COMPARISON OF HOST-FEEDING PATTERNS BETWEEN *ANOPHELES QUADRIMACULATUS* SIBLING SPECIES A AND B[1]

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ABSTRACT. Bloodmeal sources for sympatric species A and B of *Anopheles quadrimaculatus* in an area of Mississippi were identified using an indirect enzyme-linked immunosorbent assay. Females had fed only on 6 species of mammals including dog/fox, pig, opossum, raccoon, cottontail rabbit and white-tailed deer. The latter species was the predominant host, representing 96.7 and 89.5% of the bloodmeals taken by species A and B, respectively. No marked difference in feeding patterns was found.

The occurrence of a species in sympatry with a close relative is dependent on their possessing differences in some aspects of their biologies. This ability to coexist is no less true for sympatric sibling species than for species which are morphologically distinct. Historically, recognition of ecological and ethological differences provided the basis for the recognition of mosquito sibling species. For example, distinctions between saltwater and freshwater, and zoophilic and anthropophilic "races" provided evidence leading to the recognition of sibling species in the important malaria vectors, *Anopheles maculipennis* Meigen and *An. gambiae* Giles. Recently, application of molecular genetic techniques to studies of the systematics and population genetics of mosquitoes has led to the recognition of sibling species for which no information on differences in their basic biologies is available. It has recently been discovered that *An. quadrimaculatus* Say is actually a complex of at least 4 sibling species (Lanzaro 1986,[4] Kaiser et al. 1988, Narang et al. 1989). To date, there is no comparative information on the biologies of these species. We report here a comparison of host-feeding patterns of 2 of these species, *An. quadrimaculatus* species A and B.

Collections of blood-engorged adult female *An. quadrimaculatus sensu lato* (*s.l.*) were made from 2 small utility sheds at Noxubee Wildlife Refuge, Noxubee County, MI, during June to August, 1988. The 2 sheds were located at the margin of a wooded area and were approximately 20 m apart. The composition and relative abundance of host species in the adjacent area were unknown. Bloodfed females, collected with a mouth aspirator, were placed in cardboard ice cream containers and transported to the laboratory where mosquitoes were chilled and sorted. Females containing fresh bloodmeals were dissected so that the head and thorax of each specimen was separated from the abdomen. Each of these body portions was immediately placed in a separate 1.5 ml Eppendorf tube and frozen at −70°C for subsequent electrophoretic and serologic analyses. Tubes containing the body parts of each mosquito were assigned the same code number.

Identification of mosquitoes to species was achieved using established electrophoretic procedures (Lanzaro 1986,[4] Narang et al. 1989). These procedures involved electrophoresis of individuals followed by staining and scoring gels for 2 loci, *Idh-1* and *Idh-2*, coding for the enzyme isocitrate dehydrogenase (IDH, Enzyme Commission number 1.1.1.42). Lanzaro et al. (1990) reported that the loci coding for these enzymes are not linked, and therefore serve as independently assorting genetic markers which are diagnostic for sibling species in the *An. quadrimaculatus* complex. When the two IDH loci are used concurrently, *Anopheles quadrimaculatus* species A and B can be distinguished at a probability of 99.98% (Lanzaro et al. 1990). The *Idh-2* locus can distinguish species D from species A and the *Idh-1* locus can separate species B from species D, in both instances with a probability of correct identification of >99% (Kaiser et al. 1988).

Bloodmeal proteins, extracted in phosphate buffered saline, were initially tested with a modified capillary precipitin procedure (Tempelis and Lofy 1963) to generally classify each mosquito as having fed on a mammal, a bird or a reptilian/amphibian host. Specific mammalian hosts from which bloodmeals were taken were identified with an enzyme-linked immunosorbent assay using antisera produced in New Zealand white rabbits to the blood serum proteins of 11 different mammals. These procedures have

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[4] Lanzaro, G. C. 1986. Use of enzyme polymorphism and hybridization crosses to identify sibling species of the mosquito, *Anopheles quadrimaculatus* (Say). Ph.D. dissertation, University of Florida, Gainesville, FL, 92 pp.

been described by Irby and Apperson (1988), except that analyses involving anti-cottontail rabbit sera were allowed to react for 5 h after addition of substrate before reading, because of the low titer of this antiserum.

A total of 353 of 357 specimens of *An. quadrimaculatus s.l.* were successfully identified of which 115, 236 and 2 were females of species A, B and D, respectively. Of this number, the bloodmeal sources of 298 females were tested and successfully identified. Results presented in Table 1 are for 294 specimens because some females were not subjected to either electrophoretic analysis or serologic testing. Mosquitoes were determined to have fed on 6 mammals, with white-tailed deer being the predominant host. No bloodmeals were taken from reptilian or avian hosts. The host-feeding patterns of species A and B were not markedly different; however, species B did feed on a wider variety of mammalian species. Admittedly, this difference probably resulted from the larger number of specimens of species B that we analyzed. Albeit, a chi-square test indicated that differences in the host-feeding patterns between species A and B were not statistically significant ($\chi^2 = 8.403$, df = 5, $P > 0.1$). Investigations of the feeding patterns of other sibling species of *Anopheles* mosquitoes have focused on their comparative involvement in the transmission of malaria parasites. For example, significant differences in human blood indices have been reported for sibling species of *An. gambiae* (White et al. 1972, White and Rosen 1973) and *An. culicifacies* Giles (Joshi et al. 1988) complexes. To our knowledge, comparable investigations of the relative utilization of hosts other than man and domestic animals by sibling species of *Anopheles* have not been conducted.

Table 1. Host-feeding patterns[a] of sympatric sibling species[b] of *Anopheles quadrimaculatus* collected in 2 utility sheds on the Noxubee Wildlife Refuge, Noxubee County, MI, during June to August, 1988.

Blood source	No. (%) of specimens, species A	No. (%) of specimens, species B
Dog/fox	0 (0.0)	1 (0.5)
Pig	0 (0.0)	1 (0.5)
Opossum	1 (1.1)	0 (0.0)
Raccoon	0 (0.0)	6 (3.0)
Cottontail rabbit	2 (2.2)	13 (6.5)
White-tailed deer	88 (96.7)	180 (89.5)
Total	91 (100)	201 (100)

[a] Two specimens of species D fed on white-tailed deer.

[b] Differences between species A and B were not significant ($P > 0.1$) by a chi-square test ($\chi^2 = 8.403$, df = 5).

Mosquito feeding patterns are largely regulated by host availability and preference (Washino and Tempelis 1983). The large proportion of females of both species that we found to have fed on deer probably reflected the local high density of this host and a tendency of *An. quadrimaculatus* to feed on large mammals. Utilization of different hosts may provide a means by which sympatric sibling species of mosquitoes coexist. However, this ecologically appealing construct remains to be demonstrated for the sibling species of *An. quadrimaculatus*.

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