

Occurrence of *Anopheles hermsi* (Diptera: Culicidae) in Arizona and Colorado

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J. Med. Entomol. 38(2): 341–343 (2001)

ABSTRACT Historically, malaria was a significant cause of morbidity and mortality throughout the western United States, and *Anopheles freeborni* Aitken was thought to be the vector west of the Continental Divide. In 1989, *Anopheles hermsi* Barr & Guptavanij was described and subsequently found to be an effective laboratory vector of *Plasmodium*. The adults of these two species are morphologically indistinguishable, and therefore polymerase chain reaction was used to analyze the DNA from 48 mosquitoes collected in Arizona and Colorado (identified morphologically as *An. freeborni*). All specimens were identified as *An. hermsi*. This was the first report of *An. hermsi* in Arizona and Colorado and indicated that this *Anopheles* species historically may have been a malaria vector in these two western states.

KEY WORDS *Anopheles hermsi*, *Anopheles freeborni*, malaria, vector, constructed wetland

HISTORICALLY, MALARIA WAS a significant cause of morbidity and mortality throughout the United States. Although the hyperendemic level in the Mississippi, Ohio, and Missouri River Valleys and along the Atlantic Coast epitomized the American malarial experience (Barber 1929, Boyd 1941, Ackerknecht 1945, Drake 1964), the disease also has an important legacy in the western United States (Faust 1941, 1945, 1951), where it was endemic along the Pacific Coast as well as in towns, military posts, mining camps, and Indian reservations on the American Frontier. Despite Arizona's arid climate, malaria was a frequent problem throughout the state during the 19th century. United States Army medical records, for example, reported that "intermittent fever," a common medical term for malaria during this period, was one of the most frequent causes of morbidity among troops at several Army posts (Billings 1870, 1875; Fink 1998). Discerning the cause of an 1876 outbreak of the disease at Camp Lowell, located near Tucson, was one of the first duties of a young physician named Walter Reed (Quebbemen 1966). Detailed historic accounts suggested that many civilian communities, including Tucson, Wickenburg, and Obed, also were affected (McClintock 1921, Quebbemen 1966).

The incidence of endemic malaria declined sharply after World War I, and ceased to be a major public health problem in the United States after World War II. However, sporadic autochthonous cases continue to occur. Between 1957 and 1994, 56 outbreaks involving one or more cases of mosquito-transmitted malaria

were reported in the United States (Zucker 1996). California alone accounted for 79 cases between 1974 and 1990 (CDC 1991, Roberto 1991).

Although human malaria does not appear to have been present in the New World before 1492 (Dunn 1965), endemic transmission was facilitated by effective native anopheline vectors (Simmons 1941, Aitken 1945), such as *Anopheles freeborni* Aitken in the western United States (King and Bradley 1941, Aitken 1945, Bohart and Washino 1978). However, recent studies associated with autochthonous malaria transmission in California (Zucker 1996) indicate that *Anopheles hermsi* Barr & Guptavanij may be a more important malaria vector than *An. freeborni* (Fritz and Washino 1993). This species was described in 1988 (Barr and Guptavanij 1988) and initially was thought to be restricted geographically to areas in California south of the Tehachapi Mountains (Porter and Collins 1990). Subsequently, its distribution was found to include much of northern California and New Mexico (Fritz and Washino 1993). Laboratory studies have shown *An. hermsi* to be a competent vector of *Plasmodium vivax* (Porter and Collins 1990), indicating that *An. hermsi* may have been involved in recent outbreaks of malaria in California as well as in historical outbreaks along the Rio Grande River in New Mexico (Roberto 1991, Fritz and Washino 1993).

Adults of *An. hermsi* and *An. freeborni* are morphologically indistinguishable (Barr and Guptavanij 1988, Collins et al. 1990, Fritz et al. 1991, Porter and Collins 1991), confounding previous distribution records. Recent advances in molecular genetics provided improved tools for the identification of these two mosquito species. In particular, sequence analysis of DNA amplicons generated by polymerase chain reaction (PCR) may be used for differential identification, be-

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Table 1. Mosquito collection locations and results

Site	State	Lat:Long	No. specimens
Ruins of Camp Wallen (near Elgin) ^a	Arizona	N31 37' 30" W110 30' 00"	11
Hereford ^a	Arizona	N31 22' 30" W110 00' 00"	2
Cholla Lake (near Joseph City) ^b	Arizona	N33 22' 30" W113 45' 00"	11
Joseph City ^b	Arizona	N34 52' 30" W110 15' 00"	3
Canelo ^b	Arizona	N31 22' 30" W110 30' 00"	8
Fort Collins area ^b	Colorado	N40 30' 00" W105 00' 00"	4
Trail Canyon (near Cortez) ^b	Colorado	N37 07' 30" W108 15' 00"	6
Mancos River (near Mancos) ^b	Colorado	N37 15' 00" W108 15' 00"	3
		Total	48

^a Collected in 1995.

