MAN-MOSQUITO CONTACT AT KOWANYAMA NORTHERN QUEENSLAND, AUSTRALIA

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ABSTRACT. A method employing ANOVA is presented to quantify man-mosquito contact at Kowanyama, based on human bait collections indoors in light, indoors in darkness and outdoors in darkness. No shifts in feeding behavior were noted during the transitional, dry or wet seasons of 1974–75. *Anopheles bancroftii* fed in significantly greater numbers indoors in darkness; *An. amictus, An. annulipes, An. farauti, An. meraukensis, Aedes normanensis, Ae. kochi, Ae. lineatopennis, Ae. vigilax* and *Culex annulirostris* fed outdoors; *Mansonia uniformis* fed equally well in the 3 situations tested whereas *Cx. quinquefasciatus* primarily fed indoors.

INTRODUCTION

Kowanyama, northern Queensland (15° 28'S, 141° 45'E) was a major site of study of arbovirus epidemiology (Doherty 1974) in which *Culex annulirostris* Skuse was recognized as an important vector of Australian arboviruses, including the Alphavirus, Ross River and Flaviviruses, Murray Valley encephalitis and Kunjin, of medical importance. Aspects relating to the high vector potential of *Cx. annulirostris* were studied in subsequent years: abundance and diel periodicity (Standfast 1965, Kay 1979a), age structure (Kay 1979b), host-preference and feeding patterns (Kay et al. 1979b) and resting habits (Kay 1983).

Previous studies of the biting behavior of Australian mosquitoes have been largely descriptive and anecdotal which makes comparison from locality to locality difficult. In Cairns, Roberts and O'Sullivan (1948) compared the numbers of anophelines collected simultaneously from indoors and outdoors. In Papua New Guinea, Peters and Christian (1963) described biting behavior qualitatively using + to +++ gradings.

During the development of the "Feeding Index," a method for quantitating the feeding patterns of mosquitoes on vertebrate hosts (Kay et al. 1979a), it became necessary to quantitate host-vector interactions indoors and outdoors. This paper describes that method, thus providing data on several species regarded as being actual or potential vectors of either arboviruses or *Plasmodium*.

MATERIAL AND METHODS

DIEL ACTIVITY. Collections were made during nights at Kowanyama in the dry season (December 1974) and wet season (February 1975) to confirm previous findings on mosquito biting activity (Standfast 1965, Standfast and Kay, unpublished data). Each of 3 collectors acted as bait in 4 hr shifts starting at 1800 hr and aspirated mosquitoes which were biting their arms and legs. Data from this preliminary work facilitated selection of a suitable collection time in the main study detailed below.

MAN-VECTOR INTERACTIONS INDOORS AND OUTDOORS. The biting habits of mosquitoes were investigated at Kowanyama indoors in lighted surroundings, indoors in darkness and outdoors in darkness. Two rooms 2.5 m^3, 5 m apart were constructed from insulation sheeting (Sisalation 420). These were built on the verandah of the Queensland Institute of Medical Research field station against an existing wall and ceiling. A sheet of insulation was draped horizontally from the ceiling across the front of each room leaving access through an 1.2 m high opening. A 75 W bulb lit one room when required. The outdoors site on open lawn was 8 m away from the nearest room in line with both sites.

Two collectors (BHK and IDF) collected host-seeking mosquitoes from 1900 to 1945 hr, using a 12 night schedule listed below, on each of 4 visits: April (transitional), July (dry), November 1974 (dry) and February 1975 (wet season). This collecting schedule was of complete block design which was duplicated and randomized as follows:

Night 1	$A_1 + B_2$	Night 7	$A_2 + B_1$
2	$B_1 + C_2$	8	$A_1 + B_2$
3	$A_2 + B_1$	9	$A_2 + C_1$
4	$A_2 + C_1$	10	$B_1 + C_2$
5	$B_2 + C_1$	11	$A_1 + C_2$
6	$A_1 + C_2$	12	$B_2 + C_1$

where A = indoors in light
B = indoors in darkness
C = outdoors in darkness
1 = collector 1
2 = collector 2

ANALYSIS OF RESULTS. In order to minimize collector bias, each situation evaluated contained an equal number of collections by each collector. Thus, for the 12 night program, each situation, A, B and C, was analyzed on the basis

of 8 collections each, i.e., 4 each by collectors 1 and 2. The total numbers collected for each of the 3 sites were transformed to log (n + 1) to normalize the data, and entered across the top 3 cells of a 3 × 4 matrix (situation × season). Equivalent scores for each site on the 3 subsequent trips were entered accordingly and subjected to 2-way ANOVA to analyse any differences between sites (2 degrees of freedom), changes in abundance among trips (3 df) and their interaction (6 df). Any seasonal shifts in biting behavior were indicated by an interaction significant to $P \leq 0.05$. There were 12 df for error. Significance was tested by an F test and differences within each category, e.g., sites, by a Student's *t* test.

