Spring 2013

Microsatellite Analysis of Gene Flow between Culex tarsalis Populations Connected by Irrigation in Yellowstone County, Montana

Mattie Casey
Carroll College, Helena, MT

Microsatellite Analysis of Gene Flow between *Culex tarsalis* Populations Connected by Irrigation in Yellowstone County, Montana

Submitted in partial fulfillment of the requirements for graduation with honors from the Department of Natural Sciences at Carroll College, Helena, Montana

Mattie Casey

April 2013

This thesis for honors recognition has been approved for the Department of Natural Sciences by:

Jennifer Geiger — 4/11/13

Dr. Jennifer Geiger, Director — Date

Grant Hokit — 4/11/13

Dr. Grant Hokit, Reader — Date

Holly Zullo — 4/11/13

Dr. Holly Zullo, Reader — Date

Acknowledgements

I would like to thank Dr. Jennifer Geiger for all of her support and guidance and for the opportunity to understand the true meaning of "research." Her encouragement and persistence has fueled the countless hours I have spent in the lab. I thank Dr. Grant Hokit for going out into the field to gather the samples for this thesis as well as providing a map of their locations. I also thank him for his assistance in helping me calculate and analyze the results. I thank Dr. Gerald Shields for his critiques for the initial writing stage of this thesis. I thank MT-INBRE for their financial backing. This research was supported by a grant from the National Center for Research Resources (P20 RR16455-09), a component of the National Institutes of Health. I thank Kellie O'Rourke for her mentoring and continued inspiration after her graduation. I thank Keeli Nelson for her assistance in gathering data this past summer. I thank Paige Esposito and Max Bernt for their support and camaraderie for the past two years. I thank Carroll College for providing research lab facilities. Finally, I thank my friends and family for their continued encouragement and optimism.

Table of Contents

Abstract

The purpose of this study was to determine whether population connectivity by irrigation enhances gene flow between *Culex tarsalis* populations in the state of Montana. Four populations of *Cx. tarsalis* were collected along the Yellowstone and Bighorn rivers and allele frequencies obtained from PCR amplification of four microsatellite loci, visualized using the QIAxcel Advanced System, were used to assess the genetic structure of the populations. Results of the four loci indicate lower pairwise F_{ST} values between only two of three populations connected through waterway, which suggests a disparity in the data. If the genetic similarity between these populations reflects rates of gene flow, these results suggest that higher degrees of gene flow may not be due to irrigation but rather to connectivity by any waterway. Further analysis of additional polymorphic microsatellite loci needs to be performed to determine if a higher degree of gene flow does indeed occur between populations connected by irrigation.

List of Tables

List of Figures

Introduction

Background

West Nile Virus (WNV) (*Flaviviridae: Flavivirus*) is an arbovirus that was first detected in Uganda in 1937 (Clements 2012; Smithburn *et al.* 1940). The first case of WNV in the United States was discovered in 1999 in New York (CDC 1999) and by 2002 it was detected in Montana (Huhn *et al.* 2003). The strain of West Nile Virus detected in New York in 1999 was determined to be most similar to the Israeli strain (Lanciotti *et al.* 1999), and it is believed that WNV was brought to the United States through the import of exotic birds because two zoos in New York had infected exotic birds in the summer of 1999 (Clements 2012). The main vectors of WNV are mosquitoes, however not all mosquitoes are competent vectors of the virus. For a mosquito to be a competent vector of WNV it must come into contact with the virus while taking a bloodmeal. Once the mosquito ingests the virus it needs to pass through the midgut epithelium cells, replicate, and enter into the salivary glands (Clements 2012). From there the virus must eventually make its way into the salivary ducts where it can then infect its next host by being released while the mosquito takes a bloodmeal (Clements 2012).

Most people that are infected with WNV are asymptomatic, while some experience symptoms much like the flu. Symptoms, however, can be as severe as inflammation of the brain and nerve cells along with cell death and localized bleeding (Marra *et al.* 2004). WNV infection outcomes can be proportioned to: 100 asymptomatic: 30 febrile: 1 neuroinvasive (Clements 2012). Since 1999 there have been a total of 36,801 cases of WNV in the United States with 1,506 of those cases leading to death. Montana has seen 508 cases of WNV with only nine fatalities since its introduction to the state (CDC 2012). Because the majority of people are asymptomatic, however, these infection numbers could actually be much higher.

