*Turdus migratorius*). However, they also acknowledged that a shift in genetic composition toward populations with higher *Cx. pipiens* form molestus ancestry, with the corresponding changes in propensity to feed on humans, could explain the shift independently of host availability. Here we present the results of a genetic analysis using microsatellite markers of the same mosquitoes examined previously from Washington, DC, and Maryland[23] to investigate whether genetic predisposition influences the propensity of *Cx. pipiens* mosquitoes to feed on humans and other mammals. We used models to examine the general impact of feeding patterns of vectors that feed on both humans and another host on the invasion of pathogens.

## MATERIALS AND METHODS

We collected engorged mosquitoes from six urban, residential, and park sites in Washington, DC, and Maryland in 2004 and 2005 using CDC light and gravid traps and by aspirating mosquitoes using a large backpack mounted aspirator. The urban sites were 1) the National Mall in Washington, DC, between 7th and 12th Streets, 2) ~350 m northeast of the Watergate Hotel in Foggy Bottom, DC, and 3) 300 m southwest of Camden Yards in Baltimore, MD. The two residential sites were in Takoma Park, MD, bounded by Spring Park and Opal Daniels Park, and in Bethesda, MD, along Wilson Lane from Selkirk Drive to Bradley Boulevard, and the final site was a park within an urban setting, Fort Dupont Park, DC.[22,23] A standard polymerase chain reaction (PCR) proto-

* Address correspondence to A. Marm Kilpatrick, Consortium for Conservation Medicine, 460 W. 34th St., 17th Floor, New York, NY 10001. E-mail: kilpatrick@conservationmedicine.org or Dina M. Fonseca, Rutgers University, Center for Vector Biology, NJ 180 Jones Ave., New Brunswick, NJ 08901. E-mail: dinafons@rci.rutgers.edu

col was used to distinguish *Cx. pipiens* mosquitoes from *Cx. restuans* and *Cx. salinarius*.[24] We identified the source of blood meals using PCR to amplify DNA from the cytochrome B gene using class specific primers and sequenced the resulting product and matched it to known sequences on GenBank by conducting a BLAST search to identify the host.[22]

We attempted to determine the genetic ancestry of all the mosquitoes from the previous analysis, but because of degradation of the DNA in 12 specimens, we were only able to include 136 of the 148 blooded mosquitoes originally examined (all were collected in 2004), which left only two samples from one of the sites, Fort Dupont Park. As a result, we removed Fort Dupont Park from the site comparisons, leaving five sites. To increase the power of our analysis, we examined the feeding and genetics of an additional 38 engorged *Cx. pipiens* collected from the same sites in 2005, using identical methods. The results of the analyses presented below are qualitatively identical if we restrict our sample to the 136 from 2004 or use all 174 specimens from 2004 and 2005, but are statistically stronger using the larger sample. We used these samples both to examine the influence of genetic ancestry on feeding and to test for a shift in genetic composition over the transmission season. To increase our sample size for the latter analysis, we also examined genetic ancestry in an additional group of 110 non-engorged *Cx. pipiens* collected between 8 June and 28 September 2005 at one of the sites, the National Mall in Washington, DC, for which we do not have feeding data. Finally, because our sampling locations are below or near parallel 39° N, the proposed northern limit of the hybrid zone between *Cx. pipiens* and *Cx. quinquefasciatus*,[25] we also examined the influence of *Cx. quinquefasciatus* ancestry on feeding patterns.

