

# Spatial and Temporal Variation in Abundance of *Anopheles* (Diptera: Culicidae) in a Malaria Endemic Area in Papua New Guinea

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**ABSTRACT** Abundance of anophelines in 10 villages in the Wosera area of Papua New Guinea was monitored during 1990–1993. Of 85,197 anophelines collected in 1,276 paired indoor and outdoor landing catches, 40.4% were *Anopheles koliensis* Owen, 36.7% *An. punctulatus* Donitz, 14.3% *An. karwari* (James), 4.9% *An. farauti* s.l. Laveran, 3.1% *An. longirostris* Brug, and 0.7% *An. bancroftii* Giles. Maps of average indoor biting rates were produced using a Bayesian conditional autoregressive model which allowed for heterogeneities in sampling effort over time and space. Differences in spatial distributions among species were observed among and within villages and were related to the distribution of larval habitats and vegetation. Abundance of *An. punctulatus* and *An. koliensis* decreased with distance from the main waterway and probably from a sago swamp forest at 6 villages in North Wosera. Abundance of *An. punctulatus* was associated negatively with those of *An. farauti* s.l., *An. longirostris*, and *An. bancroftii*. The latter 3 species also had relatively low ratios of indoor-to-outdoor biting rates, and earlier biting times than *An. punctulatus*. Human blood indices of at least 0.79 were observed for all species except *An. bancroftii*. Abundance of all 6 species was correlated temporally with recent rainfall, but *An. koliensis*, *An. karwari*, and *An. longirostris* showed greater temporal variability than the other species. *An. punctulatus* and *An. koliensis* tended to occur together in time and space (index of association,  $I = 0.85$ ). Weaker associations were seen between *An. farauti* s.l. and *An. longirostris* ( $I = 0.44$ ) and *An. koliensis* and *An. karwari* ( $I = 0.34$ ). The most frequently collected species occurred together and were concentrated near the Amugu river; the remaining species tended to occur together but in different parts of the Wosera area. The importance of understanding ecological requirements of the different *Anopheles* vectors and their association with key household and landscape features are discussed in relation to malaria transmission and control.

**KEY WORDS** *Anopheles* spp., ecology, biting cycles, conditional autoregressive model, spatial analysis, malaria

MALARIA OCCURS THROUGHOUT the coastal lowlands of Papua New Guinea but has been most intensively studied in Madang and East Sepik Provinces near the north coast. The Madang area is highly endemic for both *Plasmodium falciparum* and *P. vivax* (Cattani et al. 1986a). In the Wosera area of East Sepik province, *P. malariae* also is frequently encountered (Genton et al. 1995). Extensive entomological studies in preparation for malaria vaccine trials have been carried out near Madang (Burkot et al. 1987, 1988b). The Wosera area also has been the site of recent epidemiolog-

ical studies in preparation for vaccine trials (Alpers et al. 1992, Genton et al. 1995), but the ecology of *Anopheles* spp. has not been studied since a pilot DDT trial during 1958–1959 (Peters 1960a, b; Peters and Standfast 1960).

The anopheline situation in Papua New Guinea is complex, both taxonomically and ecologically. The main vectors are members of the *Anopheles punctulatus* complex. Recent allozyme studies have shown that there are at least 6 species within this group in Papua New Guinea (Foley et al. 1993), including *An. punctulatus* s.s. Donitz, *An. koliensis* Owen, and 4 sibling species of *An. farauti* Laveran s.l. The 2 former species and at least 1 member of the *An. farauti* complex are important malaria vectors in Papua New Guinea (Burkot et al. 1988b). Two more species with distributions centering around New Guinea have been considered to be minor vectors—de Rook (1929) and Metselaar (1957) found low infection rates in *An.*

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*bancroftii* Giles, and *An. longirostris* Brug was suspected to be a minor vector by Peters (1965). The latter species feeds predominantly on pigs (Charlwood et al. 1985) in the Madang area, but there may be several sibling species (D.H. Foley, personal communication) with different ecologies. In addition, *An. karwari* and *An. subpictus*, 2 secondary malaria vectors whose distribution centers on the Indian subcontinent, have been found locally in Papua New Guinea (Peters and Christian 1963, Lee et al. 1987).

Variation in vector ecology is one of the most important factors affecting heterogeneity in the microepidemiology of malaria (Greenwood 1989). Both in the Western Province of Papua New Guinea (Nakazawa et al. 1994), and near Madang (Burkot et al. 1987, 1988b; Cattani et al. 1986b; Charlwood et al. 1983, 1986), considerable spatial variation in levels of malaria endemicity, transmission rates, and mosquito abundance and survival rates have been found, even between villages within a few kilometers of each other. The known malaria vectors near Madang are *An. punctulatus*, *An. koliensis*, and *An. farauti* No. 1. The latter is 1 of the 6 sibling species within the *An. farauti* complex and has been given the non-Linnean designation (Foley et al. 1993). *An. punctulatus* larvae occur in small sunlit bodies of water and are distributed widely around Madang (Charlwood et al. 1986), whereas *An. koliensis* breeds in semipermanent habitats at forest margins (Belkin 1962) and has a more focal distribution (Charlwood et al. 1986). *An. farauti* No. 1 favors brackish water and streams in lowland coastal areas.

Rainfall is spread relatively evenly throughout the year in most of Papua New Guinea, so that there is no season without any mosquitoes and densities can reach very high levels (Spencer 1970, Afffi et al. 1980). However, patterns of seasonality differ among mosquito species (Burkot et al. 1988b), and this may be important in the epidemiology of malaria because of the interspecific variation in transmission potential. For instance, in the Madang area, the peak abundance of *An. farauti* No. 1 occurs at the end of the dry season, when the larvae are less likely to be flushed out of riparian breeding sites (Charlwood et al. 1986).

Small-area variations in contact rates between mosquitoes and man may have a profound effect on the true vector potential of a mosquito population (Dye and Hasibeder 1986) and on the immunological responses in the host population (Forsyth et al. 1989). Limited mixing of mosquito populations between villages also tends to perpetuate heterogeneities in transmission of plasmodial parasites (Charlwood et al. 1988).

To determine whether the pattern of small-area variation near Madang was reproduced elsewhere in Papua New Guinea, we studied the extent and scale of spatial and temporal heterogeneity in the anopheline populations in the Wosera area. Peters and Standfast (1960) found that *An. punctulatus*

was the primary vector throughout the year, with lower densities of *An. farauti* s.l. and *An. koliensis*, but did not carry out detailed studies of small-area variation. Using a Bayesian model of spatial and temporal variability developed in analyses of malaria vectors in Tanzania (Smith et al. 1995), we estimated how abundance of *An. punctulatus* complex mosquitoes and 3 other anophelines varied geographically during a 34-mo period in 10 Wosera villages. We then attempted to relate the different spatial and temporal patterns observed for different anophelines to their ecology and to consider the implications for malaria transmission.

