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Behavioural heterogeneity of *Anopheles* species in ecologically different localities in Southeast Asia: a challenge for vector control

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Summary In Southeast Asia the biodiversity of *Anopheles* species in the domestic environment is very high. Only few species are considered major vectors throughout the region, whereas the vector status of other species varies from area to area. Often it is difficult to identify an *Anopheles* species as a malaria vector in areas with low malaria incidence. The behaviour of *Anopheles* species largely determines their vector status, and insights into their behaviour are essential to evaluate the appropriateness of vector control measures. This study was conducted in six ecologically different localities in Southeast Asia to rank the different *Anopheles* species in terms of anthropophily and endophagy in order to estimate their current epidemiological importance. Concurrently, the biting and resting behaviour of the vectors was analysed to evaluate the appropriateness of insecticide-impregnated bed nets and residual house spraying in vector control. *Anopheles dirus* A was highly anthropophilic at all sites where it occurred. By contrast, the degree of anthropophily exhibited by *An. minimus* A depended on availability of cattle. *Anopheles campestris*, *An. nimpe*, *An. sinensis*, *An. maculatus*, *An. aconitus* showed a high degree of anthropophily in certain villages, indicating their potential of participating in malaria transmission, although the actual incidence of malaria in the study villages can be fully explained by transmission of the major vectors (*An. dirus* A, *An. minimus* A and *An. sundaicus*). Late biting of *An. minimus* A and biting activity throughout the night of *An. sundaicus* favour bed nets as a control method for these species, whilst exophilic and outdoor biting in combination with early feeding behaviour of *An. dirus* A will make both insecticide-impregnated bed nets and indoor residual spraying less suitable for controlling this species. Spatial variation in biting and resting behaviour was observed within almost all *Anopheles* species. These heterogeneities may result in the differences in epidemiological importance and in response to vector control of *Anopheles* species in different areas. Moreover, environmental changes and changes in human practice are expected to influence the behaviour, hence the role of the different species in malaria transmission. The effect of environmental changes on vector behaviour should be followed up carefully.

keywords malaria control, behaviour, *Anopheles*, Southeast Asia

Introduction

In Southeast Asia, malaria control programmes are faced with a complex vector system caused by the number of *Anopheles* species potentially involved in malaria transmission. Besides the major vectors *Anopheles dirus* s.l., *An. minimus* s.l. and *An. sundaicus*, a large number of other anopheline species occur in the vicinity of human dwellings. Assessing the potential role of these species in malaria transmission is often difficult in areas with low malaria incidence (Trung *et al.* 2004). Hence, knowledge of their behaviour, which largely defines their vectorial status and the efficacy of vector control methods, is of crucial importance.

The proportion of feeds taken on humans is an important factor in determining the malaria vector status of *Anopheles* species. This proportion is influenced by both host preference and host availability (Klowden 1996). Consequently, changes in host abundance may influence malaria transmission (Giglioli 1963; Bruce-Chwatt *et al.*

1966; Gillies 1988). Indoor feeding increases the chance of human–vector contact and thus the epidemiological role of *Anopheles* species. However, the importance of outdoor biting has been emphasized in discussions on the persistence of malaria transmission in areas where indoor-insecticide use was well implemented (Elliott 1968). In Southeast Asia, biting rhythm and resting behaviour of the vectors are two major elements determining the appropriateness of the most commonly used vector control measures, i.e. insecticide-impregnated bed nets and indoor residual spraying. In Vietnam, Laos and Cambodia, insecticide-impregnated bed nets are now the main vector control measure. House residual spraying is limited to epidemic situations and to newly settled zones (Hinh 2000). However, one cannot expect great success of insecticide-impregnated bed nets in areas where the vector bites outdoors and/or early in the evening when people are still active. Likewise, house residual spraying in areas where the vector does not rest indoors will not be very effective.

Insights into vector behaviour and the influence of alternative hosts, environment and housing conditions on behaviour have direct practical implications for malaria control. We conducted an entomological study in six ecologically different localities in Southeast Asia to rank the different *Anopheles* species for their trend in anthropophily and endophagy, their biting and resting behaviour.

Materials and methods

Study sites

Six study villages were selected for mosquito collections in different geographical areas of the region. In Vietnam, one village was chosen in the hilly forested northern part of the country, two others in the hilly forested area of the Centre and one in the Mekong delta. In Cambodia, the study site was located in the north-eastern part of the country and in Laos about 100 km north of Vientiane. The study village are described in Table 1 and their location shown in Figure 1. The climate is tropical humid except at the study site of northern Vietnam, which has a winter (monthly mean temperature of 17 °C) from November to February. The rainy season generally lasts from May to October. Malaria transmission and incidence in the study villages have been described elsewhere (Verlé *et al.* 1998; Erhart *et al.* 2004a,b; Trung *et al.* 2004). Malaria was previously endemic in Khoi village, but it is currently free of malaria. In Lang Nhot, Village 3 and Cha Ong Chan, malaria remains an important health problem with relatively high transmission. In Na Ang and Van Duc A, malaria transmission still occurs but at low level. During the entomological study period and the last 2 years preceding our mosquito collections, no specific vector control measures were organized in the study villages.

