Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 87, Suppl. III, 371-383, 1992

ECOLOGY OF MALARIA VECTORS IN THE AMERICAS AND FUTURE DIRECTION

ROBERT H. ZIMMERMAN

Oficina Regional de la Organizacion Mundial de la Salud, Oficina Sanitaria Panamericana, Organizacion Panamericana de la Salud, Apartado 2171. Maracay, Aragua, Venezuela

The resurgence of malaria in the Americas has renewed interest in Anopheles *biology.* Anopheles darlingi, An. albimanus, An. nuneztovai *and* An. aquasalis *are reconfirmed as major malaria vectors and other species are playing important roles in regional malaria transmission. Adult biting activity and larval ecology are discussed in detail. Seasonal abundance and daily biting activity of Anophelines vary considerably among species and geographically for the same species.* Anopheles albimanus *has the least amount of variation in biting activity over its range and* An. darlingi *has the greatest. All species studied are more exophilic and exophagic than endophlic and endophagic.* Anopheles darlingi *is more anthropophilic, endophilic and endophagic than other Anophelines. Larval studies remain more descriptive than comprehensive. Research on Anophelines is becoming more integrated and biologists are using new biochemical techniques and ecological principles to answer critical questions. This "pluralization" will help us understand species complexes, population dynamics and malaria transmission. Integrated control programs will require more regional, in-depth ecological studies.*

Key words: Anophelines – malaria – Americas

Resurging malaria in the Americas is creating new interest in malaria vector biology and control, and causing us to rethink our approach to the study of vectors, their niche and their role in the disease's transmission.

For almost three decades prior to the 1980s, researchers neglected vector biology in the Americas or limited their efforts to specific projects (e.g. Central American Research Project, CAMRS, DC, on sterile male release of *An. albimanus*). This lack of interest can be blamed on the temporary success of insecticides, in particular DDT, the use of chloroquine and the decrease in malaria. We also cannot discount the concurrent shift away from field oriented biology to molecular and cell biology (Wilson, 1989).

However, malaria's comeback is reawakening interest in vector biology and control. Both national and international agencies are contributing to the study of *Anopheles*. New scientific tools such as immunoassay detection of parasites, DNA probes, computer aids and remote sensing are just beginning to find applications here.

These tools, coupled with developments in ecological theory and health care, are creating a new wave in research. More ecological in approach, it crosses among different fields of biology and will give us more viable solutions for control programs.

In this presentation I will focus on the results of this new wave of research, and discuss its significance.

THE VECTOR SPECIES OF MALARIA IN THE AMERICAS

The major vectors of malaria in the Americas have been summarized by Forattini (1962) and Fleming (1986). The taxonomic works of Faran (1980) and Linthicum (1988) summarize the importance of the vectors in the Albimanus and Argyritarsis sections of the subgenus *Nyssorhynchus*. As of 1991 (*Anopheles deaneorum* included) this subgenus includes at least 5 major vectors and 4 or more local or suspected vectors. Deane (1986) has presented an excellent update on the vectors of malaria in Brazil. During and subsequent to these reviews new studies have reinforced the importance of known malaria vectors and have implicated other Anopheline species in specific geographical regions that play major roles in the transmission of malaria (Table I).