As *Aedes normanensis* (Taylor) was only collected during April 1974 and February 1975, a 3×2 matrix was used for its analysis. Because data for *Anopheles annulipes* Walker and *Mansonia uniformis* (Theobald) were similar for each trip, and because of their low numbers, the replicates were combined and interaction used as the estimate of error. A further 6 species were not numerous enough to warrant analysis as above so χ^2 was used; 9 uncommon species were not analysed statistically.

To determine whether the number of trapping nights could be reduced to 6 nights for each visit, the relative numbers collected for each site were converted to proportions and analysed by χ^2. Similarly, numbers collected for nights 1–6 were compared with those of nights 7–12 to determine if there had been a depletion effect.

RESULTS

Some 6,466 mosquitoes comprising at least 21 species were collected (Table 1). There were significant differences in seasonal abundance of *An. bancroftii* Giles, *Ma. uniformis, Ae. normanensis, Cx. annulirostris* and *Cx. quinquefasciatus* Say ($P < 0.01$) and *An. annulipes* ($P < 0.05$). However for all species tested by ANOVA, the interactions (seasons × sites) were non-significant. Because this suggested that there were no seasonal shifts in biting behavior, data on site differences were combined for each trip and presented in Table 2 for analysis.

Significantly different numbers of *Anopheles bancroftii, An. annulipes, Cx. annulirostris* and *Cx. quinquefasciatus* were collected in the 3 situations studied: indoors in light, indoors in darkness and outdoors in darkness (Table 2). *Culex annulirostris, An. annulipes* and *Ae. normanensis* were strongly exophagic (biting outdoors) and avoided illuminated indoors situations but occa-

Table 1. Number of mosquitoes collected from indoors in light (A), indoors in darkness (B) and outdoors in darkness (C) on 4 trips to Kowanyama.

	April 1974			July 1974			November 1974			February 1975			
Species	A	B	C	A	B	C	A	B	C	A	B	C	Total
An. bancroftii	19	210	72	64	194	81	40	66	34	9	28	11	828
An. amictus (s.l.)*			7		1	23		1	40		2	24	98
An. annulipes	1	13	54	2	2	36		1	4	1	12	35	161
An. farauti												25	25
An. meraukensis			28		1				1		2	13	45
Cq. crassipes				1									1
Ma. uniformis	9	13	11	48	29	56	1	1	2				170
Ad. catasticta							1						1
Ae. alternans												1	1
Ae. normanensis		1	4							11	43	573	632
Ae. vigilax						2		4	21				27
Ae. kochi		1									3	11	15
Ae. notoscriptus											1		1
Ae. tremulus		1						1					2
Ae. alboscutellatus			1								1		2
Ae. lineatopennis			4							1		9	14
Cx. (Lophoceraomyia) spp.	1				1					1			3
Cx. annulirostris	10	41	489	16	59	268	8	25	189	26	84	574	1789
Cx. bitaeniorhynchus											6	2	8
Cx. quinquefasciatus	344	792	47	66	147	16	9	15	1	315	845	42	2639
Cx. starckeae										1	2	1	4
Number collected	384	1072	717	197	434	482	59	114	292	365	1029	1321	6466
Number of collections		24			24			24			24		96

* Contains both *An. amictus* and *An. hilli.*

Table 2. Mean catch* and tests of significance in Analysis of Variance of mosquitoes collected from situation A (indoors in light), b (indoors in darkness) and C (outdoors in darkness) at Kowanyama.