We examined the genetic ancestry of mosquitoes using highly polymorphic microsatellite markers as previously described.[14] We used CQ11F2/R3, CQ26F2/R, CxqGT4F3/R, CxqGT6bF/R, EmmaF/R, CxpGT12F2/R2, and CxpGT46F/R and removed from the analysis CxpGT9F2/R because recent studies have revealed the presence of null alleles in US populations where hybridization of *Cx. pipiens* with *Cx. quinquefasciatus* is prevalent (Fonseca and others, unpublished data). Analyses of mosquito families have shown that all the microsatellite loci used in this study are inherited in a mendelian fashion and are not sex-linked.[26,27] Microsatellite loci were amplified and sized as described previously.[28] We assigned individuals to three clusters (taxa) based on their multilocus genotypes with a maximum likelihood algorithm implemented in the program Structure 2.0.[29] We used 100,000 burn-in steps and 1,000,000 runs with a model of uncorrelated allele frequencies allowing admixture (gamma = 0.34, calculated at K = 1). In this analysis, the origin of each specimen is not disclosed, but the number of clusters (K) is decided *a priori* for each run. We used K = 3 because we knew from prior analyses that both forms of *Cx. pipiens* and *Cx. quinquefasciatus* had significantly distinct genetic signatures.[14] To assess the consistency of the analysis, we performed an exhaustive comparison of 10 runs scoring the similarity coefficient described in Rosenberg and others.[30] We also included pure populations of *Cx. pipiens* form pipiens (16 specimens from Nonnenweier and Altrip, Germany), *Cx. pipiens* form molestus (12 from Wiesbaden and Altenheim, Germany and 12 from Philadelphia, PA), and *Cx. quinquefasciatus* (12 from the Cayman Islands and 12 from Archer, FL) in the multilocus genotype analyses as benchmarks. Details on how these pure populations were evaluated for membership in each taxa are given elsewhere.[14,28,31]

We determined the effect of feeding on humans instead of other hosts on disease emergence by using an expression for the population growth ratio of a vector-borne pathogen, $R_0$, (adapted from[32,33]),

$$R_0 = \sqrt{\frac{a^2bcm}{r\mu}}.$$

In this expression, $a$ is the vector biting rate, $b$ and $c$ are the probabilities of hosts and vectors becoming infected after contact with an infectious vector or host, respectively, $m$ is the vector-to-host ratio, $r$ is the reciprocal of the host infectious period, and $\mu$ is the mosquito mortality rate. Because humans do not generate infectious viremias and are therefore dead-end hosts,[34] feeding on humans with probability $F_h$ reduces the biting rate $a$ on amplification hosts by a factor $1 - F_h$, and decreases $R_0$ by $(1 - F_h)$.[2] Below we explore the impact on $R_0$ of different values of $a$, as influenced by vector genetics and temporal trends in feeding behavior.

## RESULTS

The probability that mosquitoes fed on humans and mammals (including humans) increased with the fraction of *Cx. pipiens* form molestus ancestry based on microsatellite analysis [humans, logistic regression with each mosquito as a data point: const. = –2.91; coeff. = 2.73 ± SD 0.76; $N = 174$; $P < 0.001$ (Figures 1 and 2); mammals, logistic regression: const. = –2.53; coeff. = 2.35 ± 0.72; $N = 174$; $P = 0.001$; Figure 1]. There was no indication of a threshold response between ancestry and feeding on humans (Figure 2). The probability that mosquitoes fed on humans and mammals decreased with the fraction of *Cx. pipiens* form pipiens ancestry (Figure 1; humans, logistic regression: const. = –0.40; const. = –2.54 ± 0.75; $N = 174$; $P = 0.001$; mammals, logistic regression: const. = –0.41; coeff. = –2.13 ± 0.71; $N = 174$; $P = 0.003$). However, because the genetic composition sums to 1.0, these results are not independent. The probability of feeding on humans and mammals was uncorrelated with the probability of *Cx. quinquefasciatus* ancestry (Figure 1; $P = 0.44$ and 0.34, respectively), which was low (< 1 0%) in most (93.2%) of the sampled mosquitoes.