TABLE I

Anopheles species recently found positive for *Plasmodium* in the Americas

Location	*Anopheles* sp.	Method used	Author
Chiapas, Mexico	*An. vestitipennis*[1]	ELISA	Loyola et al. (1991)
Chiapas, Mexico	*An. albimanus*[3]	filtration	Ramsey et al. (1986)
Nicaragua	*An. albimanus*[1]	ELISA	PAHO (1991)
Guatemala	*An. albimanus*		PAHO (1991)
Junin, Dept., Peru	*An. trinkae* *An. oswaldoi* *An. pseudopunctipennis* *An. rangeli* *An. nuneztovari* *An.* sp. nr. *fluminensis*	salivary glands	Hayes et al. (1987)
Anodoas, Peru	*Nyssorhynchus*[1]	ELISA	Need (pers. comm.)
Charambirá, Colombia	*An. neivai*[1,2]	IRMA	Carvajal et al. (1989)
Colombia	*An. albimanus*[1]	IRMA	Herrera et al. (1987)
Colombia	*An. albimanus*[2] *An. darlingi* *An. neomaculipalpus* *An. allopha*	IRMA	Herrera et al. (1987)
Bajo Calima, Colombia	*An. nuneztovari*[3]	salivary glands	Fajardo & Alzate (1987)
Caqueta - Putamayo, Colombia	*An. rangeli*[1]	ELISA glands neg.	Suarez et al. (1991)
La Lengueta, Barinas, Venezuela	*An. nuneztovari*[1] *An. albitarsis* *An. oswaldoi*	ELISA	Rubio (1991)
Sucre State Venezuela	*An. aquasalis*[1]	ELISA 1 gland pos.	Caseres (pers. comm.)
Payapal, Bolivar, Venezuela	*An. darlingi*[3]	salivary glands	Berti (pers. comm.)
Pará State, Brazil	*An. darlingi*[2]	IRMA & ELISA	Arruda et al.[a] (1986)
Brazil	*An. darlingi*[1] *An. nuneztovari* *An. albitarsis* *An. triannulatus* *An. oswaldoi*		Arruda et al.[a] (1986)
Rondônia State, Brazil	*An. darlingi*[2] *An. albitarsis* *An. braziliensis* *An. oswaldoi*	IRMA	Oliveira-Ferreira et al. (1990)

Cont.

Location	*Anopheles* sp.	Method used	Author
Rondônia State,	*An. darlingi*[1] *An. triannulatus* *An. strodei* *An. albitarsis* *An. braziliensis*	IRMA	Oliveira-Ferreira et al. (1990)

1 - *Plasmodium vivax*, 2 - *P. falciparum*, 3 - *Plasmodium* sp.
a: salivary glands were found positive for sporozoites, except for *An. oswaldoi.*

United States – When malaria was endemic in the United States the three major vectors were *An. albimanus, An. freeborni* and *An. quadramaculatus*. Now malaria transmission occurs infrequently, but since 1986, 7 outbreaks of vivax malaria have occurred in Southern California. The presumed vector was none of the 3 mentioned above. It was a newly described species, *An. hermsi*. (Hunt et al., 1990).

Mexico & Central America – *Anopheles albimanus* continues to be the major malaria vector in the coastal lowlands of this region with An. *pseudopunctipennis* considered of major importance in the foothills (Rodriguez & Loyola, 1989). Reconfirmation of *An. albimanus* as the coastal vector has recently been made in Mexico (Ramsey et al., 1986), and in Guatemala and Nicaragua (PAHO, 1991). Forattini (1962) mentioned that An. *verstitipennis* was a probable vector in Central America, but only recently has it been implicated by enzyme-linked immunoabsorbent assay (ELISA) as a vector of *Plasmodium vivax* in southern Mexico. It could be the cause of several thousand cases of malaria each year (Loyola et al., 1991). *Anopheles darlingi* is present in a restricted area of Central America, but has not been incriminated directly by sporozoites or immunoassy as a vector. But, in Guatamala it was the only potential vector present during several malaria outbreaks in the early 1970s.

Caribbean – Until 1991, Hispaniola was the only island in the Caribbean with active malaria transmission and the major vector is *An. albimanus*. Recently, Saint-Jean & colleagues discovered *An. pseudopunctipennis* in Haiti, and it too may become involved in malaria transmission (Zimmerman & Ranjel, 1990). For the first time since erradication of malaria in Trinidad in 1965, there has been reported 11 autochthonous cases of vivax malaria in 1991. The presumed vector is *Anopheles aquasalis* (Chadee, pers. comm.).