### Materials and Methods

**Study Site.** The study was carried out as part of the Papua New Guinea Malaria Vaccine Epidemiology and Evaluation Project (Alpers et al. 1992) in the Wosera area (143° E, 52° S) of East Sepik Province (Fig. 1). The area is divided into 2 census divisions, North and South Wosera, comprising 469 and 333 km<sup>2</sup>, respectively. Its major feature is the Amugu (Screw) River, a permanent stream of greatly variable discharge arising in mountains to the north. The altitude is ≈180 m above sea level. The climate is wet tropical (Genton et al. 1995) with a mean annual rainfall of 1,847 mm during 1990–1993 (range, 1,759–1,936 mm). Approximately 58–65% of annual precipitation falls during the wet season (October–April); the dry season occurs from May to September.

All villages and hamlets were accessible by dirt roads and footpaths. Village populations ranged from 100 to 800 residents and were subdivided into geographically distinct hamlets. Traditional Wosera houses consist of a timber frame (inverted V) covered with sago leaf roofs that reach the ground. More recently, houses with elevated floors, corrugated iron roofing, and walls of split bamboo or sago palm stems have appeared.

House positions were recorded using a handheld global positioning system (GPS) device (Trimble Navigational Europe, London). The resulting map was validated against sketch maps of the village. The heights of houses and other architectural features also were recorded in annual surveys of the village. The software system MapInfo (MapInfo 1990) was used to compile a digitized map using the coordinates collected with the GPS system, showing the houses sampled, roads, rivers, creeks, potential *Anopheles* breeding sites, and other landscape features. Numerous seasonal streams exist throughout the area and their flow corresponds with periods of heavy rainfall, usually from October through April. The central part of the study area consisted of the flood plain of the Screw River and its tributaries (Fig. 2). Most of the land is mixed regrowth with patches of sago palm, *Metroxylon sagu*, swamp (Haanjen et al. 1968). Allen et al. (1993) distinguished 2 agricultural systems in use. Apusit, Kunjinigini-1, Nindi-