As we found previously,[23] using an overlapping data set, the probability that mosquitoes fed on humans and mammals increased significantly with date (Figure 1; humans, logistic regression: const. = –5.64; coeff. = 0.016 ± 0.008; $N = 174$; $P = 0.040$; mammals, logistic regression: const. = –5.79; coeff. = 0.014 ± 0.007; $N = 174$; $P = 0.012$). However, the genetic composition of mosquitoes showed no temporal trend over the season (Figure 1; % "molestus" = 0.126 + 0.0001 × date; $R^2 = 0.0\%$; $P = 0.88$; % "pipiens": $P = 0.85$; % *Cx. quinquefasciatus*: $P = 0.85$). Analysis of 110 additional non-engorged *Cx. pipiens* mosquitoes collected from the National Mall in 2005 confirmed that there was no change in genetic ancestry over the season (% "molestus": 0.277 – 0.000780 × date; $R^2 = 1.6\%$; $P = 0.19$; % "pipiens" = 0.714 + 0.000738 × date; $R^2 = 1.3\%$; $P = 0.24$; % *Cx. quinquefasciatus* = 0.0086 + 0.000043; $R^2 = 0.2\%$; $P = 0.69$).

We examined the simultaneous effects of genetic background and seasonality on feeding patterns by building a fit-

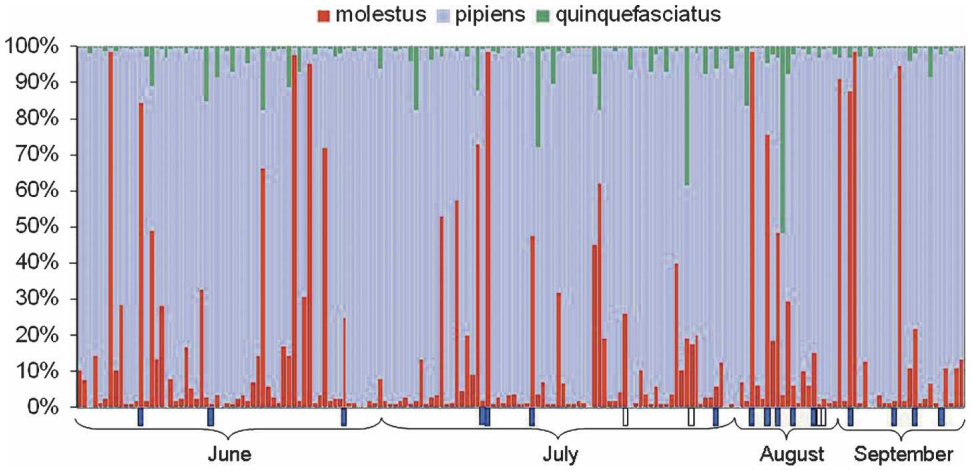


FIGURE 1. Results of a Bayesian cluster analysis. Each of the 174 individuals included in the analysis is represented by a thin vertical line, partitioned into three colored segments that represent the individual's probability of belonging to one of the three genetic clusters (*Cx. quinquefasciatus*, top segment of each column, *Cx. pipiens* form pipiens, middle segment; *Cx. pipiens* form molestus, bottom segment). Small bars under the graph denote mosquitoes that fed on mammals. Filled bars refer to specimens that obtained a human blood meal. The remaining specimens fed on birds. This figure appears in color at www.ajtmh.org.

ted regression model containing the probability of ancestry from type molestus, the observed correlation between genetic background and feeding preferences, and the changes in feeding preferences with date (Figure 3; const. = –6.54 ± 1 SD = 1.87; % molestus coeff. = 2.80 ± 0.79, $P = 0.001$; date coeff. = 0.018 ± 0.0086, $P = 0.039$). The average probability of ancestry from molestus for all mosquitoes examined was 0.146, giving a fitted or modeled probability of feeding on humans of 0.064 in mid-July and 0.17 in mid-September (Figure 3). The fitted relationship suggests that the probability of pure *Cx. pipiens* molestus mosquitoes feeding on humans would be 0.43 in mid-July and 0.69 in mid-September, whereas for pure *Cx. pipiens* pipiens the human-feeding probabilities would be 0.044 in mid-July and 0.12 in mid-September (Figure 3).

The genetic predisposition to feed on humans (for WNV, a dead-end host) led to a substantial decrease in the reproductive ratio of the pathogen, $R_0$ (Figure 3), which determines the probability of pathogen invasion.[32,35] The difference in the probability of feeding on humans between pure molestus and pure pipiens mosquitoes in our model resulted in a 2.8-fold reduction in $R_0$, in mid-July, and an 8.2-fold difference in mid-September (Figure 3).