South America – In Colombia, *An. albimanus, An. nuneztovari* and *An. darlingi* are considered the major malaria vectors (Herrera et al., 1987). Using an immunoradiometric assay (IRMA), Herrera et al. (1987) found *An. albimanus, An. allopha, An. darlingi,* and *An. neomaculipalpus* infected with *P. falciparum* and *An. albimanus* infected with *P. vivax* (Table I). In addition, recent studies have shown that *An. neivai* along the Pacific coast and *An. rangeli* in southern Colombia are suspected vectors (Carvajal et al., 1989; Suarez et al., 1991, respectively). Other potential "bromeliad malaria" vectors in Colombia are *An. boliviensis* and *An. lepidotus* (Astaiza et al., 1989).

Few studies have been done recently along the Pacific coast of South America, and over their range *An. albimanus* and *An. psuedopunctipennis* still appear to be the most important vectors. Forattini (1962) mentioned *An. rangeli* as a vector in Ecuador. In Southcentral Peru, Hayes et al. (1987) have implicated a new species, *An. trinkae* as the primary vector. Other species were also found positive (Table I). Members of the subgenus *Nyssorhynchus* have been incriminated as vectors in the Peruvian Amazon near the Ecuadorian border (J. Need, pers. comm.).

In Bolivia, *An. darlingi* and *An. pseudopunctipennis* are considered the major vectors, but no recent research is available to confirm this.

In Chile, malaria has not been detected for more than 40 years, but Ulloa (1984) alerts us to the great deal of migration of workers from malarious zones of neighboring countries and that *An. psuedopunctipennis* is still abundant in northern Chile.

In Venezuela, the resurgence of malaria mandated a review of the vectors and their role. Both *An. nuneztovari* in western Venezuela and *An. aquasalis* in eastern Venezuela were confirmed as vectors of *P. vivax* by the ELISA technique (Y. Rubio, 1991; R. Casares, pers. comm., respectively). In the study on *An. nuneztovari*, other Anophelines were also ELISA positive (Table I). No mosquitos were dissected to look for sporozoites in western Venezuela, but in eastern Venezuela 400 *An. aquasalis* were dissected and one was found to be positive. Also, in eastern Venezuela *An. psuedopunctipennis* could be playing a major role as a vector during the dry season. No salivary gland dissections nor immunoassays have been made yet on this species. The recent discovery of *P. falciparim* in eastern Venezuela for the first time ever suggests that this species in playing a greater role in malaria transmission than previously thought. *Anopheles darlingi* in southern Venezuela is presently under study and salivary glands have been found positive for sporozoites (Berti, pers. comm.).

In the Guyanas, the main vectors by implication are *An. darlingi* and *An. aquasalis* with possible secondary vectors being *An. nuneztovari* and *An. braziliensis* (Rosendaal, 1990). No recent studies have been made to determine sporozoite rate of these vectors by dissection nor immunoassay.

In Brazil, several research projects have been carried out during the last 8-10 years. This research has reconfirmed *An. darlingi* as the main vector of malaria in inland Brazilian with *An. aquasalis* a coastal vector from northern Brazil to São Paulo (Deane, 1986). The "bromeliad malaria" is caused by *An. cruzi, An. bellator* and *An. homonculus*. Deane (1986) mentioned *An. albitarsis* as a weak vector in restricted areas, but the most widespread species in Brazil. Since the review by Deane a separate species has been named from this "species complex" *An. deaneorum* (Rosa-Freitas, 1989). This species has been implicated as a vector of both *P. falciparum* and *P. vivax* near the Bolivian border in Costa Marques (Klein, pers. comm.). Kline suggested that *An. mediopunctatus* in forested areas, may be a vector of malaria to immigrant populations living in or near the forest. He also said that *An. albitarsis* maybe a seasonal secondary vector of *P. vivax* and its vector potential influenced by its zoophilic tendencies.

In the state of Para, in northern Brazil, Arruda et al. (1986) compared salivary glands, immunoradiometric assay (IRMA), and the ELISA with *Anopheles* captured in the region and concluded that *An. darlingi* was the major vector of *P. falciparum*. The results of their study suggest that in addition to *An. darlingi*, other species may be vectors of *P. vivax* Table I).