^b Collected in 1997.

cause *An. freeborni* and *An. hermsi* have unique differences in their ribosomal DNA (rDNA) regions, specifically in the restriction fragment patterns of rDNA (Porter and Collins 1991).

The distribution of *An. hermsi* outside of California and New Mexico has never been investigated. Because malaria was a common health problem in Arizona, we decided to determine the geographic distribution of *An. freeborni* and *An. hermsi* in this state using PCR.

Materials and Methods

Mosquito Collection and Initial Identification.

Mosquitoes were collected from July 1995 to September 1997 from the sites listed in Table 1. Arizona collection sites were selected on the basis of historic accounts of malaria transmission. Mosquito samples from Colorado were collected as part of another project.

Mosquitoes were collected with CDC miniature light traps baited with dry ice and transported to the Medical Entomology Laboratory at the University of Arizona where they were identified to species using the keys in Darsie and Ward (1981). Those identified as "*An. freeborni*" were retained for PCR analysis.

DNA Extraction and Diagnostic PCR. The phenol-chloroform DNA extraction and PCR procedures were described by Porter and Collins (1991). A 350-bp and a 900-bp product were diagnostic for *An. hermsi* and *An. freeborni*, respectively.

Positive controls of *An. freeborni* and *An. hermsi* for PCR analysis were supplied by D. A. Lemenager (Sutter-Yuba Mosquito Abatement District, California) and K. K. Fujioka (San Gabriel Valley Mosquito Abatement District, California), respectively.

Results and Discussion

Mosquito Collections. Forty-eight mosquitoes were collected at eight sites throughout Arizona and Colorado, identified morphologically as *An. freeborni* (Table 1), and analyzed by PCR.

Species Diagnostic PCR. The PCR assay initially was executed using the primers and reaction conditions described previously by Porter and Collins (1991). Preliminary studies using positive controls for both species resulted in a 350-bp product for *An. hermsi*, but

no discernible product for *An. freeborni*. Because results could be attained for *An. hermsi*, it was surmised that one of the primers designed to amplify *An. freeborni* DNA was confounding the assay. Multiple reactions using different parameters and primer combinations resulted in the same negative result and indicated that the *An. freeborni* ITS2 (5'-TTAC-CCAACCACACACTG-3') primer was confounding the reaction. A new primer for the *An. freeborni* ITS2 region (5'-GAAGCACCTACTCCG-3') based on the published *An. freeborni* DNA sequence (Genbank accession #M64484) was designed to amplify across the target region of the original primer allowing for direct sequencing and comparison. Upon use of the new primer, a 950-bp band was obtained for the *An. freeborni* control. This PCR product was sequenced and conformed well to the published *An. freeborni* sequence.

All 48 wild-caught mosquitoes were identified as *An. hermsi*. We surmised that in Arizona and Colorado, *An. hermsi* was found in environments similar to those where it was found in New Mexico and California. Existing literature reported that *An. freeborni* was distributed widely throughout Arizona and southern Colorado (Aitken 1945); however, Fritz and Washino (1993) suggested that these specimens actually were *An. hermsi*.

Anopheles freeborni was not found in our samples from Arizona or Colorado. It is possible that we merely failed to collect *An. freeborni*, and therefore its occurrence in Arizona and Colorado cannot be completely ruled out. Alternatively, previous reports of *An. freeborni* from these states (Aitken 1945) were actually *An. hermsi* as suggested by Fritz and Washino (1993). Our results supported this hypothesis, and we believe that *An. hermsi* may have been the vector of malaria in Arizona during the late 19th century. Three of our collection sites previously were considered malarious. In 1867, troops stationed at Camp Wallen suffered from an outbreak of malaria (Fink 1998), whereas the old Mormon settlement of Obed, located near present day Joseph City and Cholla Lake, probably was abandoned in 1877 due to the disease (McClintock 1921).

Because of the recent advent and interest in constructed wetlands as well as the revitalization of riparian areas in Arizona, these records provide insight on the potential receptivity for malaria in these areas.

Of paramount importance is the construction of wetlands in rural settings, where the vector has remained present at low levels. The mosquito habitat associated with wetlands could provide the significant reemergence of a historical nuisance pest and competent malaria vector.

Acknowledgments

The authors thank A. G. Enyart for calling our attention to the *An. freeborni/hermsi* problem. Several individuals were helpful in the collection of mosquito samples in Arizona and we extend our appreciation to Carol Ellick, Juan Alegria, Bob Bixby, the late Bill Brophy, Frank Brophy, Mira Leslie, Craig Levy, and Mary Beth McGrath. In addition, we thank Helen Jost, Stephen Billington, J. Kevin Moulton, and Dawn Bueschel for technical support. We also thank Debra A. Lemanager and Kenn K. Fujioka for supplying positive control samples.

This research was funded by the University of Arizona Honors Program.