## DISCUSSION

The feeding patterns of vectors play a key role in the transmission of vector-borne pathogens and can determine both the probability of pathogen invasion and the subsequent exposure of each host to the pathogen.[11,36–38] Here we have considered the impact of feeding behavior on transmission of vector-borne pathogens that are transmitted between two groups of hosts that may or may not amplify the pathogen and re-infect vectors. This includes a large group of important pathogens that are transmitted by an increasing number of introduced vectors with variability in genetic background and consequent variability in feeding behavior.[39,40]

Our results suggest that the feeding of *Cx. pipiens* mosquitoes may be influenced by their genetic predisposition. However, the genetic ancestry of our study populations did not change over the season, whereas their feeding patterns did.

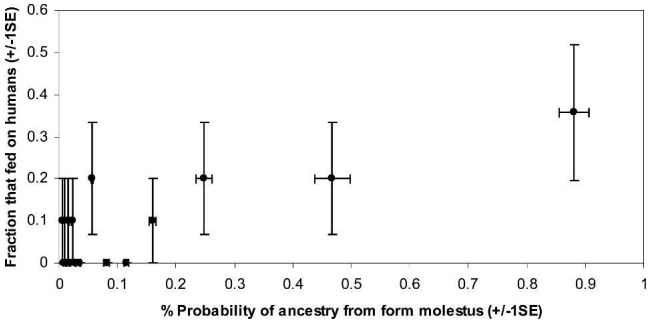


FIGURE 2. Feeding of *Cx. pipiens* mosquitoes plotted against their genetic ancestry. Each point is the average (± SE) of the feeding and genetic background of 10 individual mosquitoes, except for the top right most point that is the average of 14. Statistical analysis of the relationship treated each mosquito as a single point (logistic regression: const. = –2.91; coeff. = 2.73; $N = 174$; $P < 0.001$).

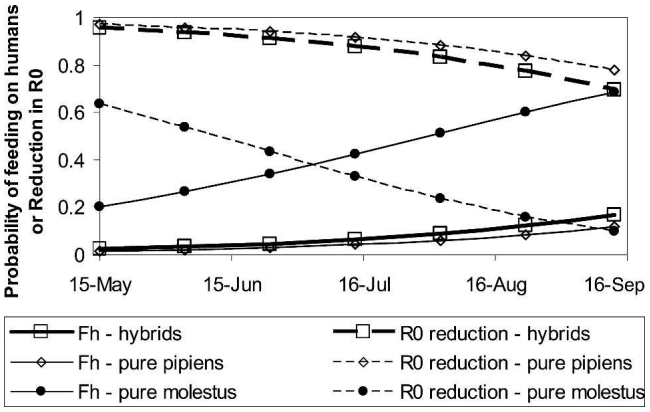


FIGURE 3. The modeled impact of genetics and date on the probability of *Cx. pipiens* feeding on humans, $F_h$, and the reduction in the probability of pathogen invasion, $R_0$.

Together these results reconcile earlier claims and provide empirical support for three hypotheses: 1) host availability plays an important role in *Cx. pipiens* feeding[23]; 2) the opportunistic feeding habits of North American *Cx. pipiens* may result from this mosquito's hybrid ancestry and likely contribute to intensified WNV epidemics[11,14]; and 3) North American WNV epidemics are intensified by a shift in the feeding of *Cx. pipiens* from birds to humans as a result of decreases in the abundance of *Cx. pipiens*' preferred host, American robins.[22,23]

Evidence of the simultaneous influence of host availability and a mosquito's genetic predisposition on feeding comes from the statistical strength of the data in Figure 2 and the fact that we found mosquitoes of primarily (> 90% probability) pipiens ancestry that fed on humans and other mammals and mosquitoes of primarily molestus ancestry that fed on birds (Figure 1). This suggests that although feeding preferences may be influenced by genetics, the feeding patterns of a mosquito are strongly affected by the availability of hosts. Thus, changes in host availability[41] may lead to different patterns of feeding, and this has important implications for the transmission of West Nile virus and other pathogens. Similarly, introduction of vectors and pathogens to new regions[42,43] may result in vector populations with different genetic backgrounds than the native populations, different feeding preferences, and altered patterns of pathogen transmission.