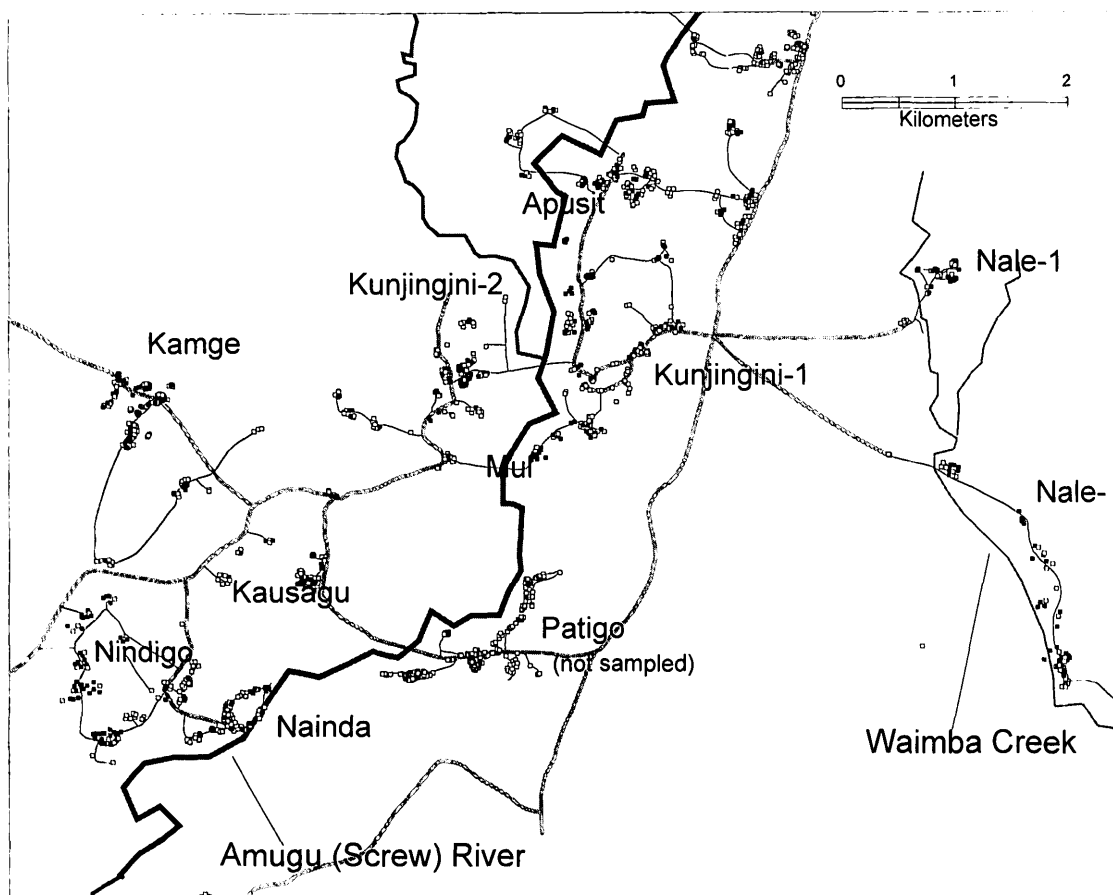


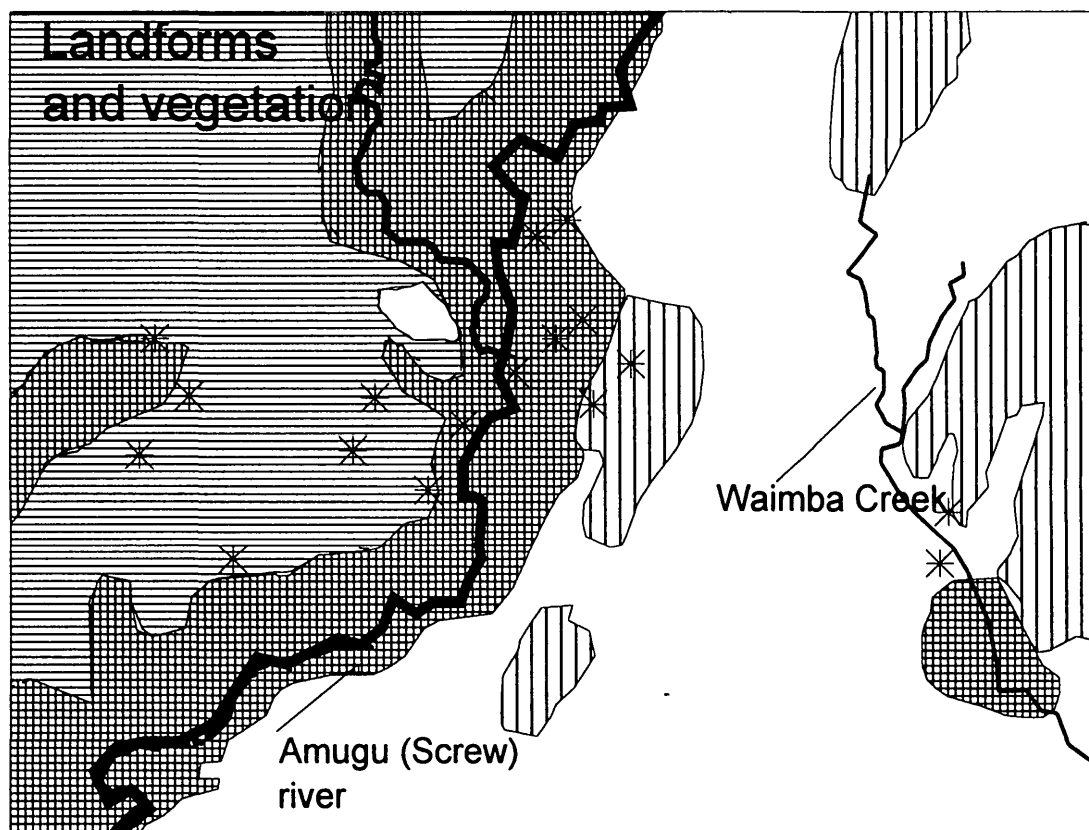
Fig. 1. Map of Wosera area, showing sampled (filled boxes) and unsampled (open boxes) houses, all-weather roads (==), footpaths (—), Amugu (Screw) River, and Waimba creek.

go, Kausagu, and Nainda form part of the most intensively cultivated area in East Sepik Province and depend on silt from river floods to sustain fertility with a short fallow period. Yams, *Dioscorea esculenta*, were the most important crop, but planted sago and taro, *Colocasia esculenta*, also are important. In Mul, Kunjingini-2, and Kamge, where there is less silt from river flooding, land use is less intensive, with a longer period of regrowth.

Much of Nale village (comprising Nale-1 and Nale-2), which is separated from the rest of the study area by a wide plain, is dominated by *Imperata* spp. grassland or *kunai*. In Nale, the forest is cultivated less intensively than in the valley of the Amugu (Screw) River, and gardens extend into the neighboring grasslands.

**Sampling of Anophelines.** In June 1990, 7 villages (Apusit, Kunjingini-1, Nale-1, Nale-2, Kunjingini-2, Mul, Kamge) in North Wosera and 3 villages (Kausagu, Nindigo, Nainda) in South Wosera were selected for entomological monitoring. Sampling of Nale-2 and Mul commenced in October 1991 and February 1992, respectively; sampling of Nainda was suspended temporarily during Febru-

ary–August 1992; sampling ceased on September 1993. All-night human landing collections were made monthly in these villages by resident adults who gave informed consent after receiving consent protocols and verbal explanations in Neo-Melanesian language on the rationale and purpose of entomological monitoring, including possible hazards. Ethical guidelines of the Papua New Guinea Medical Research Council were followed. Each of the 8 villages were divided geographically into 5 sectors (A–E), whereas Kamge and Nainda were divided into 6 sectors (A–F). Within each sector, a random individual was selected each month, and the house inhabited by that individual was, where possible, sampled for mosquitoes. The different sectors were sampled on 5 consecutive nights; sectors E and F were sampled concurrently on the 5th night. Paired indoor and outdoor landing catches were made by 4 adult male collectors working in 2 teams of 2, 1 team working from 1800 to 2400 hours and the other from 2400 to 0600 hours. One member of each team collected mosquitoes which landed on him inside the selected house; the 2nd collected outside. Searches for engorged resting mosquitoes were carried out from



**Fig. 2.** Vegetation and land systems map of the Wosera area. Names for the land systems are those used by Haanjens et al. (1968) for forest and grass-covered hills with garden regrowth (▤); rolling grassland, largely *Imperata* spp. (▥); flat to gently undulating grassland with minor swamp vegetation (▦); flood plains and low river terraces, with mixed flood plain forest and garden regrowth (▧); and *Anopheles* breeding sites (\*).

0630 to 0730 hours inside the bedrooms of the house used for the landing catch, and in 3 other neighboring houses. At the same time, outdoor collections were made from naturally occurring resting sites around the perimeters of these houses.

In the 3rd yr, immature stages of *Anopheles* were sought from accessible aquatic sites that could possibly serve as potential breeding places. These included rainwater surface pools, pools left by the receding streams, sago swamps, ground seepage, and surface areas in which the level of water fluctuates with that of the streams. Standard dipping using enamel bowls was used for sampling larvae from accessible sites. The approximate positions of these sites were entered into maps obtained from household surveys.

**Identification of Anophelines.** Mosquitoes were taken to the field laboratory, and the anophelines were identified morphologically (Belkin 1962, Lee et al. 1987). Representative females obtained from landing and resting collections were sent to the Queensland Institute of Medical Research for confirmation using cellulose acetate electrophoresis (Foley et al. 1993) and  $^{32}\text{P}$ -labeled species specific DNA probes (Cooper et al. 1991).

**Human Blood Index.** The midguts of semi- and fully engorged female *Anopheles* mosquitoes from indoor and outdoor resting collections were squashed onto filter papers and later tested by a direct enzyme-linked immunosorbent assay (ELISA) using antihost immunoglobulin (IgG) conjugates against human, pig, dog, cat, and goat (Beier et al. 1988). Each filter paper sample was eluted individually in 50  $\mu\text{l}$  PBS (pH 7.4), diluted with 1 ml PBS and stored at  $-20^\circ\text{C}$  until testing by direct ELISA. Cross-reactivity was decreased by the addition of 1:500 dilutions of heterologous sera to the conjugate solutions. Because 70% of the blood sources could be detected after 24 h of digestion (Burkot et al. 1988a), we did not compare the level of cross-reactivity with blood meals from other hosts.

**Statistical Model for Spatial and Temporal Variation in Densities.** Maps of average mosquito densities and estimates of day-to-day variation were produced separately for each *Anopheles* species, as described by Smith et al. (1995) for mapping of mosquito densities in Tanzania. In brief, a Bayesian conditional autoregressive Poisson model was used to estimate expected average indoor den-

**Table 1.** Number of indoor landing catches per house, August 1990–September 1993

No. nights	No. houses	
	North Wosera	South Wosera
1	201	78
2	111	34
3	49	18
4	20	11
>4	29	13
Totals	410	154

sities in each house, allowing appropriately for imbalances in the sampling effort and for day-to-day variation in mosquito populations.

