Analysis of post-blood meal flight distances in mosquitoes utilizing zoo animal blood meals

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Abstract

We assessed the post-blood meal flight distance of four mosquito species in a unique environment using blood meal analysis. Mosquitoes were trapped at the Rio Grande Zoo in Albuquerque, NM, and the blood source of blood-engorged mosquitoes was identified. The distance from the enclosure of the animal serving as a blood source to the trap site was then determined. We found that mosquitoes captured at the zoo flew no more than 170 m with an average distance of 106.7 m after taking a blood meal. This is the first study in which the flight distance of wild mosquitoes has been assessed using blood meal analysis and the first in which zoo animals have served as the exclusive source of blood meals.

Keywords

Aedes; *Culex*; blood meal; mosquito; flight distance

INTRODUCTION

Flight range has long been recognized as a factor in the importance of a given mosquito species in disease transmission (Reeves 1965, Rowley et al. 1968). The ability to disperse, along with its host preferences, biting frequency, and transmission rates are crucial components of vector capacity and the ability to efficiently transmit a particular pathogen (Saegerman et al. 2008). Traditionally, the primary method used to determine flight range in mosquitoes has been to mark them in some way, release them at a known point, and then try to recapture the marked mosquitoes at trap sites a known distance from the release point. This technique has been attempted repeatedly, but only a small fraction of the released mosquitoes are often recaptured (Russell et al. 1944, Wolfinsohn and Galun 1953, Quraishi et al. 1966, Muir and Kay 1998, Russell et al. 2005, Midega et al. 2007). A flight mill system developed in 1968 and subsequently improved by incorporating computer analysis in 1984 has been used successfully to determine the flight range of flying insects (Rowley et al. 1968, Clarke III et al. 1984). This system has been effective in assessing flight range, speed, and duration of flight. It did not, however, mimic the natural environmental conditions to which mosquitoes are normally exposed. In 1984, an alternative method utilizing rubidium-marked eggs was developed (Kimsey and Kimsey 1984). Its efficacy was first assessed with

Aedes aegypti in 1995 where the flight distance post-blood meal was found to be a maximum of 441 m (Reiter et al. 1995). Most investigations of mosquito flight distance have utilized labreared mosquitoes. While such mosquitoes give us an idea of flight range, they do not, however, necessarily behave as wild-type mosquitoes would.

The importance that zoo animals can play as indicators of recent, emerging infectious disease was highlighted by the emergence of West Nile Virus in 1999. The deaths of a Chilean flamingo and other exotic bird species at the Bronx Zoo were important early indicators that a new pathogen was spreading in North America (Lanciotti et al. 1999). Upon further testing, West Nile virus, previously only found in Africa and Europe, was identified as the infecting virus (Anderson et al. 1999). Other vector-borne pathogens have also been recognized as problematic in zoological parks. Avian malaria in particular has been identified in captive birds in zoos around the world, including the United States, New Zealand, Brazil, and Japan (Cranfield et al.1994, Derraik 2004, Ejiri et al. 2009, Bueno et al. 2010). Penguins, which have no natural resistance to *Plasmodium,* are at especially elevated risk, and penguin deaths due to avian malaria have been reported from zoos in the United States and Brazil (Cranfield et al. 1994, Bueno et al. 2010).

The sources of mosquito blood meals have been identified by a variety of methods (Irby and Apperson 1988, Gingrich and Williams 2005, Kay et al. 2007, Molaei et al. 2006, 2007, Sawabe et al. 2010, Barrera et al. 2011). None of these studies, however, incorporated a trapping area that encompassed a zoo containing many exotic species. Here we report on a blood-meal analysis of mosquitoes trapped at the Rio Grande Zoo, in Albuquerque, NM. Many of the blood meal sources have been identified as exotic species that were housed at the zoo. Because we can precisely measure the distance from an animal's enclosure to the site where a particular mosquito was trapped, we have been able to estimate the post-blood meal flight distance for several mosquito species, some of which transmit arboviruses of veterinary or medical significance. The presence of a variety of relatively confined hosts within the zoo, along with many readily available water sources for oviposition, make zoos a unique and interesting setting for the study of mosquitoes. In this study, we report on the post-blood meal flight distance of mosquitoes located in a zoo habitat. Our findings indicate that the Rio Grande Zoo may be acting as a self-sustaining mosquito habitat. Limited dispersal within this habitat has important implications for arbovirus transmission.