Finally, we showed how genetically influenced feeding behavior may have significant impacts on the probability of invasion of a pathogen. Increased feeding on humans is likely to decrease $R_0$ for WNV and other zoonotic vector-borne pathogens for which humans are a dead-end host. At the same time, increased human feeding by infected mosquitoes obviously also results in an increase in human exposure. Thus, feeding on humans can both increase and decrease the transmission of pathogens to humans, with the net impact depending on other aspects of transmission such as vector abundance and survival, and host and vector competence. This contrasts with pathogens where humans are an important amplification host (e.g., malaria, dengue virus), in that feeding on humans increases both the probability of an epidemic and subsequent exposure of humans. Understanding the factors that determine the feeding behavior of vectors is of paramount importance for reducing the impact of disease caused by vector-borne pathogens.

Received March 14, 2007. Accepted for publication July 2, 2007.

Acknowledgments: The authors thank Carolyn Bahnck, Amy Farrell, Tapan Ganguly, and the staff of the University of Pennsylvania Sequencing Facility for GeneScan analyses. We also thank the field crews of 2004 and 2005 (especially H. Brightman, J. Norton, and R. Peters); participants of the Smithsonian's Neighborhood Nestwatch program, and residents in Foggy Bottom, Bethesda, and Takoma Park; the staff of Fort Dupont Park, the Smithsonian National Museum of Natural History, the National Gallery of Art, and the Hirshhorn museum for permission to use their property; the Tuckers for providing logistical support, and the Wadsworth Center Molecular Genetics core for DNA sequencing and primer preparation. We are indebted to Norbert Becker, George O'Meara, and William Petrie for sending us specimens of *Cx. pipiens* complex from Germany, Florida, and the Cayman Islands, respectively.

Financial support: This work was funded by NIAID Contract NO1-AI-25490, National Fish and Wildlife Foundation Grant 2003-0209-000, NSF Grant EF-0622391 as part of the joint NSF-NIH Ecology of Infectious Disease program, and core funding to the Consortium for Conservation Medicine from the V. Kann Rasmussen Foundation.

Authors' addresses: A. Marm Kilpatrick and Peter Daszak, Consortium for Conservation Medicine, 460 W. 34th St., 17th Floor, New York, NY 10001, Telephone: 212-380-4473, E-mails: kilpatrick@conservationmedicine.org, daszak@conservationmedicine.org. Laura D. Kramer and Matthew J. Jones, Wadsworth Center, New York State Department of Health, 5668 State Farm Rd., Slingerlands, NY 12159, Telephone: 518-869-4524, E-mails: ldk02@health.state.ny.us, mjj05@health.state.ny.us. Peter P. Marra, Smithsonian Migratory Bird Center, National Zoological Park, 3001 Connecticut Avenue NW, Washington, DC 20008, Telephone: 202-633-1594, E-mail: marrap@si.edu. Dina M. Fonseca, Rutgers University, Center for Vector Biology, NJ 180 Jones Ave., New Brunswick, NJ 08901, Telephone: 732-932-3146, E-mail: dinafons@rci.rutgers.edu.

Reprint requests: A. Marm Kilpatrick, Consortium for Conservation Medicine, 460 W. 34th St., 17th Floor, New York, NY 10001. E-mail: kilpatrick@conservationmedicine.org or Dina M. Fonseca, Rutgers University, Center for Vector Biology, NJ 180 Jones Ave., New Brunswick, NJ 08901. Telephone: 732-932-3146, E-mail: dinafons@rci.rutgers.edu.