In Rodônia State, Brazil, using immunoassay, Oliveira-Ferreira et al. (1990) incriminated *An. darlingi* again as the most important vector in the state. Other species found positive were assumed to play minor roles in the transmission of malaria (Table I).

Recently malaria has become an increasing concern in Paraguay where *An. darlingi* is presumed the major vector (D. Bown, pers. comm.).

Finally, *An. pseudopunctipennis* is implicated as the major vector of malaria in northwestern Argentina due to its permanent presence there. In northeastern Argentina, by its presence and circumstancial evidence, *An. darlingi* is the presumed vector.

BIOLOGY OF THE ANOPHELINES IN THE AMERICAS

Forattini's review (1962) is considered the classic reference on Anophelines in the Americas. All subsequent reviews have drawn heavily from it (Faran, 1980; Faran & Linthicum, 1981; Fleming, 1986;' Fredrickson, 1988; Linthicum, 1988). In this presentation I will mainly concentrate on recent field studies concerning adult biology and larval ecology. Not all current research will be discussed. Therefore, I would like to call attention to the recent reviews on insecticide resistance by Brown (1986) and mosquito nets and curtains by Rozendaal (1988). The research papers presented at the "Simpósio Latinoamericano sobre Biologia y Control de Vectors de Enfermedades Tropicales" demonstrate current research and thought on Anophelines biology in the Americas (Zimmerman & Ranjel, 1990).

In addition, a series of enlightening papers have been presented in relation to vectorial capacity (Dye, 1986), host choice (Kingsolver, 1987; Burkot, 1988), and disease persistence (Hasibeder & Dye, 1988).

Adult Biology – Giglioli (1956) stated quite clearly that "The control of malaria by modern insecticidal techniques rests fundamentally on the biting and resting habits of the Anopheline species responsible for its transmission". Several studies have again emphasized this line of research.

The seasonal abundance of Anophelines is generally considered to be regulated by the presence and absence of breeding sites which is directly related to rainfall. The effect may be immediately apparent or have a time delay (e.g. PAHO, 1991; Fredrickson, 1988 for *An. albimanus;* Rubio, 1991 for *An. nuneztovari, An. albitarsis, An. oswaldoi* and *An. triannulatus;* Berti & Zimmerman, pers. comm., for *An. aquasalis* Rozendaal (1990); Klein & Lima (1990) for *An. darlingi*). The same is also true for *Kertesia* (Astaiza et al., 1988). There are exceptions as is the case for *An. pseudopunctipennis*. This species is in greater abundance when rivers have less water flow and breeding sites are exposed along the edge.

Within a single species, abundance can vary regionally and locally (Charlwood & Hayes, 1978; Hudson, 1984; PAHO, 1991). Klein & Lima (1990) have suggested that geographical variation in adult abundance of *An. darlingi* is affected by seasonal temperatures, rainfall, river level and a combination of natural water impoundments and quality of aquatic vegetation. They positively correlated adult abundance with river level. In the same study other Anophelines appeared to show the same trends. In Suriname, Rozendall (1990) examined the relation between the presence and absence of breeding sites of *An. darlingi* and malaria transmission. He also concluded that fluctuations in river height and rainfall determined the presence or absence and location of breeding sites. This directly affected adult abundance. This dynamic interplay was correlated with malaria transmission. Although not statistically significant, his results are encouraging considering the difficulties encountered in collecting data for each variable.

In another vector species, *An. nuneztovari*, Rozendaal (1990) found an adult peak in the dry season, but in Venezuela Rubio (1991) found this species more abundant during the rainy season. The difference may be related to the type of habitat. In Suriname, the breeding sites of *An. nuneztovari* occur mainly in flooded areas after the rivers have subsided. In Venezuela, however this species is found in flooded pastures in rolling terrain and is not affected by rapid changes in river height which would destroy breeding sites during the rainy season.