Mosquito collections

Human landing collections were made by one collector inside and two collectors outside each house from 18:00 to 06:00 hours using two shifts of collectors. Two houses were selected in each village. One cattle-shed was selected for cattle-bait collections performed by two collectors, from 21:00 to 24:00 hours each night. Indoor morning resting collections were conducted in another 10 houses from 06:00 to 10:00 hours by two collectors. During each entomological survey, 10 nights of mosquito collections were conducted. Each night-mosquito collection was followed by an indoor morning resting collection.

In Vietnam, eight entomological surveys were carried out concurrently in the four villages (April, August and November 1998; April and November 1999; April, August and November 2000). Collections on cattle and morning resting collections took place during the first four surveys only. All collection methods were applied in Cambodia and Laos during the three surveys (March, July and October 1999). In the study site of Laos, cattle-bait collections were only possible during the second survey (July 1999) because no cattle was present during survey 1 (March 1999) and survey 3 (October 1999).

Mosquito processing

Anopheline mosquitoes were identified morphologically to species level using an illustrated key for anophelines of Southeast Asia (modified from IMPE 1987). Specimens of *An. minimus* s.l., *An. dirus* s.l. and *An. sundaicus* were stored in liquid nitrogen or on silica gel. Members of the *An. minimus* complex were identified using the polymerase-chain reaction restriction fragment length polymorphism (PCR-RFLP) assay developed by Van Bortel *et al.* (2000). Members of the *An. dirus* complex were identified following Manguin *et al.* (2002).

Data analysis

Indoor and outdoor human landing rates were calculated as number of mosquitoes per man per night (all surveys pooled). Similarly, rates of mosquitoes biting cattle were estimated as the numbers of mosquitoes caught on cattle by a collector (all surveys pooled). Anthropophily of the *Anopheles* species was evaluated by the ratio of outdoor human landing rates to cattle landing rates, and the endophagic trend was estimated by the ratio of indoor

Table 1 Description of six study sites in Vietnam, Laos and Cambodia

Sites	Landscape	Dominant ethnic group/activities	Houses	Abundance of cattle
Khoi village, Hoa Binh Province, northern Vietnam (20°38′N, 105°10′E)	Village in a U-shaped valley with rice field surrounded by forested mountains.	Muong, Farmers	Large wooden houses, built on stilts, 1.0–1.5 m above the ground, closed walls, tiled roofs or thatched roofs.	++++
Lang Nhot village, Khanh Hoa Province, central Vietnam (12°14′N, 108°56′E)	Village surrounded by primary and secondary forest on hills, deforestation.	Rac Lay, Farmers exploiting plots in the forest	Very small, built mainly directly on the ground, very open with incomplete walls from split bamboo, large eaves and thatched roofs.	+
Village 3, Binh Thuan Province, central Vietnam (10°55′N, 107°40′E)	Village surrounded by primary forest, orchards (cashew nuts), deforestation.	Kinh, Farmers and forest workers	Medium sized houses in forest, built directly on the ground, quite closed with corrugated iron or thatched roofs.	+++
Cha Ong Chan village, Rattanakiry Province, north-eastern Cambodia (13°43′N, 106°59′E)	Village located on top of a hill surrounded by fragmented forest.	Kring, slash-and-burn exploitation, dry or upland rice	Multi-family large houses, built on stilts at 1.0–1.5 m above the ground, walls covered with plaited leaves and thatched roofs.	+++ with seasonal variations
Na Ang village, Vientiane Province, Laos (18°33′N, 101°59′E)	Village located in large open valley with a permanent river and a hilly forest 2 km away.	Lao Loum, Farmers	Large houses, walls covered with plaited leaves, built on stilts, about 2.0 m above the ground.	(+)
Van Duc A village, Bac Lieu Province, southern Vietnam (9°11′N, 105°18′E)	Coastal plain. Canals with nipa palm trees (*Nipa fruticans*).	Kinh, Agriculture (rice) and shrimp farming	Relative small houses, built directly on the ground, walls and roof covered with nipa palm leaves. Most of the walls are covered inside with plastic sheeting.	++

++++, 100% of households with one to six animals; +++, around 50% households with two to four animals; ++, around 10% of the households with one to two animals; +, <5% of households with one animal; (+), <5% of households with one animal in July.