MATERIALS AND METHODS

Collections

Mosquitoes were collected between 2006 and 2010 at the Rio Grande Zoo in Albuquerque, NM (Figure 1). The Rio Grande Zoo is located in a riparian zone adjacent to the Rio Grande River. As such, there are many standing water pools serving as ideal locations for mosquito oviposition. Furthermore, standing water in the form of water features and drinking containers is ubiquitous throughout the zoo. In 2006, only one trap site was present at the zoo. The number of trap sites was increased to two in 2008 and to four in 2010. Mosquitoes were trapped once a week throughout the mosquito season, May through mid-October, for approximately 24 consecutive weeks. Both a CDC light trap and gravid trap were placed at each trapping site for each trapping occasion. Two control trapping sites were placed in nearby areas where mosquito activity is known to be high: one to the north and one on the adjacent west side of the Rio Grande River, each approximately 3.5 km from the Rio Grande Zoo. CDC light traps were suspended 1.5 m from the ground and baited with approximately 1.5 kg of dry ice, and gravid traps were baited with dechlorinated water infused with horse manure, grass clippings, and bacterial culture (Propump Liquid Live Bacteria High Count, Ecological Laboratories, Freeport, NY, U.S.A.), which was allowed to ferment for two weeks. Traps were set in the afternoon and were collected the following morning. The

collected mosquitoes were immediately placed on dry ice until they could be stored in a −80° C freezer. Mosquitoes were identified to species according to dichotomous keys (Carpenter and LaCasse 1955, Pratt and Barnes 1959, Darsie and Ward 1981). Mosquitoes containing blood meals were set aside for blood meal analysis.

Blood meal analysis

Mosquitoes with a visible blood meal were individually placed on a microscope slide. The abdomen and midgut were removed using a razor blade and sterilized forceps under a dissecting microscope. Forceps were sterilized and a new slide and razor blade were used for each mosquito. Genomic DNA was then extracted from the midgut and abdomen using a modified DNAzol BD (Molecular Research Center, Cincinnati, OH, U.S.A.) protocol (Molaei et al. 2006).

The blood meal source was determined by subjecting each genomic DNA sample to two subsequent PCR reactions. Each reaction employed primers specific for either an avian or a mammalian portion of the *cytochrome b* gene (see Table 1 for primer sequences and cycling conditions). If the first set of reactions did not amplify the target DNA with either avian or mammalian primers, a second set of reactions was attempted. The sample was archived after two failed sets of reactions. Each 50 μl reaction contained 200–400 ng of genomic DNA serving as template, 5 μl of 10× buffer (Roche Applied Science Indianapolis, IN, U.S.A.), 8 μl dNTPs (200 μL of each, Applied Biosystems, Foster City, CA, U.S.A.), 8 μL $MgCl_2$ (4 mM, Roche Applied Science Indianapolis, IN, U.S.A.), 5μl forward primer (0.5 μM), 5 μl reverse primer (0.5 μM), and 0.25 μl TaqGold Polymerase (1.25 U per reaction, Roche Applied Science Indianapolis, IN, U.S.A.). Sterile water (Sigma-Aldrich Irvine, CA, U.S.A.) was added to bring the total reaction volume to 50 μl. All reactions also included a positive control containing either mammalian (*Mus musculus*) or avian (*Zenaida macroura*) genomic DNA serving as a template. A negative water control, lacking template, was included with all PCR reactions. A second negative control was also included and consisted of a DNAzol extract, lacking mosquito midgut and abdomen.