WNV epidemics arise when wet springs are followed by hot, dry summers (Marra *et al.* 2004). Likewise, higher temperatures shorten the extrinsic incubation period (EIP), which is the time after receiving an infectious bloodmeal that females become infected and are able to transmit the virus (Reisen *et al.* 2006). Thus early onset peak temperatures

lead to increased amplification of the virus and subsequently increased human incidence rates (Reisen *et al.* 2009). *Culex tarsalis* mosquitoes, the primary vector in the western US, are an enzootic vector, infecting avian amplifying hosts, as well as a bridge vector, meaning they can transmit the virus from an amplifying host to a dead-end host (Clements 2012). In the early summer season Kent *et al.* (2009) found that *Cx. tarsalis* primarily feed on the American Robin (*Turdus migratorius*) and Mourning Doves (*Zenaida macroura*) and shift to feeding on mammals near the end of the season due to a lack of preferred avian hosts. This behavior may be the explanation for greater human rates of WNV in the latter part of the season. The speed with which WNV spread across the United States caused scientists to believe that migratory birds were responsible for the movement (Rappole *et al.* 2000), however for the 2000 and 2001 outbreaks Rappole and Hubalek (2003) indicated that the pattern of WNV movement did not show long-distance "leap-frog" motions, but rather radiated outward from all directions. Due to this information, more recent suggestions have indicated that the virus spread more through the random movement of resident birds (Reisen *et al*. 2004). Venkatesan and Rasgon (2010), however, found evidence of high gene flow across large geographic distances for *Cx. tarsalis*, which in combination with *Cx. tarsalis'* ability to travel up to four km in a day (Reisen and Reeves 1990), may suggest a role for *Cx. tarsalis* in the dispersal of the virus across the United States.

Cx. tarsalis populations are typically more abundant at elevations between 1200 and 1450 m (Barker *et al.* 2009). This species also prefers rural habitats (Epstein and Defilippo 2001), and breeds in newly created standing water that is high in organic material (Beehler and Mulla 1995). River corridors near irrigated lands, which may be effective breeding grounds for *Cx. tarsalis*, are now being considered as a means for dispersing these mosquitoes into nearby populations (Eisen *et al.* 2010). These findings have led to more studies looking at the importance of irrigation to the development of WNV. The same study by Eisen *et al*. (2010) showed that there is a meaningful positive affiliation between shorter distance to irrigated agriculture and elevated WNV prevalence in Colorado where the landscape is similar to Montana. Likewise, positive correlations between rural agricultural settings and WNV incidence were found in Iowa (DeGroote *et al.* 2008), California (Reisen *et al.* 1992), and Texas (Cardenas *et al.* 2011). From 2002 to

2007, Montana saw a jump from 1.967 million acres of irrigated land to 2.013 million acres (USDA 2007). The majority of Montana inhabitants reside in rural communities, many of which center around the agricultural industry. This landscape provides ideal habitat for *Cx. tarsalis* development as well as bird hosts (Cardenas *et al.* 2011). Because of this, risk levels should be higher near irrigation due to the number of females feeding on infected birds and later on dead-end hosts. This study utilizes microsatellites to determine gene flow of *Cx. tarsalis* and WNV through irrigated land.