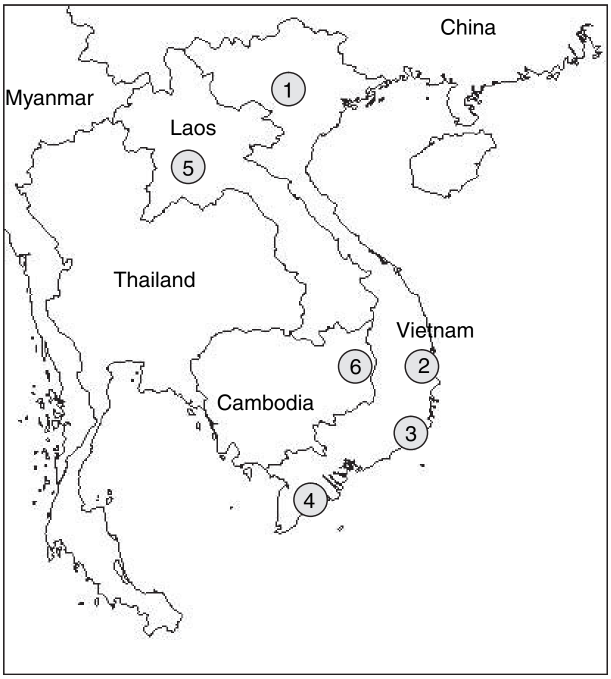

Figure 1 Map with the six study sites (1. Khoi, 2. Lang Nhot, 3. Village 3, 4. Van Duc A, 5. Na Ang, 6. Cha Ong Chan).

human landing rates to outdoor human landing rates. The ratio of the number of mosquitoes collected by indoor morning resting collections to total number of mosquitoes collected by indoor, outdoor human landing and cattle landing collections was used to evaluate the endophilic trend. Confidence intervals of ratios (95%) and comparisons between ratios were calculated and analysed by multiplicative Poisson regression (Egret analysis module version 0.26.6, Statistics and Epidemiology Research Corporation). This software programme was also used to calculate the relative risk to be bitten by an *Anopheles* species during one hour before 22:00 hours compared with one hour after 22:00 hours (indoor and outdoor collections combined and all surveys pooled). For anthropohily, endophagy and biting risks, ratios and relative risks are compared within study sites by assigning them to groups based on similarity ($P > 0.05$).

Results

Anthropophily

The ratio of outdoor human to cattle landing rates for the six study sites are given in Table 2. Almost all *Anopheles* species were attracted to both human and cattle. *Anopheles dirus* A, an efficient malaria vector in Southeast Asia, showed an extremely high preference for humans at all sites where it occurred. By contrast, *An. minimus* A showed clearly spatial variation in anthropophily. In Lang Nhot (Khanh Hoa Province), and Na Ang (Vientiane Province) *An. minimus* A was classified among the most anthropophilic species. Both *An. minimus* A and *An. minimus* C in Khoi (Hoa Binh Province) and *An. minimus* A in Cha Ong Chan (Rattanakiry Province) were more attracted to cattle than to humans, and were less anthropophilic than some non-confirmed vector species. In Van Duc A village (Bac Lieu Province), *An. sundaicus* was predominantly anthropophilic.

Anopheles campestris, *An. sinensis* and *An. nimpe*, three species incriminated as potential vectors in southern coastal areas of Vietnam, were highly anthropophilic in Van Duc A (Bac Lieu Province). In contrast, *An. aconitus* and *An. maculatus*, potential vectors in many parts of forested mountainous areas in Southeast Asia, were relatively zoophilic, except in Na Ang (Vientiane). Generally, the degree of anthropophily was more pronounced among *Anopheles* species in Van Duc A (Bac Lieu) and Na Ang (Vientiane), whereas in Khoi (Hoa Binh) all species were more attracted to cattle than to humans.

Endophagy

At each study site, the major vectors (*An. minimus* s.l., *An. dirus A* and *An. sundaicus*) were among the most endophagic species, except for *An. dirus* A in Village 3 (Binh Thuan Province) (Table 3). However, differences in behaviours between the study sites were found. *Anopheles minimus* A was the most endophagic in Lang Nhot (Khanh Hoa Province) compared with the other sites. *Anopheles dirus* A was also more endophagic in Lang Nhot than in Village 3 (Binh Thuan Province). In Khoi village *An. minimus* C was nearly three times less endophagic than *An. minimus* A. In Van Duc A, four species showed highly endophagic behaviour, especially *An. sundaicus* the main malaria vector and *An. campestris*. The other *Anopheles* species showed relatively low endophagy.