The (natural) logarithm of the expected number of mosquitoes ( $E_{ij}$ ) was analyzed as a function of space and time as  $\ln E_{ij} = \Psi_i + \Theta_j$ , where  $\Psi_i$  is the effect associated with house  $i$ , and  $\Theta_j$  is the effect associated with night  $j$ .

To allow for spatial autocorrelation and to provide estimates of densities for houses that were not sampled, a spatially smooth function  $\Psi_{x,y}$  was defined, corresponding to the mean of  $\Psi_i$  for all houses within a radius of 150 m ( $r = 150$ ) from the point  $(x,y)$ , excluding any house at precisely point  $(x,y)$ . The model then treated the effect for a given house  $i$ , at position  $(x,y)$ ,  $\Psi_i$ , as drawn from a normal distribution with mean  $\Psi_{x,y}$ .  $\Psi_i - \Psi_{x,y}$  then measures the difference (on a log scale) between the estimated numbers of mosquitoes in a house and the numbers that would be expected given its position.

The value of  $r$  (150 m) was chosen to allow for short-range autocorrelation. A lower limit to  $r$  is imposed because isolated houses (with no neighbors at distances  $< r$ ) cannot be included in the model. The value of 150 m for  $r$  allowed inclusion of a total of 564 sampled houses.

The night effects,  $\Theta_j$ , also were treated as random effects. This made it possible to compare the levels of both spatial and temporal variation in densities for the different species while allowing for sampling variation (Smith et al. 1995). The model was fitted by Gibbs sampling using the program BUGS (Thomas et al. 1992).

**Associations Between Species.** Southwood's (1978) index of association between species was

calculated as  $I = 2 [J/(A + B) - 0.5]$ , where  $J$  is the number of individuals of both species in samples where they occur together, and  $A$  and  $B$  are the total numbers of individuals of the 2 species in all samples (Service 1993).  $I$  lies between the normal range of  $-1$  (no association) to  $+1$  (complete association).

## Results

**Sampling Pattern.** Fig. 1 shows the locations of 564 houses included in the analyses and other unsampled houses. 72.7% ( $n = 410$ ) of the sampled houses were located in North Wosera. The distribution of houses and number of sampling occasions per house is shown in Table 1. There was a high level of household participation in implementing the original sampling plan. The number of indoor landing catches was similar during 1990–1991 and 1992–1993 (623 versus 653, respectively).

**Species Abundance and Endophily.** Morphologically identified *An. koliensis*, *An. punctulatus*, *An. karwari*, *An. farauti* s.l., *An. longirostris*, and *An. bancroftii* comprised 40.4, 36.7, 14.3, 4.9, 3.1, and 0.7%, respectively, of the 85,197 *Anopheles* specimens collected during the 1,276 paired landing catches. Allozyme studies confirmed the presence of both *An. punctulatus* s.s. ( $n = 27$ ) and *An. koliensis* ( $n = 118$ ). The *An. farauti* s.l. differed electrophoretically ( $n = 9$ ) and by DNA probe analysis ( $n = 127$ ) from *An. farauti* sibling species Numbers 1, 2, and 3, but a positive identification of these mosquitoes was not obtained.

The ratios of numbers of landing mosquitoes caught indoors/outdoors are given in Table 2. All these ratios differed significantly from 1:1 except for that of *An. karwari* ( $\chi^2 = 2.2$ ,  $P > 0.05$ ).

**Spatial Pattern.** Table 3 gives mean human landing rates by village for each anopheline species, for the houses and time periods sampled. There were substantial differences in both species composition and abundance (numbers relative to a collector) among villages. These averages did not allow for imbalances in the sampling effort, nor did they indicate the extent of variation within the villages. However, Fig. 3 shows contour maps of the indoor bites per person per night, averaged over

**Table 2.** Comparison of anopheline species

	<i>An. punctulatus</i>	<i>An. koliensis</i>	<i>An. karwari</i>	<i>An. farauti</i> s.l.	<i>An. bancroftii</i>	<i>An. longirostris</i>
Total caught	31,263	34,376	12,162	4,156	611	2,629
In/out ratio	0.87	0.95	0.96	0.72	0.69	0.65
Total tested for human blood	926	2,097	313	111	38	31
Human blood index	0.90	0.79	0.79	0.88	0.40	0.84
Empirical variance of CAR model estimates						
House effects ( $\psi_i$ ) <sup>a</sup>	1.42	5.60	3.49	2.42	2.90	2.03
Night effects ( $\theta_j$ )	1.00	1.57	2.99	1.00	0.54	1.67

CAR, conditional autoregression.

<sup>a</sup> Isolated groups of houses in which the species did not occur are not included in this analysis.

Table 3. Human landing rates by village and species

Village	Person-nights	<i>An. punctulatus</i>	<i>An. koliensis</i>	<i>An. karwari</i>	<i>An. farauti</i> s.l.	<i>An. bancroftii</i>	<i>An. longirostris</i>	Totals
Kamge	280	3.1	1.8	0.2	1.8	0.3	4.8	12.0
Nindigo	260	3.9	10.1	0.6	2.0	0.3	1.5	18.4
Nale-2	174	4.1	14.6	11.7	7.4	0.5	2.0	40.3
Nale-1	300	5.6	15.0	26.0	1.8	0.4	0.3	49.1
Kunjingini-1	298	9.6	9.9	2.8	0.9	0.2	0.5	23.9
Nainda	216	11.4	11.5	0.7	1.0	0.1	0.3	25.0
Kausagu	252	11.6	2.7	0.6	1.4	0.3	0.4	17.0
Mul	180	14.7	20.0	0.5	1.0	0.2	0.1	36.5
Apusit	339 <sup>a</sup>	23.1	23.2	2.2	0.4	0.1	0.2	49.2
Kunjingini-2	252	32.8	26.3	0.5	0.5	0.1	0.2	60.4

Data tabulated are the mean numbers of mosquitoes captured from 1800 to 0600 hours per exposed mosquito collector per night over all sampling nights. Both indoor and outdoor catches are included.

<sup>a</sup> One night's sampling from Apusit consisted of indoor landing data only.

time and smoothed in space, allowing appropriately for imbalances in the sampling scheme using the conditional auto regression models. The models included data for 164 nights of sampling, in which 564 houses were represented and a total of 1,141 landing catches made.