The Utility of Microsatellites

Microsatellites are tandem repeats of one to six nucleotides that have been useful determinants in other studies to estimate migration and individual relatedness (Selkoe and Toonen 2006). The number of alleles per locus can indicate the diversity of a population (Allendorf 1986), which can be valuable information when determining if gene flow occurs between two distinct regions. Rasgon *et al.* (2006) characterized six polymorphic microsatellite loci for *Cx. tarsalis* that were in Hardy-Weinberg equilibrium. Three of these loci were used in this study to determine genetic diversity between different populations in the Yellowstone County with the addition of one locus from Venkatesan *et al.* (2007). The loci used were: CutC6, CutD120, CutD107, and CutC201. Microsatellites have yet to be used to determine gene flow between populations of *Cx. tarsalis* in areas of irrigated land. Previous investigations analyzed land cover data, human incidence rates, and prevalence of *Cx. tarsalis* near areas of agricultural activity. However, in terms of measurement of gene flow, previous studies have utilized microsatellite markers to calculate F_{ST} values which can provide a measurement of population divergence (Neigel 2002).

The West Nile Virus Project at Carroll College

The goal of the West Nile Virus project at Carroll College is to produce a risk assessment model for the state of Montana. To develop this model geographic information systems and molecular characterization and detection methods are being used. The model will include information such as elevation, temperature, bird hosts,

precipitation, vegetation, virus and vector frequency, and vector genetic diversity (Hokit *et al.* 2008).

The specific aim of this study is to more deeply examine gene flow between *Cx. tarsalis* populations to see if irrigation enhances gene flow and thus exchange of WNV. My hypothesis is that populations connected by irrigation systems will be more genetically similar than those that are disconnected. Likewise, populations that are connected through a nearby river but not connected through irrigation will be more genetically alike than those disconnected through both irrigation and nearby waterways. There are already known barriers to gene flow such as mountain ranges, arid conditions, and large geographic distances (Gimnig *et al.* 1999), however, enhancers to gene flow are relatively unknown with waterway connectivity and short geographic distances among the few actually studied. Based upon previous research, however, irrigation could be a new measure of enhanced gene flow.

Materials and Methods

Mosquito samples were collected using CDC CO_2 baited traps from four fishing access sites along the Yellowstone and Bighorn rivers: Gritty Stone (Yellowstone River), Voyagers Rest (Yellowstone River), Bundy Bridge (Yellowstone River) and Arapooish (Bighorn River). The Voyagers Rest and Bundy Bridge locations are not only connected by waterway, but are also connected by the same flood irrigation zone. Gritty Stone is connected to these two sample sites by the same waterway, however, it is not connected by irrigation, and Arapooish is not connected to any of these sample sites by either waterway or irrigation (Figure 1). Collected mosquitoes were stored at -20° C overnight and sorted based upon identification. A minimum of 30 *Cx. tarsalis* mosquitoes were required for a population.