Biting rhythm on humans

Spatial differences in biting rhythm were found in *An. minimus* A and *An. dirus* A. The relative risk of being bitten an hour before 22:00 hours by *An. minimus* A was significantly lower in Lang Nhot (Khanh Hoa Province) than elsewhere; whereas the relative risk of being bitten in an hour before 22:00 hours by *An. dirus* A in Lang Nhot was significantly higher than in Village 3 (Binh Thuan Province) and Cha Ong Chan (Rattanakiry Province). Generally, in Lang Nhot, Na Ang and Village 3 most

Table 2 Rank of anthropophily: ratios of outdoor human (OH)-to-cattle (OC) landing rates of *Anopheles* at six sites in Vietnam, Laos and Cambodia

Sites	Species	Ratio OH/OC	95% Confidence interval	Significant group*
Van Duc A (Bac Lieu, Vietnam)	*An. campestris*	(16/0)		
	An. nimpe	23.07	3.15–165.20	a, b
	An. sundaicus	11.53	10.09–13.18	a
	An. sinensis	4.78	4.46–5.12	b
	An. tessellatus	3.96	2.93–5.34	b
	An. subpictus	0.55	0.51–0.60	c
	An. vagus	(0/42)		
Khoi (Hoa Binh, Vietnam)	*An. splendidus*	0.47	0.36–0.63	a
	An. maculatus	0.31	0.18–0.54	a–c
	An. sinensis	0.25	0.21–0.29	b
	An. kochi	0.17	0.13–0.25	b–d
	An. philippinensis	0.16	0.13–0.22	b–d
	An. annularis	0.15	0.13–0.18	d
	An. jeyporiensis	0.12	0.07–0.19	c–e
	An. minimus A	0.10	0.09–0.12	e
	An. minimus C	0.09	0.08–0.11	e
	An. aconitus	0.09	0.05–0.16	d, e
	An. tessellatus	0.08	0.04–0.20	c–f
	An. vagus	0.02	0.01–0.04	f
Village 3 (Suoi Kiet, Binh Thuan, Vietnam)	*An. dirus A*	35.17	5.09–264.60	a
	An. sinensis	2.83	0.62–12.89	a, b
	An. peditaeniatus	1.51	0.91–2.49	b
	An. tessellatus	0.56	0.21–1.51	b, c
	An. barbirostris	0.38	0.13–1.06	b–d
	An. maculatus	0.25	0.21–0.30	c
	An. splendidus	0.20	0.13–0.29	c, d
	An. pampanai	0.16	0.13–0.23	c, d
	An. vagus	0.13	0.10–0.17	d, e
	An. karwari	0.12	0.05–0.27	c–f
	An. philippinensis	0.10	0.05–0.23	c–f
	An. nivipes	0.09	0.05–0.17	d–f
	An. aconitus	0.07	0.05–0.11	e, f
	An. varuna	0.02	0.01–0.07	f
Lang Nhot (Khanh Hoa, Vietnam)	*An. dirus A*	13.00	1.83–99.35	a
	An. minimus A	8.10	3.28–19.99	a
	An. maculatus	0.12	0.09–0.16	b
	An. peditaeniatus	0.08	0.05–0.13	b
	An. crawfordi	0.06	0.02–0.14	b
	An. philippinensis	0.05	0.01–0.86	b, c
	An. aconitus	0.01	0.00–0.01	c
	An. kochi	(0/23)		
	An. vagus	(0/645)		
Cha Ong Chan (Rattarakiry, Cambodia)	*An. dirus A*	(25/0)		
	An. umbrosus	2.50	0.72–8.64	a–c
	An. barbirostris	1.64	1.01–2.08	a
	An. barbumbrosus	1.10	0.83–2.59	a, b
	An. kochi	0.95	0.68–1.33	a–c
	An. aconitus	0.61	0.46–0.82	c, d
	An. jamesii	0.60	0.39–0.92	b–d
	An. minimus A	0.47	0.23–0.95	b–e
	An. vagus	0.42	0.35–0.50	d
	An. maculatus	0.24	0.19–0.29	e, f
	An. philippinensis	0.16	0.13–0.20	f, g
	An. karwari	0.08	0.05–0.13	g

Table 2 Continued

Sites	Species	Ratio OH/OC	95% Confidence interval	Significant group*
Na Ang (Vientiane, Laos)	*An. dirus A*	(6/0)		
	An. maculatus	(24/0)		
	An. minimus A	10.11	7.96–12.82	a
	An. umbrosus	6.67	2.06–21.55	a, b
	An. aconitus	3.93	2.52–6.13	b
	An. barbirostris	2.64	1.73–4.01	b, c
	An. philippinensis	2.32	1.73–3.11	b, c
	An. hyrcanus	1.53	1.29–1.83	c, d
	An. tessellatus	1.30	0.46–3.65	b–e
	An. sinensis	0.69	0.28–1.71	d–f
	An. nivipes	0.47	0.23–0.95	e, f
	An. kochi	0.46	0.29–0.73	e, f
	An. vagus	0.07	0.02–0.29	f
	An. pallidus	(0/10)		

Figures in brackets: Absolute numbers of mosquitoes collected by outdoor human landing/on cattle at night.
* Within a study site, ratios are not significantly different ($P > 0.05$) between species having common letters.