Substantial variation in anopheline abundance was evident even within the villages, and because the peripheries of 4 villages (Kunjingini-1, Mul, Kunjingini-2, Apusit, Nindigo, and Nainda) were more or less contiguous, the spatial patterns often did not segregate by village boundaries (Fig. 3). The variances of the distributions of  $\Psi_i$  (Table 2) measure the overall spatial variability of each species. All species had focal distributions within the study area. Although *An. koliensis* had the highest variance of  $\Psi_i$ , the spatial distributions of the 2 most abundant species were quite similar, with both *An. punctulatus* and *An. koliensis* abundance concentrated along the banks of the Amugu river and its tributaries in Apusit, Mul, and Kunjingini-2 (Fig. 3). *An. karwari* was most frequently collected in Nale village but also was relatively abundant in the parts of Apusit and Kunjingini which were nearest to Nale (and hence farthest from the Amugu river). *An. farauti* s.l. showed a non-overlapping distribution with *An. punctulatus* (Fig. 3) with relatively high densities in Nale-2 and Nindigo and low densities in Apusit, especially near the river. Although *An. longirostris* was relatively and infrequently collected in most villages, it was the commonest human biter in Kamge village, which formed an isolated group of houses where the other species were least abundant. It also was collected relatively frequently in the other villages along the western fringe of the study area and in Nale-2. The distribution of *An. bancroftii* was similar to that of *An. longirostris*, although it was not abundant.

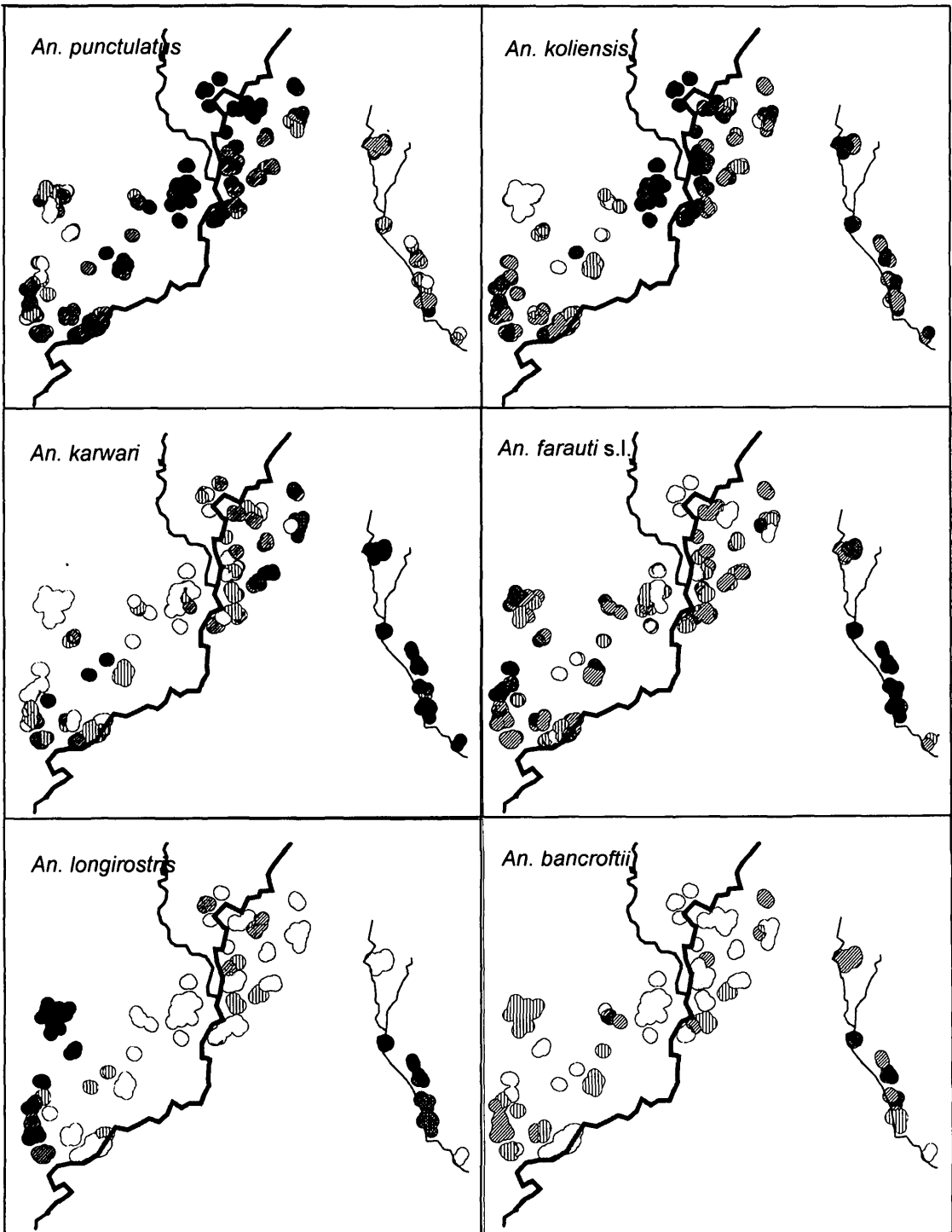
**Changes in Landing Rates over Time.** Fig. 4 shows the average mosquito abundance in the sampled houses predicted from the conditional auto regression model for each day of sampling, which comprised 8, 61, 50, and 45 d in 1990, 1991, 1992, and 1993, respectively. Similar patterns were

observed for the unadjusted geometric mean densities (not shown). Ranges of  $\exp(\Theta_j)$  were 0.1–70.27 females per person per night for *An. koliensis* (Fig. 4A), 0.25–26.09 for *An. punctulatus* (Fig. 4A), 0.02–33.82 for *An. karwari* (Fig. 4B), 0.07–5.75 for *An. farauti* s.l. (Fig. 4B), 0.02–4.82 for *An. longirostris* (Fig. 4C) and 0.04–1.52 for *An. bancroftii* (Fig. 4C). The variances of the night effects of  $\Theta_j$  (Table 2) give measures of the overall temporal variability for each species. The magnitude of maximum and minimum peaks in landing rates showed that *An. koliensis* (Fig. 4A), *An. karwari* (Fig. 4B), and *An. longirostris* (Fig. 4C) have the highest degree of variability over time. There was much less temporal variability in landing rates of *An. punctulatus* (Fig. 4A), *An. farauti* s.l. (Fig. 4B) and *An. bancroftii* (Fig. 4C).

The abundance of all 6 anopheline species increased following increases in rainfall (compare Fig. 4 A–C and Fig. 4D), but there were no regular annual cycles in abundance over time. Spearman correlation coefficients between values of  $\Theta_j$  and mean daily rainfall measured during the 4 wk preceding day  $j$  were similar for each of the 6 species, ranging from +0.18 (*An. karwari*) to +0.34 (*An. koliensis*) (all  $P < 0.03$ ).