Amplified PCR products were purified with one of several methods including E-Gel® SizeSelect™ gels (Invitrogen, Carlsbad, CA, U.S.A.), PCR purification kit (Qiagen, Valencia, CA, U.S.A.), minielute column (Millipore, Billerica, MA, U.S.A.), or exo-sap (Affymetrix, Cleveland, OH, U.S.A.). Amplicons were directly sequenced with a Big Dye 3.1 sequencing kit, using the big dye step protocol PCR regime (Platt et al. 2007). Samples were then sequenced on an ABI 3130 DNA Sequencer (Applied Biosystems, Foster City, CA, U.S.A.) at the University of New Mexico, Department of Biology Molecular Facility. Sequences were edited using Sequencher version 4.10.1 (Gene Codes, Ann Arbor, MI, U.S.A.) and identified to species through a blast search comparison with the GenBank DNA database (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Measuring flight range

If the source of a blood meal was identified as an exotic species not normally present in New Mexico but found at the Rio Grande Zoo, it was included in our analysis. The distance from the center of the animal's enclosure to the mosquito trap site was determined to the nearest meter using GPS. Flight distances of the different species were compared for significance using a t-test. Mosquitoes that took blood meals from native species that may or may not have been within the zoo were excluded from analysis.

RESULTS

A total of 4,917 mosquitoes, representing 12 different species, was recovered from the four zoo trap sites (Table 2). Of these mosquitoes, 70.8% were trapped with CDC light traps, while 29.2% were recovered from in gravid traps.

A total of 106 mosquito blood meal sources were identified from the zoo (Table 3). Of these, 44 (41.5%) were unambiguously from animals in exhibits. Only these mosquitoes were utilized in the flight distance analysis. Other identified blood meal sources were non-exotic birds, coyotes, small mammals, or domestic animals such as dogs, which are common in the area immediately surrounding the zoo. Of the 44 mosquitoes included in our study, 27 (61.4%) were *Aedes vexans*, ten (22.7%) were *Culex quinquefasciatus*, five (11.4%) were *Cx. tarsalis,* and two (4.5%) were *Culiseta inornata* (see Table 3). The average post-blood meal flight distance was 109.2 m for *Ae. vexans* (Figure 2). For *Cx. quinquefasciatus* this value was 91.4 m, while for *Cx. tarsalis* and *Cu. inornata* it was 115.2 m and 128 m, respectively. The difference in flight distance between *Cx. quinquefasciatus* and *Ae. vexans* was not significant. Because of low sample size, *Cx. tarsalis* and *Cu. inornata* were not included in the statistical analysis. The average flight distance for all mosquito species was 106.7 m. No mosquitoes engorged on exotic species were collected from control-trapping sites outside the grounds of the Rio Grande Zoo.

DISCUSSION

We have estimated the post-blood meal flight distance for four mosquito species, utilizing blood meal analysis of mosquitoes trapped in a zoological park, where mosquito flight distance from blood meal source to trap site could be easily determined. The majority of the mosquitoes collected were caught in CDC light traps (Table 2). However, for one important species in our study, Cx. quinquefasciatus, gravid traps were found to be the more effective trap type. This highlights the need to use more than one trap type to more accurately describe a particular area's mosquito fauna.

The mosquito species that we assessed flew an average of less than 107 m following a blood meal. Because mosquitoes vary their flight paths (Bidlingmayer 1975, Bidlingmayer and Hem 1979) it cannot be assumed that the mosquitoes in our study flew directly in a straight line to trap sites. Thus, our data represents flight dispersal distance as opposed to an actual linear measurement of flight. Furthermore, once trapped, mosquitoes are obviously unable to fly further. Because untrapped mosquitoes might ordinarily alight repeatedly, we may be underestimating the actual distance traversed by a blood-fed mosquito.

Several other factors that limit the precision of our data must be addressed. Our measurements of flight distance were made from the center of an animal enclosure. Obviously, animals may have been anywhere within their enclosure when fed upon. This would, however, alter our results by, at the most, only several meters. We also have no knowledge of how many mosquitoes fed upon zoo animals and likewise how many mosquitoes were not trapped. Finally, we were unable to account for the effects of wind, rainfall, or other phenomena that may affect mosquito flight.