Anopheles species bite early in the night, i.e. before 22:00 hours, whereas in Van Duc A the risk to be bitten by an *Anopheles* species before and after 22:00 hours was nearly similar (Table 4).

Endophily

Only *An. minimus* A in Khoi (northern Vietnam) exhibited a degree of endophily (ratio 0.67). The remaining species in Khoi and all species in other study villages showed a low endophilic trend (ratios <0.21; Table 5).

Discussion

In Southeast Asia, a large number of *Anopheles* species occur in the domestic environment. Only a few are considered major vectors throughout the region whereas the vector status of other species varies from area to area (Covell 1944). Overall, anopheline density is not suited as first indicator for estimating the risk of malaria transmission, particularly when malaria incidence is low (Trung *et al.* 2004). Behaviour of *Anopheles* species largely determines their status as malaria vectors (Klowden 1996; Costantini *et al.* 1999) and can only be described by evaluating it at a large geographical scale (Van Bortel *et al.* 2004). Insights into the behaviour of different *Anopheles* species and variation between sites were inferred through collection data. Comparable data were obtained by the use of a standard collection protocol at the six study sites. Standard traps can be used for studying mosquito behaviour (Service 1993). However in this study we chose studying behaviour in several ecological settings under different housing conditions, varying human behaviour and cattle abundance and opted for looking at the relative position of a species for an indicator (e.g. endophagy) to that of other anopheline fauna. The degree of anthropophily is usually based on the identification of the blood meal origin of resting mosquitoes, expressing the proportion on feeds on humans. The main constraint of this approach lies in the difficulty of obtaining unbiased samples of resting mosquitoes mainly when they rest outside (Gillies 1988). The present ranking approach based on the proportion of human/cattle biting density is also valid, although limited to only two hosts.

Intraspecific behavioural differences were observed among populations of most *Anopheles* species. However, *An. dirus* A showed a high degree of anthropophily at all study sites where it was found. This is consistent with the fact that this species is the most important vector in Southeast Asia (Scanlon & Sandhinand 1965; Rosenberg & Maheswary 1982). *Anopheles minimus* s.l. has been characterized as anthropophilic (Vien 1974; Harrison 1980). However, in our study, the preference of *An. minimus* A to feed on humans was only found in the study sites of central Vietnam and in Laos, whereas *An. minimus* A from northern Vietnam and Cambodia were more attracted to cattle. One possible reason causing the spatial heterogeneity in host choice of *An. minimus* A is the difference in abundance of cattle between study villages. In the Cambodian site, and especially in the site of northern Vietnam, cattle are abundant and mainly kept under the floors during the night, attracting large proportions of *An. minimus* A. But where cattle were scarce, *An. minimus* A was found to be relatively attracted to humans. The abundance of cattle also affected the host choice of other *Anopheles* species. However, an exception was observed in

Table 3 Rank of endophagy: ratios of indoor (IH)-to-outdoor (OH) human landing rates of *Anopheles* at six sites in Vietnam, Laos and Cambodia