Several authors have summarized the biting activity of Anopelines in the Americas (Table II). For *An. darlingi* it seems to be the general conclusion that the variation in biting activity of this species is due to geographical variation or the existence of a species complex. Forattini (1987), in a thoughtful presentation, makes two important observations about *An. darlingi;* 1) when one looks at the studies of at least one year's duration, there are two fundamental biting rhythms for *An. darlingi;* unimodal around midnight and bimodal with a crepuscular rhythm, and 2) that "beyond a doubt" distinct populations do exist (from polymorphism studies), but we are lacking data that relate to behavioral characteristics like biting rhythm with genetic polymorphism.

More recent data from two ongoing studies in the Amazon Region of Venezuela show that *An. darlingi* is bimodal and crepuscular in activity (Berti & Zerpa, pers. comm.). Lourenço de Oliveira et al. (1989) found *An. darlingi* with a unimodal peak in northern Rondonia State, Brazil.

Forattini emphasizes that we do not know what is the real reason for variation in the biting rhythm of *An. darlingi* because our studies have many times been only precise moments in time. We may not see the true variation in biting rhythm or lack of it. This conclusion is equally applicable for other Anophelines. A good example of this is the study in Costa Marques, Brazil by Klein & Lima (1990). If *An. darlingi* were captured only from January to March 1987, one would conclude that nocturnal activity began at 1900 hrs and continued all night with no clear peak, not crepuscular as one concludes after seeing all the data. In addition, the effect of population density on activity pattern has been discussed by Elliott (1972) and in particular for *An. darlingi* by Roberts et al. (1987). They both agree that density causes variation and that behavioral studies may be misinterpreted due to density.

Also, there has been a critical lack of studies relating landscape and habitat with biting activity. Recent unpublished data from Venezuela on *An. aquasalis* clearly demonstrate that this vector can be very efficient at low densities (Berti & Zimmerman, pers, comm.). This is contrary to the reported information that *An. aquasalis* is a vector only when densities are high (Deane, 1986). The apparent reason for this difference in vectorial capacity is the landscape of habitat difference between sites. The study area with low relative density had high parous rates compared to a site with very high relative densities. The high malarious area is at the confluence of two rivers next to the sea, has many springs and is always humid. The breeding sites are close to the houses and there are no large animals to take advantage of this species, reported zoophilic behavior. The area with low malaria transmission was drier, major breeding sites were greater than 5 km away and large animals were present. In this case the landscape and habitat use dictated vector capacity, not relative density.

Tadei et al. (1988) studied the biology of *Anopheles* in Ariquemes, Rondônia, Brazil and concluded that the town structure, and breeding site location influenced the number and diversity of *Anopheles* species collected as well as malaria cases.

Other species also show geographical variation in nocturnal biting behavior (Table II) and if one could critically evaluate past data for other species one may see variation due to habitat, location, density and collection methods.

The recent multinational study in Central America on *An. albimanus* had as one of its priorities the observations of the biting activity of this species (intra and domiciliary), its resting behavior and its human blood index (PAHO, 1991). The results were surprisingly similar over the entire area studied. If we include the recent work of Bown and colleagues from Mexico (Fredrickson, 1988; Bown et al., 1991; Rodriguez et al., 1991) we can say that this species is more exophagic than endophagic, an opportunistic feeder and shows greater exophily than endophily.

There are a few studies presently underway on *An. psuedopunctipennis*, but it is too early to mention results. However, in relation to exophily and exophagy, Loyola et al. (1990) showed that insecticide type influenced the number of mosquitos resting inside as well as season of the year.

Anopheles vestitipennis was collected slightly more inside than outside in Mexico (n = 40-59, 54.4%) (Loyola et al., 1991) and more outside than inside (n = 405. 88%) by Mekuria et al. (1990) in the Dominican Republic. More data is necessary in order to conclude more about its endophily or anthropophily.

In Colombia, *An. neivai* was much more exophagic than endophagic (Astaiza et al., 1988).

