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SCIENTIFIC NOTE

BITING SITES OF *ANOPHELES KOLIENSIS* ON HUMAN COLLECTORS IN PAPUA NEW GUINEA[1]

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ABSTRACT. A field study was conducted to determine the preferred biting site of *Anopheles koliensis* on the lower limbs of humans. This species showed a preference for biting the feet and ankles, rather than the legs, and when denied access to the feet and ankles, this species showed a preference for legs without hair, rather than legs with hair.

KEY WORDS *Anopheles koliensis,* biting sites, human bait, attractants

In Papua New Guinea, malaria control strategies have relied on the application of indoor residual insecticides to reduce the longevity of the vectors (Sweeney 1983). As part of the evaluation of these control efforts, adult anophelines were collected in whole-night human biting catches (Spencer 1965, 1977). The collectors used for this work were required to bare their lower legs and, more importantly, their feet and ankles, as it was believed that leg hair discouraged anophelines from landing and that the feet and ankles, being hairless, were more attractive feeding sites. The rationale for this dogma has never been tested. Recent studies on anopheline biting sites indicate that certain species have a preference for specific parts of the body; studies also indicate that *Anopheles gambiae* Giles s.s. shows a preference for the foot and ankle areas because of the presence of odor-emitting eccrine sweat glands (De Jong and Knols 1995). This odor is believed to be similar to that of Limburger cheese, and on the feet and ankles of humans, this odor is caused by fatty acids produced by certain types of skin bacteria (Knols et al. 1997).

To investigate the attractiveness of feet and legs, both with and without hair, to anopheline mosquitoes, 2 experiments were conducted during April 1998 at Dasiama village, 20 km north of Port Moresby, Papua New Guinea. The experiments involved anopheline collections made over 2 consecutive nights between 2000 and 2200 h. On each occasion, 2 collectors sat 3 m apart. With an aspirator and torch, they collected mosquitoes and placed them into prelabeled cups. Mosquitoes were identified by proboscis morphology, but as this characteristic is not entirely reliable, a subsample of mosquitoes was identified using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis (Beebe and Saul 1995).

In the 1st experiment, 2 male collectors (collector 1: weight 102 kg, height 183 cm; collector 2: weight 67 kg, height 176 cm) wore long-sleeved shirts buttoned at the wrist, long trousers, socks, and shoes. Each collector had shaved (using water only) all the hair off 1 leg below the knee before the study began. A marking pen was used to indicate the hairline above the ankle and below the knee on both legs. At the commencement of the experiment, each collector exposed 1 leg by removing the sock and shoe and rolling the trouser leg to the knee. The collector then collected all anophelines coming to bite, and those that bit above the marked line on the ankle were held separately from those that bit the ankle and foot. Collections were made alternately from the shaved and unshaved legs. The leg not in use was covered by trousers, sock, and shoe. Each collection period lasted for 10 min, followed by a 10-min break. Six collections were made by each collector, 3 from the shaved leg, ankle, and foot and 3 from the unshaved leg, ankle, and foot.

In a 2nd experiment, conducted on the following night, both the shaved and unshaved legs of the same collectors were exposed; the feet and ankles remained covered with socks and shoes. Six collections lasting 10 min each were made by each collector, with a 10-min break between collection periods.

All mosquitoes were identified morphologically as *Anopheles koliensis* Owen, and this was verified by PCR-RFLP. This species is a member of the *Anopheles punctulatus* group, which comprises 12 closely related species, many of which are major vectors of malaria and Bancroftian filariasis in the southwest Pacific.

A 1-way analysis of variance was performed on the data after a log $(n + 1)$ transformation. In experiment 1, significantly more mosquitoes were bit-

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ing the feet and ankles than the legs (means ± SE: feet and ankles, 66.92 ± 7.91, legs, 7.92 ± 2.98; F = 16.9, df = 1, $P = 0.001$). There was no statistical difference between the shaved and unshaved legs, though more mosquitoes were collected from the shaved leg (mean ± SE: shaved, 11.67 ± 5.01, unshaved, 4.17 ± 2.87; F = 1.99, df = 1,10, $P = 0.19$). Collector 1 recorded more bites than collector 2; however, the difference was not significant (means ± SE: collector 1, 43.8 ± 11.7; collector 2, 31.1 ± 9.2; F = 1.34, df = 1,22, $P = 0.26$). In experiment 2, significantly more mosquitoes were recorded biting the shaved leg than the unshaved leg (mean ± SE: shaved, 62.3 ± 14.07; unshaved, 25.6 ± 7.39; F = 7.72, df = 1,22, $P = 0.01$). There was a significant difference in the numbers biting the collectors, with collector 1 recording more mosquitoes than collector 2 (mean ± SE: collector 1, 68.42 ± 13.40; collector 2, 19.50 ± 5.08; F = 16.1, df = 1,22, $P = 0.006$).

At the time of these experiments, the biting density of *An. koliensis* was very high. A total of 1,953 mosquitoes was collected during the 2 nights, with an average of 81.3 ± 11.4 bites per 10 min taken from the legs, ankles, and feet. During the collection period, *An. koliensis* was also found biting the hands and head and biting through clothing where it was tight against the body. The biting at these sites was not assessed because of the upper-body movement of the collectors. However, the number of anophelines attracted to these sites was small compared with those attracted to the legs and feet.

This study shows that when given a choice between lower legs and feet and ankles, *An. koliensis* prefer to bite the feet and ankles and that the presence or absence of leg hair had no significant effect on the biting site the mosquitoes chose. However, when allowed to feed only on the legs, the presence or absence of hair affected the site selected for biting, with significantly more mosquitoes biting the shaved leg.

De Jong and Knols (1995) demonstrated that foot odor attracted *An. gambiae* s.s. to the feet. In the experiments conducted here, *An. koliensis* also showed a preference for biting the feet; as with *An. gambiae,* it may be a similar odor that attracts *An. koliensis.* Attraction to the feet has also been recorded for another member of the *An. punctulatus* group. On Guadalcanal in the Solomon Islands, 86% of *Anopheles farauti* s.l. (most likely *An. farauti* Laveran s.s., this being the only anthropophilic *An. farauti* species occurring in this region [Foley et al. 1994]) bites occurred within 10 cm of the ground, i.e., on the feet and ankles. The attraction to this area of the body was not influenced by proximity to the ground, as 84% of bites still occurred in this region when the collector was raised 62 cm off the ground (Suzuki 1998).

With *An. koliensis,* the presence of a foot odor was not necessarily a prerequisite to feeding. When the feet and ankles were covered, similar numbers of *An. koliensis* fed readily on the legs. In this situation, the presence of hair acted as a deterrent to biting, with more bites being received by the shaved than the unshaved leg. Leg hair perhaps represented a physical barrier, discouraging mosquitoes from landing.

In both experiments, collector 1 recorded more mosquitoes biting than collector 2, although this was only statistically significant in the 2nd experiment. This difference was not likely to be related to the individual skill of the collectors, as both have several years' field experience in collecting mosquitoes. Rather, the preference for collector 1 may be associated with size. The larger body mass of collector 1 may translate to a higher temperature and larger odor source; the larger mass may also have resulted in higher humidity and a greater volume of exhaled carbon dioxide, making this collector more attractive to mosquitoes. Other workers have also noted that larger human hosts attract more mosquitoes than do smaller hosts (Port et al. 1980).

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