Sites	Species	Ratio IH/OH	95% Confidence interval	Significant group*
VanDuc A (Bac Lieu, Vietnam)	*An. campestris*	1.75	0.85–3.59	a, b
	An. sundaicus	1.27	1.25–1.30	a
	An. barbirostris	1.05	0.75–1.47	a, b
	An. tessellatus	1.02	0.87–1.19	b
	An. sinensis	0.88	0.86–0.91	b
	An. nimpe	0.75	0.49–1.12	b, c
	An. subpictus	0.45	0.39–0.50	c
Khoi (Hoa Binh, Vietnam)	*An. minimus A*	0.93	0.83–1.14	a
	An. minimus C	0.35	0.26–0.56	b
	An. vagus	0.25	0.03–1.99	a–d
	An. aconitus	0.20	0.05–0.86	a–d
	An. jeyporiensis	0.17	0.06–0.46	b–d
	An. tessellatus	0.17	0.02–1.28	a–d
	An. sinensis	0.17	0.12–0.23	c
	An. maculatus	0.12	0.04–0.33	b–d
	An. splendidus	0.12	0.06–0.21	c, d
	An. philippinensis	0.06	0.01–0.24	c, d
	An. annularis	0.05	0.02–0.10	d
	An. kochi	0.03	0.00–0.25	c, d
Village3 (Suoi Kiet, Binh Thuan, Vietnam)	*An. nivipes*	1.00	0.38–2.66	a, b
	An. tessellatus	1.00	0.38–2.66	a, b
	An. vagus	1.00	0.68–1.47	a
	An. sinensis	0.64	0.36–1.69	a, b
	An. philippinensis	0.57	0.19–1.74	a–c
	An. peditaeniatus	0.34	0.19–0.60	b, c
	An. barbirostris	0.33	0.10–1.13	a–c
	An. dirus A	0.32	0.21–0.50	b, c
	An. splendidus	0.25	0.16–0.40	b, c
	An. aconitus	0.21	0.15–0.31	c
	An. pampanai	0.20	0.10–0.41	b, c
	An. maculatus	0.16	0.10–0.25	c
Lang Nhot (Khanh Hoa, Vietnam)	*An. minimus A*	7.95	6.56–10.70	a
	An. tessellatus	1.61	0.67–3.88	b, c
	An. dirus A	1.30	0.98–1.73	b
	An. sinensis	0.84	0.22–3.25	b–d
	An. crawfordi	0.42	0.17–1.02	b–d
	An. peditaeniatus	0.31	0.13–0.73	c, d
	An. aconitus	0.13	0.05–0.32	d
	An. maculatus	0.12	0.05–0.27	d
Cha Ong Chan (Rattanakiry, Cambodia)	*An. dirus A*	0.64	0.29–1.42	a, b
	An. minimus A	0.62	0.37–1.69	a
	An. karwari	0.59	0.22–1.59	a, b
	An. philippinensis	0.49	0.31–0.77	a, b
	An. kochi	0.42	0.26–0.68	a, b
	An. hyrcanus	0.36	0.08–1.64	a, b
	An. vagus	0.35	0.26–0.49	a, b
	An. aconitus	0.34	0.20–0.55	a, b
	An. maculatus	0.19	0.11–0.35	b
	An. barbirostris	0.14	0.06–0.36	b
Na Ang (Vientiane, Laos)	*An. minimus A*	1.35	1.25–1.39	a
	An. dirus A	1.00	0.25–3.93	a–d
	An. aconitus	0.81	0.76–0.87	b
	An. sinensis	0.71	0.23–2.24	a–d
	An. tessellatus	0.60	0.20–1.85	a–d

Table 3 Continued

Sites	Species	Ratio IH/OH	95% Confidence interval	Significant group*
	An. kochi	0.37	0.17–0.83	b–e
	An. hyrcanus	0.37	0.32–0.43	c
	An. umbrosus	0.26	0.12–0.58	c–e
	An. nivipes	0.18	0.07–0.45	c–e
	An. pallidus	0.16	0.08–0.31	d, e
	An. barbirostris	0.13	0.07–0.26	d, e
	An. philippinensis	0.11	0.07–0.18	e
	An. maculatus	0.08	0.01–0.60	c–e

* Within a study site ratios are not significantly different ($P > 0.05$) between species having common letters.

Lang Nhot (central Vietnam) where eight of 11 species exhibited extremely high zoophily although cattle were very scarce. In the study site of the Mekong delta, besides *An. sundaicus*, some other species such as *An. campestris*, *An. nimpe* and *An. sinensis* showed a high trend in anthropophily indicating their potential in malaria transmission. They very likely contribute to maintaining malaria transmission in some coastal brackish foci in the Mekong Delta. This confirms the previous investigations incriminating *An. campestris*, *An. nimpe* and *An. sinensis* as secondary or suspected vectors in the coastal areas of southern Vietnam (Am 1993; Hinh *et al.* 1997). Similarly, the relatively high anthropophily of *An. sinensis* (Village 3 central Vietnam), *An. maculatus*, *An. umbrosus* and *An. aconitus* (Na Ang, Laos), *An. umbrosus* and *An. barbirostris* (Cha Ong Chan, Cambodia), indicates their potential to participate in malaria transmission, although their contribution is not necessary to explain the actual malaria incidence (Trung *et al.* 2004). A number of studies showed that potential malaria vectors often contribute considerably to malaria transmission after environmental changes, which could shift their feeding more towards humans and favour their survival, hence increasing their vectorial status (Amerasinghe *et al.* 1991; Maheswary *et al.* 1992).

Both *An. minimus* A and *An. dirus* A in Lang Nhot village (central Vietnam) were more endophagic than at other study sites. A large difference between the Lang Nhot study site and the other villages is the house construction. In Lang Nhot, most houses are directly built on the ground, largely open, with low and incomplete walls of split bamboo and very large eaves, whereas houses in other study sites are quite closed. The open construction of houses allows anthropophilic mosquitoes to detect attractant stimuli from human hosts easily and to enter the houses for feeding.