For the vector species *An. nuneztovari*, and *An. aquasalis*, data from Venezuela show that they are exophilic, more exophagic than endophagic, and readily come to man or animal (Rubio, 1991 and Berti & Zimmerman, pers. comm.). Choice experiments during the dry season for *An. aquasalis* showed slightly greater preference for burro compared to man. Rubio (1991) showed that *An. nuneztovari* had a human blood index resting outdoors between 18.2 and 38.9.

When examining resting and biting behavior for *An. darlingi* we see more variation than in other species. In the 1930s and 1940s this species had been said to be endophilic, endophagic, and anthropophilic (Fleming, 1986), but recently it has been found to be more exophagic, exophilic and zoophilic (Rachou, 1958; Roberts et al. 1987). Klein & Lima (1990) demonstrated its exophagic behavior using two houses, one where 82% of the *An. darlingi* were collected from human bait outside and the other where 70% were collected outside. But it was definitely more endophagic than other species collected, except, *An. deaneorum*. *Anopheles darlingi* is generally collected closer to houses than in the forest when compared to other species collected in the same studies (Roberts et al, 1987; Deane et al., 1988; Klein & Lima, 1990). Again there was an exception in the study of Arruda et al. (1986). The flight range of *An. darlingi* is also greater than other sylvatic Anophelines and this would increase its vector potential (Charlwood & Alecrim, 1989).

We do not know if *An. darlingi* adapted its biting behavior from endophily to exophily due to insecticides, the presence of man, or other

TABLE II

Recent research on the biting activity of *Anopheles* in the Americas

Location/Author	Biting activity	Observation
	An. darlingi	
Aseli Kamp & Apoma Tapoe, Suriname (Hudson, 1984)	unimodal 2100-2300	no daytime biting observed
Upper-Marowijne River, Suriname (Rozendaal, 1990)	unimodal 2300-0130	bites all night Upper-Suriname River with low density 73% caught between 1830-2030
Trou-Poissons, French Guyana (Pajot et al., 1979)[a]	trimodal 1800-1900 0100-0200 0700-0800	bites all night and during the day. peaks are not abrupt
Dourado, São Paulo Brazil (Forattini, 1987)	bimodal 1700-1900 0500-0700	bites all night and during the day
Ituxi River, Amazonas, Brazil (Roberts et al., 1987)	bimodal 1830-2100 - major peak 0600 - minor peak	bites all night and some early day biting single walled house 4 walled inside unimodal peak
Northern Rondônia, Brazil (Lourenço de Oliveira et al., 1989)	unimodal 1800-2400	bites all night, but very seldon in day
Ariquemes, Rondônia, Brazil (Tadei et al., 1988)	bimodal 1800-2200 - major peak 0400-0600 - minor peak	bites all night
Costa Marques, Rondônia, Brazil (Klein & Lima, 1990)	bimodal 1800-2100 - major peak 0500-0700 - minor peak	bites all night
Aripa & Payapal, Bolivar, Venezuela (Berti & Zerpa, pers. comm.)	bimodal 1800-2000 0500-0600	preliminary data
	An. darlingi	
La Ceiba, Atlantida, Honduras (Rivera & Nelson in Zimmerman & Rangel, 1990)	unimodal 1800-2300	bites all night
	An. deaneorum	
Costa Marques, Rondônia, Brazil (Klein & Lima, 1990)	bimodal 1800-1900 - major peak 0500-0600 - minor peak	bites all night

Cont.