**Biting Cycles.** Fig. 5 shows the biting cycles for each species. In each case, the percentage of human landing per hour was plotted separately for indoor and outdoor collections. *An. punctulatus*, *An. koliensis*, and *An. karwari* showed peak biting during the early hours of the morning, whereas *An. farauti* s.l. and *An. longirostris* were abundant early in the evening (Fig. 5). The biting activity of *An. bancroftii* occurred more or less equally throughout the night. *An. farauti* s.l., *An. longirostris*, and *An. bancroftii* all showed a slight tendency to bite earlier outdoors than indoors.

**Human Blood Index.** In total, 8,463 anophelines were obtained from indoor and outdoor resting collections in the 10 villages during 1990–1993. The specimens processed for blood meal identification comprised 95.4% of all 3,685 engorged females. Of the 3,516 specimens processed, 2,245 (46.3%) were collected resting inside houses and



**Fig. 3.** Contour plot of average indoor anopheline abundance per house in Wosera during 1990–1993. Human landing rate of *An. punctulatus* and *An. koliensis*: □, <1; ▤, 1–2; ▥, 2–5; ▦, 5–10; ■, >10. Human landing rate of *An. karwari*, *An. farauti s.l.*, *An. longirostris* and *An. bancroftii*: □, <0.1; ▤, 0.1–0.2; ▥, 0.2–0.5; ▦, 0.5–1.0; ■, >1.0.

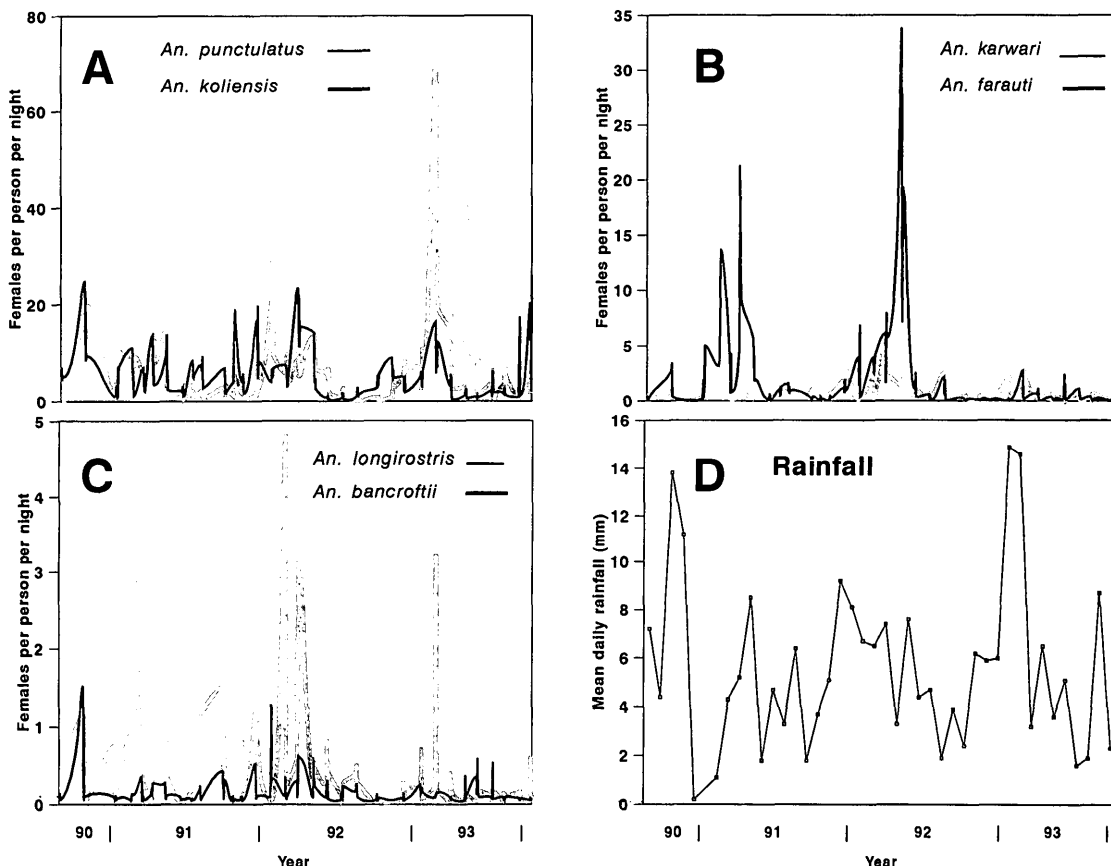


Fig. 4. Human-biting rate ( $\exp(\theta_i)$ ) of *Anopheles* mosquitoes estimated from indoor landing catches, 1990–1993. Fitted values estimated from the CAR model for the 564 sampled houses. Solid lines refer to the 1st species, the dotted line to the 2nd species. (A) *An. punctulatus* and *An. koliensis*. (B) *An. karwari* and *An. farauti s.l.* (C) *An. longirostris* and *An. bancroftii*. (D) Rainfall, by 4-wk period recorded at Kunjingini, 1990–1993.

1,271 (35.1%) were collected from outdoor natural shelters. The human blood index was calculated as the sum of indoor and outdoor numbers containing a specific host blood meal divided by the total numbers of indoor and outdoor blood-fed mosquitoes (Garrett-Jones 1964). *An. punctulatus* had the highest HBI (Table 2) followed by *An. farauti s.l.*, *An. longirostris*, *An. koliensis*, and *An. karwari*. *An. bancroftii* had a much lower HBI than the other species. Human blood indices calculated separately from indoor and outdoor resting mosquitoes for all species other than *An. koliensis* were not significantly different. 84.1% of 1,337 *An. koliensis* caught indoors had fed on humans, compared with 70.1% of 760 caught outdoors ( $\chi^2 = 57.5$ ,  $P < 0.0001$ ).

**Index of Association Between Species.** Indices of association between species reflected spatial and temporal co-occurrence. The strongest positive associations were between *An. punctulatus* and *An. koliensis* (+0.85), followed by *An. farauti s.l.* and *An. longirostris* (+0.44) and *An. koliensis* and *An. karwari* (+0.34) (Table 4). *An. punctulatus* and *An. koliensis* were strongly negatively associated with

the rarer species *An. bancroftii* and *An. longirostris*. The negative association between *An. punctulatus* and *An. farauti s.l.* was not as obvious here as shown in Fig. 3, presumably because the tendency for them to occur in different villages was somewhat offset by a tendency to occur on the same nights.

**Relationship of Numbers of Mosquitoes Caught to House Construction.** The quantity  $\Psi_i - \Psi_{x,y}$  is a measure of the deviation of mosquito abundance in house  $i$  from that expected for its location. Means of  $\Psi_i - \Psi_{x,y}$  were computed for each anopheline species separately for houses raised on stilts (104 houses) and for houses at ground level (252 houses). The form of construction of the remaining sampled houses had not been recorded. For all 6 species differences were small and not statistically significant.