## REFERENCES

1. Weaver SC, 2005. Host range, amplification and arboviral disease emergence. *Arch Virol Suppl:* 33–44.
2. Tempelis CH, 1974. Host-feeding patterns of mosquitoes with a review of advances in analysis of blood meals by serology. *J Med Entomol 11:* 635–653.
3. Turell MJ, Sardelis MR, O'Guinn ML, Dohm DJ, 2002. Potential vectors of West Nile virus in North America. Mackenzie J, Barrett A, Deubel V, eds. *Japanese Encephalitis and West Nile Viruses. Vol. 267 Current Topics in Microbiology and Immunology.* Berlin: Springer-Verlag, 241–252.
4. Mukwaya LG, Kayondo JK, Crabtree MB, Savage HM, Biggerstaff BJ, Miller BR, 2000. Genetic differentiation in the yellow fever virus vector, *Aedes simpsoni* complex, in Africa: sequence variation in the ribosomal DNA internal transcribed spacers of anthropophilic and non-anthropophilic populations. *Insect Mol Biol 9:* 85–91.
5. Arredondojimenez JI, Bown DN, Rodriguez MH, Villarreal C, Loyola EG, Frederickson CE, 1992. Tests for the existence of genetic determination or conditioning in host selection by *Anopheles albimanus* (Diptera, Culicidae). *J Med Entomol 29:* 894–897.
6. Hii JLK, Chew M, Sang VY, Munstermann LE, Tan SG, Panyim S, Yasothornsrikul S, 1991. Population genetic-analysis of host seeking and resting behaviors in the malaria vector, *Anopheles balabacensis* (Diptera, Culicidae). *J Med Entomol 28:* 675–684.
7. Powell JR, Tabachnick WJ, Arnold J, 1980. Genetics and the origin of a vector population-*Aedes aegypti*, a case study. *Science 208:* 1385–1387.
8. Tabachnick WJ, Black WC, 1995. Making a case for molecular population genetic-studies of arthropod vectors. *Parasitol Today 11:* 27–30.
9. Killeen GF, Fillinger U, Kiche I, Gouagna LC, Knols BGJ, 2002. Eradication of Anopheles gambiae from Brazil: lessons for malaria control in Africa? *Lancet Infect Dis 2:* 618–627.
10. Tatem AJ, Hay SI, Rogers DJ, 2006. Global traffic and disease vector dispersal. *Proc Natl Acad Sci USA 103:* 6242–6247.
11. Kilpatrick AM, Kramer LD, Campbell S, Alleyne EO, Dobson AP, Daszak P, 2005. West Nile virus risk assessment and the bridge vector paradigm. *Emerg Infect Dis 11:* 425–429.
12. Kramer LD, Bernard KA, 2001. West Nile virus infection in birds and mammals. *Ann N Y Acad Sci 951:* 84–93.
13. Hubalek Z, Halouzka J, 1999. West Nile fever—a reemerging mosquito-borne viral disease in Europe. *Emerg Infect Dis 5:* 643–650.
14. Fonseca DM, Keyghobadi N, Malcolm CA, Mehmet C, Schaffner F, Mogi M, Fleischer RC, Wilkerson RC, 2004. Emerging vectors in the *Culex pipiens* complex. *Science 303:* 1535–1538.
15. Harbach RE, Harrison BA, Gad AM, 1984. *Culex (Culex) molestus* Forskal (Diptera: Culicidae): neotype designation, de-