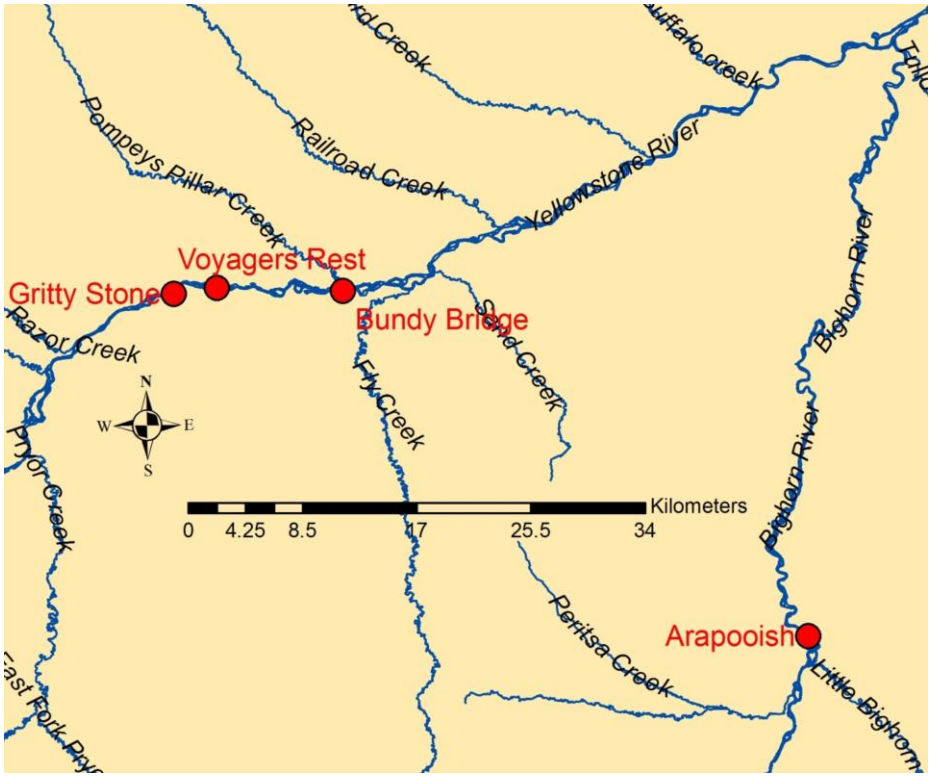

Figure 1: A map of sampling locations where Gritty Stone, Voyagers Rest, and Bundy Bridge are connected by waterway. Voyagers Rest and Bundy Bridge are also connected through irrigation, and Arapooish is not connected by either waterway or irrigation.

The DNA extraction protocol described by Black and DuTeau (1997) was performed on mosquitoes. Individual mosquitoes were placed in 1.5 mL round-bottom tubes with 25 µl of grinding buffer (0.1 M NaCl, 0.2 M Sucrose, 0.1 M Tris-HCl pH 9.1, 0.05 M EDTA, 0.05% SDS) and ground with glass pestles until insect parts could not be seen. The pestle was then washed with an additional 25 µl of grinding buffer to remove any residual DNA. The contaminated pestle was rinsed with water and HCl before repeated use. Mosquitoes were briefly centrifuged to get the homogenate down to the bottom of the tube before incubating it at 65°C for 30 minutes. Immediately following incubation, 7 µl of 8 M potassium acetate (KAc) was added to each tube and placed on ice for another 30 minute incubation. The tubes were then centrifuged at 15,000 g for 15 minutes followed by transfer of the supernatant to a new 1.5 mL tube. One hundred microliters of 100% ethanol kept in a -20°C freezer was then added to each tube before a 5 minute incubation at room temperature. Tubes were centrifuged at 15,000 g for 15 minutes, the ethanol was removed, and an additional 100 µl of 100% ethanol was added

before centrifugation at 15,000 g for 5 minutes. The ethanol was removed again and the tubes were placed in the Speed Vac to dry. The pellets were then resuspended in 200 µl of TE (0.01 M Tris-HCl pH 8.0, 1 mM EDTA) and vortexed to break up the pellet before being stored at -20°C.

The microsatellite loci used were CutC6 (Rasgon *et al.* 2006), CutD120 (Rasgon *et al.* 2006), CutD107 (Rasgon *et al.* 2006) and CutC201 (Venkatesan *et al.* 2007). PCR amplification of these microsatellite loci was conducted following the procedure of Venkatesan *et al.*, (2007). PCR was set up in 25 µl reactions containing 0.8 units of Taq polymerase, 2.5 µl of 10X Thermopol buffer (New England Biolabs), 0.2 mM of each dNTP, 10 µM of each microsatellite primer, 16.6 µl of PCR certified water, and 2.5 µl of sample DNA. All microsatellite loci were run with the following cycling conditions: 95°C for 5 min; 10 cycles of 94°C for 30 s, 57°C for 1 min, and 72°C for 30 s; 27 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 30 s; followed by a 10 minute extension at 72°C (Bio Rad MyCycler Thermocycler).

Gel electrophoresis was used to confirm amplification with 1% agarose gels loaded with 4 µl of each sample and stained with 2.5 µl of ethidium bromide (0.625 mg/mL) per 50 mL.