Species	F test	Mean catch			Student's *t*-test		
		A	B	C	A–B	B–C	A–C
An. bancroftii	13.6**	12.2	45.8	19.0	5.1**	3.5**	1.6
An. annulipes	20.11**	0.9	4.8	23.5	2.8*	3.6*	6.4**
Ma. uniformis	1.1	9.0	7.9	12.2	no	significant	difference
Ae. normanensis	4.9	1.6	3.0	21.4	0.6	2.4	3.0*
Cx. annulirostris	125.1**	6.6	23.4	169.8	5.6**	15.6**	9.9**
Cx. quinquefasciatus	19.9**	39.7	80.3	6.2	2.1	6.2**	4.1**

* Williams' mean (Haddow 1954).
Asterisks denote the following levels of significance: * $p<0.05$; ** $p<0.01$.

sionally fed on man in the darkened room. *Anopheles amictus* Edwards (s.l.), *An. meraukensis* Venhuis, *An. farauti* Laveran (s.l.), *Ae. vigilax* (Skuse), *Ae. kochi* (Dönitz) and *Ae. lineatopennis* (Ludlow) fed predominantly outdoors in darkness (χ^2, $P < 0.01$) (Table 1). *Cx. quinquefasciatus* fed less readily outdoors (average of 6.2) than indoors in either light (39.7) or darkness (80.3). The mean score for *An. bancroftii* in the darkened room (45.8) was significantly greater than in the illuminated room (12.2) or outdoors (19.0) ($P < 0.01$). *Mansonia uniformis* was collected equally indoors in light (9.0) and in dark (7.9) and outdoors (12.2).

For each of *Cx. annulirostris, Cx. quinquefasciatus* and *An. bancroftii,* the total numbers collected for nights 1–6 of each trip were converted into relative proportions A:B:C and compared with their respective duplicates (nights 7–12) to determine if a 6 night program was adequate. For 3 species tested, only 6 of 12 duplicates showed non-significant differences for visits 1–4 respectively: *Cx. annulirostris* (NS, <0.01, NS, NS), *Cx. quinquefasciatus* (<0.05, <0.01, NS, <0.05) and *An. bancroftii* (NS, <0.01, <0.01, NS). Because of this variation between replicates, a 12 night program would seem to be necessary. There was no depletion of these 3 species from nights 1–6 to 7–12 as the respective totals collected were 2658 to 2634. All χ^2 tests for the above species for each trip were non-significant.

DISCUSSION

The protocol described in this paper provides a basis for quantifying the degree of contact between man and mosquitoes in various biotopes. However, the quantification obtained must be carefully qualified as the variation in housing conditions from locality to locality may also influence the numbers of mosquitoes biting.

The similarities in the relative proportions of each species collected from each site for the 4 visits suggest that 12 nights trapping is sufficient to provide data of a general nature. That half of the duplicates were significantly different from their counterparts suggests that 6 nights trapping per visit is insufficient. Expansion of the complete block design to include a fourth situation (e.g., outdoors in light) would require 12 nights ($^4P_1$) without any duplication but would have only 8 df for error. This may, of course, be inadequate; a 24 night program may be required. This latter situation could be important in some pathogen transmission situations, e.g., outdoor barbecues. Such information may be useful in establishing the relative amounts of biting contact between a particular vector and man in different situations, and thus allows for the development of appropriate control strategies.

This method was developed to quantitate the degree of concurrence between vector and host in studies of the "Feeding Index" (Kay et al. 1979a) in order to provide a more complete analysis of host-feeding patterns based on precipitin test analyses. The host-vector concurrence (HVC) factor is calculated from data on the relative availability of hosts and the vector indoors and outdoors. This can be modified to define the overall risk to the human population. For example, from the *Cx. annulirostris* data (Table 1), the relative proportion biting indoors (mean of light + dark) to outdoors was 0.08: 0.92 (Table 2). During the early hours of the evening when *Cx. annulirostris* is most active, approximately half of the human population is indoors. From this, the risk of being infected by *Cx. annulirostris* outdoors (0.5×0.92) can be calculated as being 11 times greater than indoors (0.5×0.08). Once the relative risk has been established, appropriate action can be taken. In fact at Kowanyama, the zooprophylactic effect of community dogs interdispersed among social gatherings of aborigines sitting outdoors during this time effectively reduces the arbovirus (Murray Valley encephalitis, Kunjin and Ross River) transmission

potential, as feeding on dogs was 5 times more common than on man (Kay et al. 1979b). In Papua New Guinea, Standfast (1967) also noted that *Cx. annulirostris* fed outdoors on villagers in the early evening.