## Discussion

The 6 morphological species of *Anopheles* identified in the Wosera area of Papua New Guinea exhibited substantial differences in abundance,



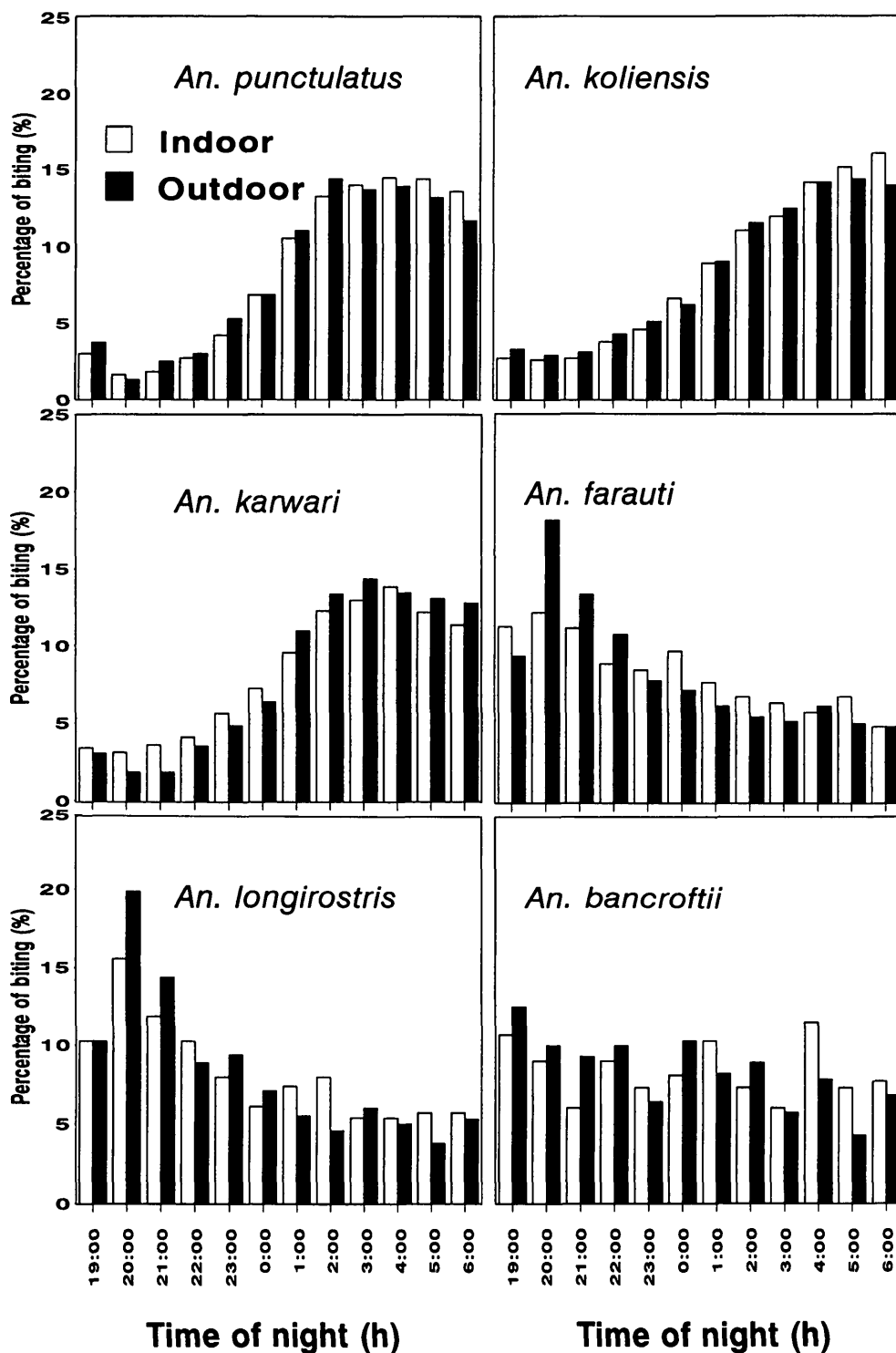


Fig. 5. Nocturnal indoor and outdoor biting cycles of females of 6 *Anopheles* species collected from 10 villages in study area. Open bars refer to indoor biting, and filled bars to outdoor biting. *An. punctulatus* ( $n = 14,505$  indoor, 16,758 outdoor); *An. koliensis* ( $n = 16,748$  indoor, 17,629 outdoor); *An. karwari* ( $n = 5,965$  indoor, 6,197 outdoor); *An. farauti* s.l. ( $n = 1,745$  indoor, 2,411 outdoor); *An. longirostris* ( $n = 1,038$  indoor, 1,591 outdoor); *An. bancroftii* ( $n = 249$  indoor, 362 outdoor).

Table 4. Measure of association (I) between *Anopheles* species

Species	<i>An. koliensis</i>	<i>An. karwari</i>	<i>An. farauti</i> s.l.	<i>An. bancroftii</i>	<i>An. longirostris</i>
<i>An. punctulatus</i>	0.85	0.12	-0.27	-0.73	-0.60
<i>An. koliensis</i>	—	0.34	-0.09	-0.68	-0.06
<i>An. karwari</i>	—	—	0.29	-0.22	-0.43
<i>An. farauti</i> s.l.	—	—	—	-0.25	0.44
<i>An. bancroftii</i>	—	—	—	—	-0.13

spatial and temporal distribution, and biting behavior. The taxonomy of these anophelines, especially of *An. farauti* s.l., is not fully resolved. Foley et al. (1993) recognized 6 species of the *An. farauti* complex in Papua New Guinea and the Solomon Islands, but new species continue to be discovered (e.g., Foley et al. 1995).

Recently described species included one sibling morphologically identical to *An. punctulatus* found near the south coast of Papua New Guinea (Foley et al. 1995). However, allozyme studies indicated that the Wosera populations belong to *An. punctulatus* s.s. The allozyme analyses also confirmed the identity of *An. koliensis* from Wosera, but we cannot exclude the possibility that some *An. farauti* No. 4 also may occur, because this taxon is morphologically indistinguishable from *An. koliensis* (Foley et al. 1993). The human blood indices for *An. punctulatus* and *An. koliensis* in our study were in good agreement with those of Burkot et al. (1988a) for the Madang area.

The abundance of *An. punctulatus* was highest along the low-lying banks of the Amugu River and adjacent tracts of sago swamp running north-south through Apusit, Kunjingini-1, Kunjingini-2, and Mul, but with its abundance decreasing with distance from the river. Because the fringes of the river are populated densely and have the most intensive agriculture, this is consistent with a high degree of vector anthropophily and with *An. punctulatus* breeding in the areas most disturbed by human activity. Although this species has been reported to disperse up to 1.8 km from a release site (Charlwood and Bryan 1987), most females remained concentrated in small areas along the banks of the Amugu River, most noticeably in Apusit and Kunjingini-2.