The QIAxcel Advanced System was then used to determine the size of alleles present at each microsatellite locus. The QIAxcel Advanced User Manual, DNA High Resolution Kit and Cartridge, QX 25-500 bp Size Marker diluted to 30 ng/µl and the QX 15 bp-600 bp Alignment Marker were used in conjunction with the QIAxcel Advanced System to base call alleles (QIAGEN 2011). Base pair peaks were considered valid if they have a minimum concentration of 2.61 ng/µl. Homozygotes were defined as individuals with one peak that has a concentration of at least double the next highest peak's concentration. Samples that had two peaks were considered heterozygotes if the lower peak was at least half the concentration of the higher peak. Only individuals with valid peaks in the region of 140 to 260 base pairs were used as data. Any samples with more than two peaks of similar concentrations were considered missing data, as well as any samples with the highest concentration peaks outside of the base pair range (Geiger

pers. comm.). FreeNA was used to analyze relatedness of individuals and thus estimate the level of gene flow between populations. This software estimates null allele frequencies for microsatellite datasets and calculates F_{ST} values that have been corrected for the presence of high null allele frequencies (Chapuis and Estoup 2007). Null alleles are those in which a mutation has occurred which either prevents PCR amplification completely or amplifies only one homologue which can lead to an overestimate of homozygotes (Callen *et al.* 1993). F_{ST} values can be defined based upon the inbreeding coefficient of a population or the differences in allele frequencies (Neigel 2002).

Results

Loci amplification did occur within the 160 to 260 base pair range. Amplification of CutC6, CutD120, and CutD107 revealed nine alleles while CutC201 contained eleven alleles (Table 1).

Table 1: Allele size per locus (bp)

CutC6 (9)	**CutD120** (9)	**CutD107** (9)	**CutC201** (11)
186	165	166	204
198	168	184	207
213	171	190	210
216	174	193	213
219	177	196	216
222	180	199	225
228	183	205	228
231	189	211	231
288	237	217	234
			237
			240

The Gritty Stone, Voyagers Rest and Bundy Bridge populations all shared the same most frequent allele size for locus 6, 107 and 201 (Table 2). All populations sampled contained at least one allele in common for each locus and four CutD201 alleles were common to all populations (data not shown). All of the populations with the

exception of Arapooish had at least one allele that was unique for a particular locus (Table 3).

Table 2: The allele size most frequently observed for each population broken down by locus. VR=Voyagers Rest, GS=Gritty Stone, BB=Bundy Bridge, AP=Arapooish

Loci	Allele size most frequent in VR	Allele size most frequent in GS	Allele size most frequent in BB	Allele size most frequent in AP
CutC6	216	216	216	219
CutD120	180	177	174	174
CutD107	193	193	193	193
CutC201	228	228	228 and 231	207

Table 3: Allele sizes that are unique to each sample site and number of times present within population.

Loci	Voyagers Rest	Gritty Stone	Bundy Bridge	Arapooish
CutC6	186 (1)	-	-	-
CutD120	-	189 (2), 237 (2)	183 (2)	-
CutD107	217 (1)	-	-	-
CutC201	-	240 (2)	-	-

Analysis Using FreeNA:

The FreeNA program was run for all sample sites at locus 6, 120, 107 and 201. Any individual samples that did not amplify at two of the four loci were removed from the data set before the analysis was performed. The null allele frequencies indicated that only the Voyagers Rest population at locus 107 was below a normal estimate for null allele frequency (Table 4). Populations were considered to have a high null allele frequency if the value was 20% or above.

Table 4: Null allele frequencies for each population at locus 6, 120, 107 and 201. The bolded value indicates the site that had a low null allele frequency. VR=Voyagers Rest, GS=Gritty Stone, BB=Bundy Bridge, AP=Arapooish

Locus	Population	Null Allele Frequency
6	VR	0.30941
6	GS	0.26563
6	BB	0.26122
6	AP	0.36676
120	VR	0.38023
120	GS	0.40501
120	BB	0.39631
120	AP	0.41120
107	VR	**0.13057**
107	GS	0.27595
107	BB	0.34953
107	AP	0.30909
201	VR	0.33297
201	GS	0.39387
201	BB	0.40773
201	AP	0.35867

FreeNA also provided an analysis of pairwise F_{ST} values that accounted for high null allele frequency. Two populations that had an F_{ST} value of less than 0.015 were considered to not be significantly different from each other (Hokit pers. comm.). All of the populations were distinct from each other except for the Gritty Stone and Bundy Bridge populations which were considered to be the same (Table 5).