Our results suggest that a blood meal imposes a heavy burden on a mosquito in terms of its dispersal ability. Non-engorged mosquitoes often fly far greater distances. Provost (1957) noted that *Aedes taeniorhynchus* are capable of flying over 24 miles. *Anopheles gambiae* and *An. atroparvus* were found to fly as far as 10 km (Kauffman and Briegel 2004). Because we lack data on the flight distances of blood-seeking mosquitoes, we are unable to make a direct comparison between engorged and non-engorged mosquitoes in our study.

Our results, however, are similar to those in other studies in which post-blood meal flight distance was assessed. Laboratory-reared *Ae. aegypti* were found to fly an average of 181 m following a blood meal, utilizing rubidium-marked eggs (Reiter et al. 1995). Russell et al. (2005), using a mark-release-recapture-technique, estimated that blood fed mosquitoes flew an average distance of 77.8 m. It is difficult, however, to draw meaningful conclusions from this study, as only 3.4% of the released mosquitoes were recaptured.

The short distances traveled by the mosquitoes may reflect to some degree the unique conditions found within the zoo environment. Although blood-fed mosquitoes might disperse further in other situations, it is entirely possible that within the zoo, flight distances are reduced. A large number of easily accessible hosts are available at the zoo in relatively confined enclosures, and various water features, ponds, and drinking water containers provide ample habitat for oviposition in close proximity to the animals. The vast majority of the non-exotic species upon which zoo mosquitoes fed are abundant within the confines of the zoo (see Table 3). Even those non-exotics not found within the zoo property, such as horses and cows, are common in the semi-rural habitat immediately adjacent to the zoo. Thus, the zoo may serve as a sort of "closed system," in which mosquitoes complete their entire life cycle without leaving the zoo premises. If this is the case, it has obvious implications for the control of arbovirus transmission within the confines of the zoo. More specifically, it suggests that focal control within the zoo may be of greater value than previously suspected, even if mosquito populations outside the zoo remain uncontrolled.

Of the 109 blood meals we identified, nine (8.49%) were identified as human. We have no conclusive means to demonstrate that these individuals were at the zoo while fed upon. Yet our results, which indicate that mosquitoes travel less than 107 m following a blood meal, as well as the high concentration of people at the zoo, suggest that the zoo may be a likely place for mosquitoes to feed upon humans. All nine human blood meals were taken by *Ae. vexans*. Molaei and Andreadis (2006) have suggested that *Ae. vexans* may be involved in bridge transmission of West Nile virus to humans, which currently presents a risk in central New Mexico for humans as well as other mammals. This mosquito species may occasionally vector other arboviruses as well such as western equine encephalitis virus, which, while rarely infecting humans in New Mexico, may occasionally present an important health risk (Clark et al. 1986).

Additionally, our data suggest that mosquitoes native to the southwest United States feed readily on exotic species. While the tendency of mosquitoes to feed on exotic species has been found before (Lanciotti et al. 1999), this is the first data set obtained entirely from zoo animals. The species we have investigated, specifically *Cx. quinquifasciatus* and *Cx. tarsalis*, are known arbovirus vectors and may play an especially important role in the transmission of West Nile virus to birds and mammals (Sardelis et al. 2001, Goddard et al. 2002, Turell et al. 2001, 2005). Our results may therefore be of value to those interested in zoo veterinary medicine. Exotic species in zoos have already proven to be excellent sentinels of newly emerging zoonotic diseases and they may suffer high morbidity and mortality as a result of infection (Lanciotti et al. 1999). Additionally, the Rio Grande Zoo has suffered significant losses of some species to West Nile virus, most notably Rainbow Lorikeets (*Trichoglossus haematodus*) in previous years (DiMenna, personal communication). The importance of vector control for the maintenance of zoo animal health has been previously noted (Derraik 2005). A better understanding of vector behavior, including post-blood meal flight, might be utilized to enhance known mosquito control methods in zoos, resulting in improved protection of zoo animals and the human population visiting these attractions.