The importance of outdoor biting has been emphasized in discussions on the persistence of malaria transmission in certain areas where indoor-insecticide use was well implemented (Elliott 1968). Our study showed that an unprotected inhabitant in Village 3 (central Vietnam) received about three times more *An. dirus* A bites outdoors than indoors. In this village inhabitants often sleep outdoors in hammocks, which are hung on posts around the houses during the first half of the night. In addition, the new introduction of electricity into the village was accompanied by an increase of outdoor activities such as open-air karaoke and video shows. These bring people into greater contact with *An. dirus* A. It may, therefore, be expected that malaria transmission in this village occurs mainly outdoors.

Biting rhythm and resting behaviour of the vectors are two major elements determining the appropriateness of the most commonly used vector control measures, i.e. insecticide-impregnated bed nets and indoor residual spraying. In Lang Nhot (central Vietnam), with open constructions, a large proportion of the bites of *An. dirus* A occurred before 22:00 hours, coinciding with various activities of people inside and outside their houses before bedtime. Consequently, the human–vector contact in this village is high. The use of bed nets, even impregnated with insecticide, may only be expected to prevent about 50% of host-seeking *An. dirus* A in this village, assuming that the normal bedtime of local people is 22:00 hours. *Anopheles minimus* A at this site was a late biter, and therefore more suitable to be controlled by insecticide-impregnated bed nets: about 90% of bites from *An. minimus* A can be prevented by using bed nets. The situation of malaria vectors in Lang Nhot reflects the complexity of malaria in localities where both *An. minimus* s.l. and *An. dirus* s.l. occur, not only because of their complementary transmission of malaria, but also the heterogeneous impact of vector control measures on these species. In other study villages, the biting rhythm of *An. minimus* A and *An. sundaicus* indicated the suitability of bed nets in malaria control. *Anopheles dirus* A was nearly absent in indoor morning resting collections in all study villages showing its extreme exophily. This is coincident with the previous observations conducted in

Table 4 Rank of early biting activity: relative risk (RR) of being bitten in an hour before 22:00 hours compared with an hour after 22:00 hours

Sites	Species	RR	95% Confidence interval	Significant group*
Van Duc A (Bac Lieu, Vietnam)	*An. nimpe*	1.48	1.00–2.17	a
	An. subpictus	1.10	0.99–1.21	a
	An. tessellatus	0.97	0.83–1.14	a
	An. sundaicus	0.80	0.78–0.82	b
	An. sinensis	0.78	0.76–0.80	b
	An. campestris	0.61	0.26–1.42	a–c
	An. barbirostris	0.50	0.33–0.74	c
Khoi (Hoa Binh, Vietnam)	*An. philippinensis*	3.31	2.03–5.38	a, b
	An. tessellatus	3.20	1.05–9.79	a–c
	An. annularis	3.14	2.52–3.90	a
	An. maculatus	3.00	1.86–4.84	a, b
	An. aconitus	1.67	0.72–3.86	a–d
	An. jeyporiensis	1.59	0.92–2.74	a–d
	An. kochi	1.28	0.76–2.16	b–d
	An. sinensis	1.17	0.99–1.39	c
	An. splendidus	1.09	0.82–1.46	c, d
	An. minimus C	1.03	0.78–1.36	c, d
	An. minimus A	0.79	0.67–0.93	d
Village 3 (Suoi Kiet, Binh Thuan, Vietnam)	*An. vagus*	4.13	2.81–6.08	a
	An. splendidus	3.57	2.63–4.85	a, b
	An. barbirostris	3.23	1.34–7.79	a–c
	An. peditaeniatus	3.12	2.06–4.71	a, b
	An. pampanai	2.71	1.81–4.06	a–c
	An. maculatus	2.51	1.96–3.23	a, b
	An. nivipes	2.49	0.98–6.30	a–d
	An. karwari	1.63	0.67–3.92	a–d
	An. sinensis	1.55	0.77–3.11	a–d
	An. aconitus	1.50	1.22–1.85	c
	An. tessellatus	0.99	0.37–2.65	b–d
	An. dirus A	0.79	0.56–1.10	d
Lang Nhot (Khanh Hoa, Vietnam)	*An. peditaeniatus*	4.29	2.27–8.08	a
	An. tessellatus	3.71	1.48–9.31	a
	An. crawfordi	3.67	1.82–7.41	a
	An. aconitus	3.52	2.23–5.55	a
	An. maculatus	3.05	2.07–4.50	a
	An. dirus A	2.23	1.69–2.94	a
	An. minimus A	0.29	0.22–0.38	b
Cha Ong Chan (Rattarakiry, Cambodia)	*An. maculatus*	3.59	2.53–5.09	a
	An. philippinensis	2.18	1.52–3.13	a, b
	An. vagus	1.92	1.53–2.42	b
	An. barbirostris	1.22	0.76–1.95	b, c
	An. kochi	1.00	0.68–1.46	c
	An. minimus A	1.00	0.40–2.66	a–d
	An. aconitus	0.87	0.60–1.28	c, d
	An. jamesii	0.86	0.47–1.57	b–d
	An. karwari	0.75	0.29–1.92	b–d
	An. dirus A	0.20	0.06–0.66	d
	An. umbrosus	0.14	0.02–1.09	c, d
Na Ang (Vientiane, Laos)	*An. umbrosus*	3.36	1.98–5.70	a, b
	An. hyrcanus	3.30	2.93–3.72	a
	An. philippinensis	2.92	2.36–3.61	a, b
	An. maculatus	2.55	1.16–5.61	a–c
	An. barbirostris	2.46	1.79–3.37	a, b
	An. kochi	2.40	1.33–4.34	a–c