Table 5: Pairwise population F_{ST} values for all loci. The bolded value is the one pairwise comparison in which the populations are genetically indistinguishable. VR=Voyagers Rest, GS=Gritty Stone, BB=Bundy Bridge, AP=Arapooish

Population	VR	GS	BB
GS	0.029403		
BB	0.030320	**-0.000830**	
AP	0.063319	0.067653	0.055176

Discussion

The objective of this study was to determine if higher degrees of pairwise gene flow existed between *Cx. tarsalis* populations that were connected by irrigation which could also suggest movement patterns of WNV. My hypothesis was that populations connected by irrigation systems are more genetically similar than those disconnected. Likewise, populations that are connected through a nearby river but not connected through irrigation are more genetically alike than those disconnected through both irrigation and nearby waterways.

The results of this study indicate that a higher pairwise gene flow between populations connected through irrigation does not exist and that populations geographically separated by waterway and irrigation are genetically different (Table 5). Statistical data analysis using FreeNA for loci 6, 120, 107 and 201 indicates that there are high null allele frequencies within this data set (Table 4). Null alleles have been known to cause deviations from Hardy-Weinberg Equilibrium (HWE) (Callen *et al.* 1993). A smaller than expected number of heterozygous individuals, as seen in this data set, can then account for populations failing to adhere to HWE.

The pairwise F_{ST} values indicated that the Gritty Stone and Bundy Bridge populations could be considered genetically indistinguishable whereas all the other populations were considered distinct from one another. Populations exhibited the greatest difference when compared to the Arapooish population (Figure 1 and Table 5). This was as expected due to the large geographic distance and degree of waterway separation between Arapooish and the other three populations. However, it would be expected that Gritty Stone and Voyagers Rest would be more genetically similar because of the short geographic distance separating them as well as their connectivity by water. Likewise, Voyagers Rest and Bundy Bridge would be expected to be genetically similar due to their connectivity by waterway and flood irrigation zone. The data indicated that Gritty Stone and Bundy Bridge were genetically similar even though they only share connectivity by waterway but do not share the additional factor of close geographic proximity or connection through a flood irrigation zone. However, due to the majority of populations exhibiting genetic distinction from one another it may be that Gritty Stone and Bundy

Bridge are indeed distinct sites. Nevertheless, it is difficult to distinguish the true relationship between these populations because all, with the exclusion of one, exhibited high null allele frequencies for each locus which could explain either the similarity between Gritty Stone and Bundy Bridge or the distinctness of the other populations. This disparity in the data obtained could additionally be due to the small population size and limited number of loci used.

As previously noted, these results are unexpected, especially upon comparison to previous studies on irrigation and WNV. For example, Cardenas *et al.*'s (2011) 2003 to 2010 case study indicated that a proximity to irrigation canals increased the risk of WNV for residents of El Paso County, Texas by six fold. He observed that 43% of human WNV cases reported during these seven years were within 330 meters of irrigation canals while only 8% of WNV cases lived greater than 7400 meters away (Cardenas *et al.* 2011). Likewise, research by Eisen *et al.* (2010) found that in Colorado a distance of less than one kilometer from irrigated agricultural land was a statistically significant risk factor for obtaining WNV during 2003 and 2007. This suggested that the larger the *Cx. tarsalis* larvae habitats, the greater the WNV incidence rate (Eisen *et al.* 2010). Eisen *et al.* (2010) also proposed that rivers near these areas of irrigated land, which are considered breeding grounds for *Cx. tarsalis*, could be the means of dispersing the vector into nearby populations. With these studies in mind, it would be expected that gene flow should be enhanced due to the high proportion of agricultural land in the state of Montana which is optimal *Cx. tarsalis* breeding ground and bird host habitat.

Barker *et al.*'s (2009) study additionally suggested that *Cx. tarsalis* disperses along waterways in Colorado. They indicated that a higher degree of pairwise gene flow existed between populations that are less than 190 kilometers apart (Barker *et al.* 2009). This suggests that if a higher degree of pairwise gene flow does not exist between populations connected through irrigation then the Gritty Stone, Voyagers Rest and Bundy Bridge populations connected through waterway should exhibit greater levels of gene flow, which was not found here.