Acknowledgments

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Figure 1.
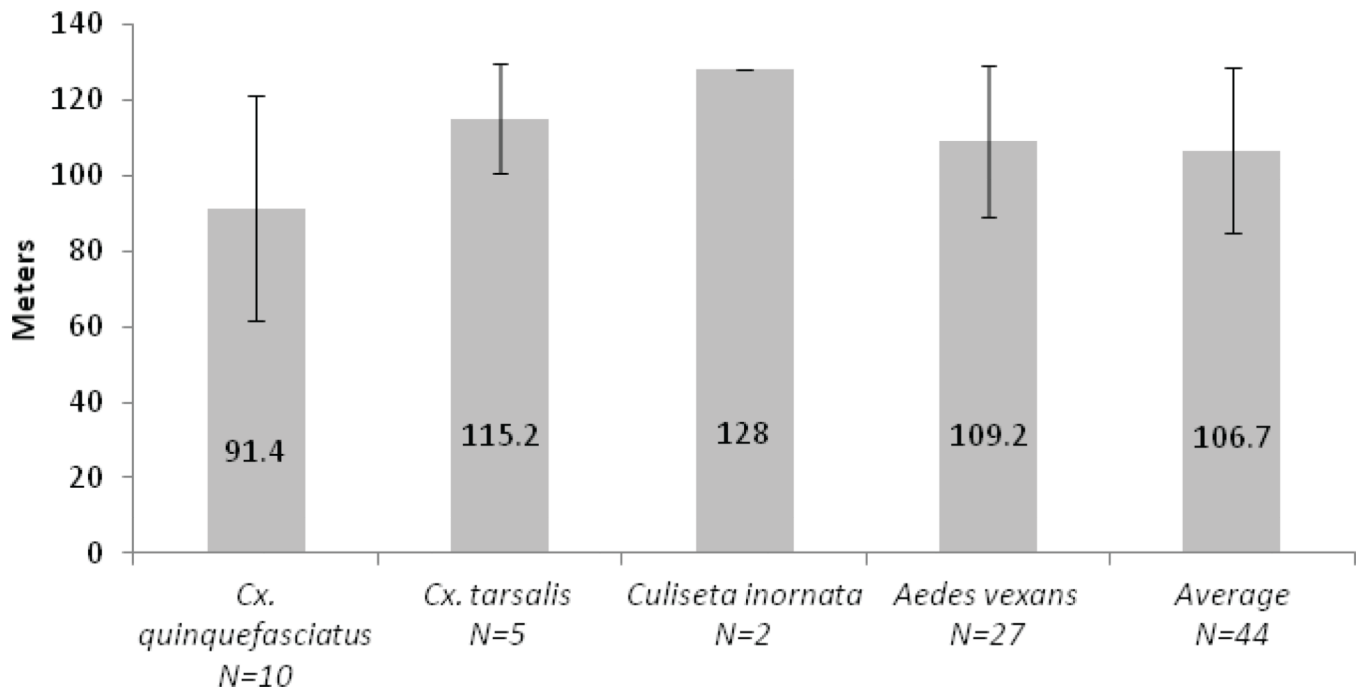Map of the Rio Grande Zoo. Numbers indicate the location of mosquito trapping sites within the zoo.

Figure 2.
Average distances of each individual species and average distance for all species combined.

Table 1

PCR primers and temperature regime.

Mammalian specific primers 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3'(f) 5'-TGTAGTTRTCWGGGTCHCCTA-3' (r)			Avian-specific primers 5'-GACTGTGACAAAATCCCNTTCCA-3'(f) 5'-GGTCTTCATCTYHGGYTTACAAGAC-3'(r)
Initial Denaturation		10 min 95° C	10 min 95° C
36 cycles	Denature	30 sec 95° C	30 sec 95° C
	Anneal	40 sec 55° C	40 sec 56° C
	Extension	50 sec 72° C	50 sec 72° C
Final extension		3 min 72° C	3 min 72° C

Table 2

Summary of mosquitoes trapped at the Rio Grande Zoo, Albuquerque, NM, from 2006 through 2010. The number and percentages of mosquito caught in each trap type is indicated.