Although *An. koliensis* reportedly exploits larval habitats different than those of *An. punctulatus* (Charlwood et al. 1986, Lee et al. 1987), we found that adults of the 2 species were strongly associated, even though *An. koliensis* showed more variation over time in landing catches and showed a decreasing gradient of abundance away from the Amugu (Screw) River. Larvae of *An. koliensis* were collected from the sago swamps among the northerly hamlets of Apusit and Kunjingini-1, which may have been major breeding sites (unpublished data), but we do not have an explanation for the relative scarcity of this species in areas around similar swamps near Kamge and Nainda. Larvae occasionally have been found in temporary habitats at the forest margin (Owen 1945, Belkin 1962).

Peters and Standfast (1960) also found *An. koliensis* to be more seasonal than *An. punctulatus* in the same area as the current study, whereas changes in population density of both species were synchronized with monthly rainfall in the Madang area (Charlwood 1985, Burkot et al. 1988b).

Landing rates of *An. farauti* s.l. were highly seasonal near Madang (Afifi et al. 1980, Charlwood et al. 1986, Burkot et al. 1988b), and in that area it was the predominant vector of malaria in coastal but not inland villages, breeding mainly in coastal streams with large temporal fluctuations in adult densities. Although not as early as in the Wosera, peak landing catches in Madang area also occurred before midnight (Charlwood 1985). In the Wosera, *An. farauti* s.l. was one of the species with the least variation over time in landing catches and has a relatively early biting cycle, in agreement with the previous study of Standfast (1967) from the Maprik district. The human blood index in our study was double that of coastal *An. farauti* s.l. reported by Burkot et al. (1988a). It occurred mostly in the villages of Nale-1 and Nale-2, but even there peak abundance was not high. This species seems to be associated with less disturbed habitats than those of *An. punctulatus*, and the *Imperata* spp. grassland would seem to provide some degree of isolation from the anopheline communities in the center of the study area. Allozyme and DNA probe results (D. Foley and N. Beebe, personal communication) indicated that the *An. farauti* s.l. in Wosera is a different species than the coastal populations which belong to *An. farauti* No. 1.

The morphotype of *An. longirostris* also may consist of several sibling species in Papua New Guinea (D. Foley, personal communication). Unlike Charlwood et al. (1985), we found *An. longirostris* to be predominantly anthropophagic; therefore we suspect that another sibling species different from that in Madang may predominate in Wosera. There is a relatively high abundance of *An. longirostris*, both in Kamge at the western side of the study area where this species accounts for the most human biting, and in Nale-2.

*Anopheles karwari*, which can be identified easily morphologically, breeds in foothill seepages and small streams (Lee et al. 1987) but has a spatial distribution in Wosera similar to that of *An. farauti* s.l. and is concentrated in Nale-1 and Nale-2, perhaps breeding in the small creeks there. In Nale-1, *An. karwari* is the most abundant anopheline biting humans. *An. karwari* showed much more temporal variation in abundance than did *An. far-*

*auti* s.l.; therefore, despite similar spatial distributions, we suspect that these species have different breeding sites.

The least abundant anopheline recorded, *An. bancroftii* also can be identified morphologically. It showed less temporal variation in abundance than the other species and is the least anthropophilic, occurring at low densities in all the villages. This species also has a low propensity for human biting in Australia, preferring to feed on domestic animals (Lee et al. 1987).

It has long been recognized (Peters and Standfast 1960) that the most intense malaria transmission in Papua New Guinea occurs where the main vectors are *An. punctulatus* or *An. koliensis*. The high human blood indices of these species are an important reason for this, but none of the Papua New Guinea species fed exclusively on humans. *An. koliensis* fed on marsupials and pigs as well as on humans, and *An. punctulatus* also fed on pigs (Foley et al. 1993). The late-biting cycles of these 2 most frequently collected species may be a specialization for human biting which they share with Afrotropical malaria vectors (Charlwood et al. 1996). However *An. karwari* also has a late-biting cycle, although it prefers to feed on bovines in Asia (Lee et al. 1987). Cattle are not found in Wosera where *An. karwari* has a high human blood index. In Nale-1, it was the anopheline with the highest human landing rate; therefore, it may be an important malaria vector in this area.

Because the more abundant anophelines tend to occur together in time and space, there is marked spatial clustering of human biting in Wosera. It may be that the clustering of malaria transmission is even more pronounced, because the minor anopheline species, whose spatial distributions to some extent complement those of the more abundant species, probably are less efficient vectors. *An. farauti* s.l., *An. longirostris*, and *An. bancroftii* are early outdoor biters and seem to be more successful in biting people that are engaged in outdoor activities before midnight. However, because they are less likely to enter houses, this would make it easier for people to avoid them. Nevertheless, all these species except *An. bancroftii* had high human blood indices; therefore, their importance as vectors of malaria remains uncertain.

It seems unlikely that human behavior plays an important role in either spatial or temporal variation in landing rates. Although there are large differences among villages in the use of mosquito bed nets (Genton et al. 1994), these nets are not impregnated with insecticide. The structure of houses and the activity patterns and life styles of the people varied little among the villages. Only the mission station at Kunjingini-1 formed a discrete area of houses, some of which were screened against mosquitoes. In contrast to the study by Charlwood et al. (1984) in coastal Madang of Papua New Guinea, we did not find a significant reduction in landing rates in raised structures, presumably be-

cause different sibling species of *An. farauti* occurred there.

The evidence presented here clearly shows that *Anopheles* populations are clustered rather than homogeneously distributed, although explanations for these patterns are not clear. The co-occurrence of *An. punctulatus* and *An. koliensis* in Wosera, despite focal distributions of both species, may lead to extreme variation in space in the transmission of malaria. The degree of concentration of anopheline abundance in Wosera might be exceptional, but given the degree of species diversity (Foley et al. 1993) and breeding site specialization (Charlwood 1985, Charlwood et al. 1986), extreme small-area variation in the composition of anopheline communities is probably a general characteristic in Papua New Guinea. The consequent large variations in malaria transmission over a few hundreds of meters very likely play an important role in keeping some parts of the human population relatively free from sporozoite inoculations and reducing the spread of *Plasmodium* variants. It also may explain the small-area epidemiological variations observed by Cattani et al. (1986b). Further analyses of our data will consider the sporozoite rates in the different anopheline species. This will make possible a direct test of this hypothesis by relating malaria in the human population to measured entomological inoculation rates.

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