References Cited

- Ackerknecht, E. H. 1945. Malaria in the upper Mississippi Valley, 1760–1900. John Hopkins Press, Baltimore, MD (reprinted 1977, Arno, New York).
- Aitken, T.H.G. 1945. Studies on the anopheline complex of western North America. Univ. Calif. Publ. Entomol. 7: 273–364.
- Barber, M. A. 1929. The history of malaria in the United States. Public Health Rep. 44: 2575–2587.
- Barr, A. R., and P. Guptavanij. 1988. *Anopheles hermsi* n.sp., an unrecognized American species of the *Anopheles maculipennis* group (Diptera: Culicidae). Mosq. Syst. 20: 352–356.
- Billings, J. S. 1870. Report on barracks and hospitals with descriptions of military posts. Surgeon General's Office Circular, Washington DC War Department 4: 458–478 (reprinted 1974, Sol Lewis, New York).
- Billings, J. S. 1875. A report on the hygiene of the United States Army with descriptions of military posts. Surgeon General's Office Circular, Washington DC War Department 8: 525–564 (reprinted 1974, Sol Lewis, New York).
- Bohart, R. M., and R. K. Washino. 1978. Mosquitoes of California. University of California, Berkeley.
- Boyd, M. F. 1941. A historical sketch of the prevalence of malaria in North America. Am. J. Trop. Med. 21: 223–244.
- Centers for Disease Control and Prevention. 1991. Mosquito-transmitted malaria—California and Florida, 1990. MMWR 40: 106–108.
- Collins, F. H., C. H. Porter, and S. E. Cope. 1990. Comparison of rDNA and mtDNA in the species *Anopheles freeborni* and *Anopheles hermsi*. Am. J. Trop. Med. Hyg. 42: 417–423.
- Darsie, R. F., Jr., and R. A. Ward. 1981. Identification and geographical distribution of the mosquitoes of North America, North of Mexico. American Mosquito Control Association, Fresno, CA.
- Drake, D. 1964. Malaria in the Interior Valley of North America. University of Illinois Press, Urbana.
- Dunn, F. L. 1965. On the antiquity of malaria in the Western Hemisphere. Hum. Biol. 37: 385–393.
- Faust, E. C. 1941. The distribution of malaria in North America, Mexico, Central America and the West Indies, pp. 8–18. In F. R. Moulton [ed.], A Symposium on Human Malaria With Special Reference to North America and the Caribbean Region. American Association for the Advancement of Science, Washington, DC.
- Faust, E. C. 1945. Clinical and public health aspects of malaria in the United States from a historical perspective. Am. J. Trop. Med. 25: 185–201.
- Faust, E. C. 1951. Malaria in the United States. Am. Sci. 39: 121–130.
- Fink, T. M. 1998. John Spring's account of "malarial fever" at Camp Wallen, A.T., 1866–1869. J. Ariz. Hist. 39: 67–84.
- Fritz, G. N., and R. K. Washino. 1993. *Anopheles hermsi*, probable vector of malaria in New Mexico. Am. J. Trop. Med. Hyg. 49: 419–424.
- Fritz, G. N., S. K. Narang, D. L. Kline, J. A. Seawright, R. K. Washino, C. H. Porter, and F. H. Collins. 1991. Diagnostic characterization of *Anopheles freeborni* and *An. hermsi* by hybrid crosses, frequencies of polytene X chromosomes and rDNA restriction enzyme fragments. J. Am. Mosq. Control Assoc. 7: 198–206.
- King, W. V., and G. H. Bradley. 1941. Distribution of the nearctic species of *Anopheles*, pp. 71–78. In F. R. Moulton [ed.], A Symposium on Human Malaria With Special Reference to North America and the Caribbean Region. American Association for the Advancement of Science, Washington, DC.
- McClintock, J. H. 1921. Mormon settlement in Arizona. Manufacturing Stationers, Phoenix, AZ.
- Porter, C. H., and F. H. Collins. 1990. Susceptibility of *Anopheles hermsi* to *Plasmodium vivax*. Am. J. Trop. Med. Hyg. 42: 414–416.
- Porter, C. H., and F. H. Collins. 1991. Species-diagnostic differences in a ribosomal DNA internal transcribed spacer from the sibling species *Anopheles freeborni* and *Anopheles hermsi* (Diptera: Culicidae). Am. J. Trop. Med. Hyg. 45: 271–279.
- Quebbem, F. E. 1966. Medicine in territorial Arizona. Arizona Historical Foundation, Phoenix, AZ.
- Roberto, R. R. 1991. Mosquito-transmitted malaria in California: 1988–1989, pp. 46–48. In S. L. Durso and L. M. Sandoval [eds.], Proceedings and Papers of the 58th Annual Conference of the California Mosquito and Vector Control Association. California Mosquito and Vector Control Association, Sacramento.
- Simmons, J. S. 1941. The transmission of malaria by the *Anopheles* mosquitoes of North America, pp. 113–130. In F. R. Moulton [ed.], A Symposium on Human Malaria With Special Reference to North America and the Caribbean Region. American Association for the Advancement of Science, Washington, DC.
- Zucker, J. R. 1996. Changing patterns of autochthonous malaria transmission in the United States: a review of recent outbreaks. Emerg. Infect. Dis. 2: 37–43.

Received for publication 31 January 2000; accepted 31 October 2000.