Mosquito species	CDC light traps non-blood fed	Gravid traps non-blood fed
Aedes dorsalis N=4	4 (100%)	0
Aedes melanimon N=8	8 (100%)	0
Aedes nigromaculis N=2	2 (100%)	0
Aedes trivittatus N=1	1 (100%)	0
Aedes vexans N=2,216	2,208 (99.6%)	8 (0.4%)
Anopheles franciscanus N=1	1 (100%)	0
Anopheles freeborni N=9	6 (66.7%)	3 (33.3%)
Culex quinquefasciatus N=2,250	844 (37.5%)	1,406 (62.5%)
Culex tarsalis N=387	371 (95.9%)	16 (4.1%)
Culiseta inornata N=37	35 (94.6%)	2 (5.4%)
Aedes dorsalis N=1	1 (100%)	0
Orthopodomyia signifera N=1	1 (100%)	0
Total mosquitoes N=4,917	3,482 (70.8%)	1,435 (29.2%)

Table 3

Mosquito flight distances measured to the nearest meter. An (*) denotes mosquito trapped at a different trap site. Flight distance for non-exotic species was not assessed.

Species	Blood meal source	Common name (number)	Distance in meters
	Hylobates syndactylus	Siamang gibbon (1)	37
	Panthera tigris	Tiger (1)	18
	Dromaius novaehollandiae	Emu (1)	67
	Trichoglossus haematodus	Rainbow Lorikeet (1)	3
	Ceratotherium simum	White Rhino (2)	140
	Potamochoerus porcus	Red River Hog (1)	122
	Struthio camelus	Ostrich (1)	168
	Unica unica	Snow Leopard (1)	72
	Vultur gryphis	Andean Condor (1)	147
	Accipiter Cooperi	Cooper's Hawk (1)	
Culex quinquefasciatus N=43	*Anas platyrhynchos*	Muscovy Duck (1)	
	Canis latrans	Coyote (1)	
	Canis lupis familiaris	Dog (2)	
	Carpodacus mexicanus	Finch (3)	
	Columba livia	Pigeon (1)	
	Geococcyx califoriaus	Roadrunner (1)	
	Mus musculus	Mouse (1)	
	Passer domesticus	House sparrow (7)	
	Pheucticus melanocephalus	Black-headed Grosbeak (1)	
	Turdus migratorius	American Robin (8)	
	Zenaida macroura	Mourning Dove (6)	
Culex tarsalis N=5	*Lynx candensis*	Lynx (1)	64
	Lama guanaco	Guanaco (4)	128
Culiseta inornata N=3	*Lama pacos*	Alpaca (2)	128
	Bos taurus	Cow (1)	
	Hippopotamus amphibious	Hippo (1)	140
	Lama glama	Llama (5)	128
	Lama pacos	Alpaca(4)	128
	Lama guanaco	Guanaco (5)	128
	Alopex lagopus	Arctic Fox (1)	76
	Ailurus fulgens	Red Panda (1)	65
Aedes vexans N=55	*Camelus bacteranus*	Bactran Camel (1)	133
	Camelus dromedaries	Dromedary camel (1)	96
	Ceratotherium simum	White Rhino (1)	161
	Ceratotherium simum	White Rhino*(1)	12
	Dromaius novaehollandiae	Emu (1)	54
	Equus grevyi	Zebra (1)	167

Species	Blood meal source	Common name (number)	Distance in meters
	Macropus rufus	Red Kangaroo (1)	49
	Panthera leo	Lion (1)	63
	Panthera tigris	Tiger (1)	18
	Potamochoerus porcus	Red River Hog (1)	122
	Bos taurus	Cow (6)	
	Canis lupis familiaris	Dog (2)	
	Equus caballus	Horse (1)	
	Homo sapiens	Human (9)	
	Sylvilagus audobonii	Cotton tail (10)	