The biting patterns of 12 mosquito taxa collected at Kowanyama are generally consistent with published information although it is difficult to evaluate purely descriptive data. At least 9 species were not collected in sufficient numbers to warrant comment. Apparent disparities do exist, e.g., in Papua New Guinea, *Cx. annulirostris* fed as readily indoors as outdoors; *An. bancroftii* fed only outdoors and *An. farauti* fed equally well indoors and outdoors (Peters and Christian 1963). In Papua New Guinea, *An. farauti* fed abundantly in dimly-lit houses but in Cairns this species was 9–12 times more abundant in human bait collections outdoors (Roberts and O'Sullivan 1948). These differences may be interspecific in relation to *An. farauti* which is known to comprise 3 sibling species (Mahon 1983). Other genetic differences (Coluzzi et al. 1977) or environmental factors (Slooff 1964[1], Kaul and Wattal 1968) may alter the behavior of geographically-isolated species.

Residual indoor spraying still remains the method of choice for *Anopheles* control in the Torres Strait, which separates Australia from New Guinea. In view of (1) the considerable degree of exophagy of the anophelines evaluated in this study and (2) their habit of resting outdoors (Roberts and O'Sullivan 1948, Kay 1983), it would be prudent to conduct a similar investigation of biting behavior on some of the islands which, in 1984, have experienced indigenous transmission of *Plasmodium vivax*.

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[1] Slooff, R. 1964. Observations on the effect of residual DDT house spraying on behaviour and mortality in species of the *Anopheles punctulatus* group. Thesis, Leyden Univ., Holland.

References Cited

Coluzzi, M., A. Sabatini, V. Petrarca and M. A. Di Deco. 1977. Behavioural divergences between mosquitoes with different inversion karyotypes in polymorphic populations of the *Anopheles gambiae* complex. Nature, Lond. 266:832–833.

Doherty, R. L. 1974. Arthropod-borne viruses in Australia and their relation to infection and disease. Progr. Med. Virol. 171:136–192.

Haddow, A. J. 1954. Studies of the biting habits of African mosquitoes. An appraisal of methods employed, with special reference to the twenty-four hour catch. Bull. Entomol. Res. 45:199–242.

Kaul, N. H. and B. L. Wattal. 1968. Studies on culicine mosquitoes IV. Influence of climate on the feeding behavior of *Culex fatigans* females in village Arthala, near Delhi. Bull. Indian Soc. Malar. Comm. Dis. 5:45–52.

Kay, B. H. 1979a. Seasonal abundance of *Culex annulirostris* and other mosquitoes at Kowanyama, north Queensland, and Charleville, south west Queensland. Aust. J. Exp. Biol. Med. Sci. 57:497–508.

Kay, B. H. 1979b. Age structure of populations of *Culex annulirostris* (Diptera: Culicidae) at Kowanyama and Charleville, Queensland. J. Med. Entomol. 16:309–316.

Kay, B. H. 1983. Collection of resting adult mosquitoes at Kowanyama, northern Queensland and Charleville, south-western Queensland. J. Aust. Entomol. Soc. 22:19–24.

Kay, B. H., P. F. L. Boreham and J. D. Edman. 1979a. Application of the "Feeding Index" concept to studies of mosquito host-feeding patterns. Mosq. News 39:68–72.

Kay, B. H., P. F. L. Boreham and G. M. Williams. 1979b. Host preferences and feeding patterns of mosquitoes (Diptera: Culicidae) at Kowanyama, Cape York peninsula, northern Queensland. Bull. Entomol. Res. 69:441–457.

Mahon, R. J. 1983. Identification of the three sibling species of *Anopheles farauti* Lavaran by the banding pattern of their polytene chromosomes. J. Aust. Entomol. Soc. 22:31–34.

Peters, W. and S. H. Christian. 1963. The bionomics, ecology and distribution of some mosquitoes (Diptera, Culicidae) in the Territory of Papua and New Guinea. Acta Trop. 20:35–79.

Roberts, F. H. S. and P. J. O'Sullivan. 1948. Studies on the behaviour of adult Australasian anophelines. Bull. Entomol. Res. 39:159–178.

Standfast, H. A. 1965. A miniature light trap which automatically segregates the catch into hourly samples. Mosquito News 25:48–53.

Standfast, H. A. 1967. Biting time of nine species of New Guinea Culicidae (Diptera). J. Med. Entomol. 4:192–196.