The results of this study are unclear and could suggest that populations connected through waterway exhibit a greater degree of gene flow or that for this particular

sampling year populations are genetically distinct from one another. Additional studies should include multiple locations connected through a variety of irrigated areas over numerous WNV seasons as well as the use of additional microsatellite loci. This study does not indicate that a higher degree of gene flow exists between populations connected through irrigation. As previously mentioned, high null allele frequencies and small population size could be reason for this result.

Literature Cited

Allendorf, F.W. 1986. Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biology* 5: 181-190.

Barker, C.M., B.G. Bolling, W.C. Black, C.G. Moore and L. Eisen. 2009. Mosquitoes and West Nile Virus along a river corridor from prairie to montane habitats in eastern Colorado. *Journal of Vector Ecology* 34(2): 276-293.

Beehler, J.W. and M.S. Mulla. 1995. Effects of organic enrichment on temporal distribution and abundance of culicine egg rafts. *Journal of the American Mosquito Control Association* 11(2): 167-171.

Black, W.C. and N.M. DuTeau. 1997. RAPD-PCR and SSCP analysis for insect population genetic studies. pp.361-373 in *The Molecular Biology of Insect Disease Vectors: A Methods Manual*. Chapman & Hall, New York.

Callen, D.F., A.D. Thompson, Y. Shen, H.A. Phillips, R.I. Richards, J.C. Mulley and G.R. Sutherland. 1993. Incidence and Origin of "Null" Alleles in the (AC)n Microsatellite Markers. *American Journal of Human Genetics* 52: 922-927.

Cardenas, V.M., J. Jaime, P.B. Ford, F.J. Gonzalez, I. Carrillo, J.E. Gallegos and D.M. Watts. 2011. Yard flooding by irrigation canals increased the risk of West Nile disease in El Paso, Texas. *Annals of Epidemiology* 21(12): 922-929.

[CDC] Center for Disease Control. 1999. Update: West Nile-like viral encephalitis – New York, 1999. *Morbidity and Mortality Weekly Report (MMWR)* 48(39): 890-892.

[CDC] Center for Disease Control. 2012. *CDC West Nile Virus Homepage*. Retrieved from http://www.cdc.gov/ncidod/dvbid/westnile/index.htm.

Chapuis, M.P. and A. Estoup. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* 24(3): 621-631.

Clements, A.N. *The Biology of Mosquitoes, Volume 3: Transmission of Viruses and Interactions with Bacteria*. (Cambridge University Press), United Kingdom, 2012.

DeGroote, J.P., R. Sugumaran, S.M. Brend, B.J. Tucker and L.C. Bartholomay. 2008. Landscape, demographic, entomological, and climate associations with human

disease incidence of West Nile Virus in the state of Iowa, USA. *International Journal of Health Geographics* 7:19.

Eisen, L., C.M. Barker, C.G. Moore, W.J. Pape, A.M. Winters and N. Cheronis. 2010. Irrigated agriculture is an important risk factor for West Nile Virus disease in the hyperendemic Larimer-Boulder-Weld area of north central Colorado. *Journal of Medical Entomology* 47(5): 939-951.

Epstein, P.R., and C. Defilippo. 2001. West Nile virus and drought. *Global Change & Human Health* 2(2): 105-107.

Gimnig, J.E., W.K. Reisen, B.F. Eldridge, K.C. Nixon and S.J. Schutz. 1999. Temporal and spatial genetic variation within and among populations of the mosquito *Culex tarsalis* (Diptera: Culicidae) from California. *Journal of Medical Entomology* 36(1): 23-29.

Hokit, D.G., S. Alvey and J. Geiger. 2008. Spatial epidemiology of West Nile Virus infection risk across Montana. Research Proposal. Department of Natural Sciences, Carroll College.

Huhn, G.D., J.J. Sejvar, S.P. Montgomery and M.S. Dworkin. 2003. West Nile Virus in the United States: an update on an emerging infectious disease. *American Family Physician* 68(4): 653-660.