Table 4 Continued

Sites	Species	RR	95% Confidence interval	Significant group*
	An. sinensis	2.29	0.83–6.30	a–c
	An. nivipes	2.14	1.29–3.55	a–c
	An. pallidus	1.94	1.38–2.73	b, c
	An. aconitus	1.55	1.45–1.65	c
	An. minimus A	0.51	0.47–0.54	d

* Within a study site, ratios are not significantly different ($P > 0.05$) between species having common letters.

Table 5 Rank of indoor resting: ratio of numbers of mosquitoes collected by indoor morning resting collections (IMR) to indoor (IH), outdoor (OH) human landing collections and collections on cattle (OC) at night combined (all surveys pooled)

Site	Species	Ratio {*IMR*/(*IH* + *OH* + *OC*)}	95% Confidence Interval
Van Duc A (Bac Lieu)	*An. subpictus*	0.15	0.13–0.16
	An. sundaicus	0.10	0.09–0.11
	An. sinensis	0.03	0.02–0.04
	An. nimpe	0.03	0.01–0.03
	An. campestris	(0/30)	
Khoi (Hoa Binh)	*An. minimus* A	0.67	0.61–0.74
	An. minimus C	0.15	0.12–0.18
	An. maculatus	0.02	0.002–0.11
	An. aconitus	(0/100)	
Lang Nhot (Khanh Hoa)	*An. minimus* A	0.08	0.05–0.11
	An. dirus A	0.02	0.003–0.15
	An. maculatus	0.004	0.001–0.02
	An. aconitus	(0/1275)	
Village 3 (Binh Thuan)	*An. minimus* A	(0/4)	
	An. dirus A	(0/73)	
	An. aconitus	(0/222)	
	An. maculatus	(0/531)	
Cha Ong Chan (Rattanakiry)	*An. minimus* A	0.21	0.10–0.45
	An. dirus A	(0/33)	
	An. aconitus	(0/212)	
	An. maculatus	(0/400)	
Na Ang (Vientiane)	*An. minimus* A	0.04	0.03–0.05
	An. aconitus	0.03	0.02–0.04
	An. dirus A	(0/9)	
	An. maculatus	(0/25)	

Thailand (Wilkinson *et al.* 1978) and Vietnam (Phan *et al.* 1973). *Anopheles minimus* s.l. and *An. sundaicus* have been characterized as endophilic in Southeast Asia (Harrison 1980; Hinh *et al.* 1987; Thuan *et al.* 1997; Phan 1998). However, in our study, they were highly exophilic in all study villages where they occurred, except in Khoi village (northern Vietnam), where *An. minimus* A exhibited a certain degree of preference to rest indoors, but not *An. minimus* C. This finding suggests that the conventional application of indoor residual spraying for controlling the vectors in malaria outbreaks may not always give the expected outcome in the interruption of malaria transmission. In the epidemic situation, and in some particular malaria hyper-endemic areas where both *An. dirus* A and *An. minimus* A are present, both house residual spraying and impregnated bed nets may be recommended. However, these vector control measures will have a low impact on *An. dirus* A because of its high exophilic behaviour combined with outdoor feeding and early biting habit. Other personal protection measures should be considered, such as impregnated hammocks.

In conclusion, the spatial variation in anthropophily, endophagy, biting cycle and endophily may result in the differences in the epidemiological importance of *Anopheles* species and in their response to vector control measures in different areas. Moreover, it can be expected that

Anopheles behaviour and related malaria transmission will be influenced by the environmental changes and changes in human practices induced by e.g. the economic development. National Malaria Control programmes in Southeast Asia must deal with this complex situation. Although the actual incidence of malaria can be explained by the transmission of the major vectors in the region, *An. dirus* s.l., *An. minimus* s.l. and *An. sundaicus*, the possible role of the other *Anopheles* species in malaria transmission should be carefully followed-up.

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