scription, variation, and taxonomic status. *Proc Entomol Soc Wash 86:* 521–542.
16. Spielman A, 2001. Structure and seasonality of nearctic *Culex pipiens* populations. *Ann N Y Acad Sci 951:* 220–234.
17. Fonseca DM, Keyghobadi N, Malcolm CA, Schaffner F, Mogi M, Fleischer RC, Wilkerson RC, 2004. Outbreak of West Nile virus in North America—response. *Science 306:* 1473–1475.
18. Urbanelli S, Silvestrini F, Reisen WK, 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.
19. Samuel PP, Arunachalam N, Hiriyan J, Thenmozhi V, Gajanana A, Satyanarayana K, 2004. Host-feeding pattern of *Culex quinquefasciatus* Say and *Mansonia annulifera* (Theobald) (Diptera: Culicidae), the major vectors of filariasis in a rural area of south India. *J Med Entomol 41:* 442–446.
20. Zinser M, Ramberg F, Willott E, 2004. *Culex quinquefasciatus* (Diptera: Culicidae) as a potential West Nile virus vector in Tucson, Arizona: blood meal analysis indicates feeding on both humans and birds. *J Insect Sci 4:* 20.
21. Apperson CS, Hassan HK, Harrison BA, Savage HM, Aspen SE, Farajollahi A, Crans W, Daniels TJ, Falco RC, Benedict M, Anderson M, McMillen L, Unnasch TR, 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.
22. Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD, 2006. Host heterogeneity dominates West Nile virus transmission. *Proceedings of the Royal Society B: Biological Sciences 273:* 2327–2333.
23. Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P, 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol 4:* 606–610.
24. Crabtree MB, Savage HM, Miller BR, 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 53:* 105–109.
25. Barr AR, 1957. The distribution of *Culex p. pipiens* and *Culex p. quinquefasciatus* in North America. *Am J Trop Med Hyg 6:* 153–165.
26. Fonseca DM, Atkinson CT, Fleischer RC, 1998. Microsatellite primers for *Culex pipiens quinquefasciatus*, the vector of avian malaria in Hawaii. *Mol Ecol 7:* 1617–1619.
27. Keyghobadi N, Matrone MA, Ebel GD, Kramer LD, Fonseca DM, 2004. Microsatellite loci from the northern house mosquito (*Culex pipiens*), a principal vector of West Nile virus in North America. *Mol Ecol Notes 4:* 20–22.
28. Smith JL, Fonseca DM, 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.
29. Pritchard JK, Stephens M, Donnelly P, 2000. Inference of population structure using multilocus genotype data. *Genetics 155:* 945–959.
30. Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, Zhivotovsky LA, Feldman MW, 2002. Genetic structure of human populations. *Science 298:* 2381–2385.
31. Bahnck CM, Fonseca DM, 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.
32. Dobson AP, Foufopoulos J, 2001. Emerging infectious pathogens of wildlife. *Philos Trans R Soc Lond B Biol Sci 356:* 1001–1012.
33. Wonham MJ, de-Camino-Beck T, Lewis MA, 2004. An epidemiological model for West Nile virus: invasion analysis and control applications. *Proc R Soc Lond B Biol Sci 271:* 501–507.
34. Biggerstaff B, Petersen L, 2002. Estimated risk of West Nile virus transmission through blood transfusion during an epidemic in Queens, New York City. *Transfusion 42:* 1019–1026.
35. Anderson RM, May RM, 1991. Infectious diseases of humans. Dynamics and control. London: Oxford University Press.
36. Woolhouse MEJ, Dye C, Etard JF, Smith T, Charlwood JD, Garnett GP, Hagan P, Hii JLK, Ndhlovu PD, Quinnell RJ, Watts CH, Chandiwana SK, Anderson RM, 1997. Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proc Natl Acad Sci USA 94:* 338–342.
37. Dye C, Hasibeder G, 1986. Population dynamics of mosquito-borne disease—effects of flies which bite some people more frequently than others. *Trans R Soc Trop Med Hyg 80:* 69–77.
38. Hasibeder G, Dye C, 1988. Population-dynamics of mosquito-borne disease—persistence in a completely heterogeneous environment. *Theor Popul Biol 33:* 31–53.
39. Besansky NJ, Hill CA, Costantini C, 2004. No accounting for taste: host preference in malaria vectors. *Trends Parasitol 20:* 249–251.
40. Ulloa A, Arredondo-Jimenez JI, Rodriguez MH, Fernandez-Salas I, Gonzalez-Ceron L, 2004. Innate host selection in *Anopheles vestitipennis* from southern Mexico. *J Am Mosq Control Assoc 20:* 337–341.
41. La Deau SL, Kilpatrick AM, Marra PP, 2007. West Nile virus emergence and large-scale declines of North American bird populations. *Nature 447:* 710–713.
42. Kilpatrick AM, Daszak P, Goodman SJ, Rogg H, Kramer LD, Cedeno V, Cunningham AA, 2006. Predicting pathogen introduction: West Nile virus spread to Galapagos. *Conserv Biol 20:* 1224–1231.
43. Kilpatrick AM, 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.