Kent, R., L. Juliusson, M. Weissmann, S. Evans and N. Komar. 2009. Seasonal blood-feeding behavior of *Culex tarsalis* (Diptera: Culicidae) in Weld County, Colorado, 2007. *Journal of Medical Entomology* 46(2): 380-390.

Lanciotti, R.S, *et al.* 1999. Origin of the West Nile Virus responsible for an outbreak of Encephalitis in the northeastern United States. *Science* 286: 2333-2337.

Marra, P.P., S. Griffing, C. Caffrey, A.M. Kilpatrick, R. McLean, C. Brand, E. Saito, A.P. Dupuis, L. Kramer and R. Novak. 2004. West Nile Virus and wildlife. *Bioscience* 54(5): 393-402.

Neigel, J.E. 2002. Is F_{ST} obsolete?. *Conservation Genetics* 3: 167-173.

QIAGEN. 2011. *QIAxcel DNA Handbook* (2 ed.). Retrieved from http://www.qiagen.com.

Rappole, J.H., S.R. Derrickson and Z. Hubalek. 2000. Migratory birds and spread of West Nile Virus in the western hemisphere. *Emerging Infectious Diseases* 6(4): 319-328.

Rappole, J.H., and Z. Hubalek. 2003. Migratory birds and West Nile virus. *Journal of Applied Microbiology* 94 (Supplement): 47S-58S.

Rasgon, J.L., M. Venkatesan, C.J. Westbrook and M.C. Hauer. 2006. Polymorphic microsatellite loci from the West Nile virus vector *Culex tarsalis. Molecular Ecology Notes* 6: 680-682.

Reisen, W.K., B.D. Carroll, R. Takahashi, Y. Fang, S. Garcia, V.M. Martinez and R. Quiring. 2009. Repeated West Nile Virus epidemic transmission in Kern County, California, 2004-2007. *Journal of Medical Entomology* 46(1): 139-157.

Reisen, W.K., Y. Fang and V.M. Martinez. 2006. Effects of temperature on the transmission of West Nile Virus by *Culex tarsalis* (Diptera: Culicidae). *Journal of Medical Entomology* 43(2): 309-317.

Reisen, W., H. Lothrop, R. Chiles, M. Madon, C. Cossen, L. Woods, S. Husted, V. Kramer and J. Edman. 2004. West Nile Virus in California. *Emerging Infectious Diseases* 10(8): 1369-1378.

Reisen, W.K., M.M. Milby and R.P. Meyer. 1992. Population dynamics of adult *Culex* mosquitoes (Diptera: Culicidae) along the Kern River, Kern County, California, in 1990. *Journal of Medical Entomology* 29: 531-543.

Reisen, W.K., and W.C. Reeves. 1990. Bionomics and ecology of *Culex tarsalis* and other potential mosquito vector species. pp. 286-288 in *Epidemiology and Control of Mosquito-Borne Arboviruses in California 1943-1987.* California Mosquito and Vector Control Association Inc., California.

Selkoe, K.A. and R.J. Toonen. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters* 9: 615-629.

Smithburn, K.C., T.P. Hughes, A.W. Burke and J.H. Paul. 1940. A neurotropic virus isolated from the blood of a native of Uganda. *The American Journal of Tropical Medicine* 20(4): 471-492.

[USDA] United States Department of Agriculture. Census of Agriculture. 2007 Census Volume 1, Chapter 1: State Level Data – Montana. Table 10. Irrigation: 2007 and 2002.

Venkatesan, M., M.C. Hauer and J.L. Rasgon. 2007. Using fluorescently labeled M13-tailed primers to isolate 45 novel microsatellite loci from the arboviral vector *Culex tarsalis*. *Medical and Veterinary Entomology* 21: 204-208.

Venkatesan, M. and J.L. Rasgon. 2010. Population genetic data suggest a role for mosquito-mediated dispersal of West Nile Virus across the western United States. *Molecular Ecology* 19(8): 1573-1584.