Location/Author	Biting activity	Observation
	An. albimanus	
Northern Haiti (Hobbs et al., 1986)	2000- peak	started at 2000 bites all night with gradual decline
Central America (PAHO, 1991)	unimodal 1800-2100	bites all night with gradual decline Guatemala had minor peak at 0100-0400
Clarines, Anzoategui, Venezuela (Barrera, pers. comm.)	unimodal 2400 - peak outside 2000 - peak inside	bites all night with gradual decline until 0800
Dajabon, Dominican Republic (Mekuria et al., 1990)	bimodal? 2100 - major peak	gradual deline until 0600
	An. nuneztovari	
Bajo Calima, Valle del Cauca, Colombia (Fajardo & Alzate, 1987)	unimodal 2130 - peak inside 2030 - peak outside	bites all night with gradual decline until 0600. Outside coll. until midnight
La Lengueta de Barinas, Barinas, Venezuela (Rubio, 1991)	unimodal 2330	bites all night gradual rise and fall in biting activity
Upper-Marowijne River, Upper Suriname River, Suriname (Rozendaal, 1990)	unimodal 1830-2030	bites all night peak abrupt
Brownsweg, Suriname (Panday, 1977)[a]	unimodal 1900 - 1900	bites some all night peak abrupt
	An. triannulatus	
La Lengueta de Barinas, Barinas, Venezuela (Rubio, 1991)	unimodal 1900	abrupt peak with some biting all night
Ariquemes, Rondônia, Brazil (Tadei et al., 1988)	unimodal 1800 - 1900	some biting during the night, numbers low
	An. albitarsis	
La Lengueta de Barinas, Barinas, Venezuela (Rubio, 1991)	unimodal 1900 - 2200	bites all night with gradual decline to 0700

Cont.

Location/Author	Biting activity	Observation
Dourado, São Paulo Brazil (Forattini, 1987)	bimodal	bites all night, low numbers, apparently crepuscular
Costa Marques, Rondônia, Brazil (Klein & Lima, 1990)	bimodal (?)	bites all night, low numbers coll.
	An. oswaldoi	
La Lengueta de Barinas, Barinas, Venezuela (Rubio, 1991)	unimodal 1900 - outside 2330 - inside	bites all nigth
Upper Marowijne river, Upper Suriname River, Suriname Rozendaal (1990)	unimodal 1830 - 2030	bites all night
	An. aquasalis	
Santa Fe & Guyana, Sucre State, Venezuela, Berti & Zimmerman, pers. comm.)	bimodal 1830 - 2230 - major peak 0500 - 0630 - minor peak	bites all night
	An. neivai	
Charambira, Colombia (Astaiza et al., 1988)	bimodal (outside) 1800 - 1900 - major peak 0600 - 0700 - minor peak	bites all night not during day low activity indoors
	An. vestitipennis	
Dajabon, Dominican Republic (Mekuria et al., 1990)	unimodal 2000 - 2300 - outiside 2000 - 2100 - inside	gradual decline until 0600

a: included for comparison.

evolutionary reasons, or if it is ecological variation. But there is no doubt that it is more endophilic, endophilic, endophagic and anthropophilic than any other *Anopheles* in the Americas.

The implications that exophily and exophagy have for control of malaria are many. The recent studies concerning indoor resting behavior are expressions of this concern (Bown et al., 1986; Quinones & Suarez, 1990; PAHO, 1991). Also, several insecticides are presently being selected for their vapor fumigant effect, and spraying may become more important outside than inside houses. Without a doubt the exophily and exophagy of present day vectors makes it imperative that more research and control projects involve the use of personal protection.

Larval Biology – Again we return to Forattini (1962) for a guide to larval characteristics of Anopheline larvae. Most research on larvae has concentrated on surveys of larval presence and absence, and general characterization of habitats. These data have been summarized by several authors (Faran, 1980; Fredrickson, 1988; Linthicum, 1988). Until recently the most complete research was done on *An. aquasalis* by Senior White (1951) and by Andrade & colleagues (Andrade, 1958). More recently Scorza & colleagues published a series of papers associating *An. nuneztovari* larvae with abiotic and biotic factors and finalized this research with a study on population ecology and recommendations for control (Scorza et al., 1981). In Colombia, Murillo et al. (1988) studied the population dynamics and distribution of *An. neivai.* They stratified the vegetation according to foliage cover from the mangrove area to the village and concluded that vegetation structure and height of bromeliads determined abundance along with amount of water in the bromeliads. Larval mortality was high with only 23.7% surviving to the fourth instar.

Rozendaal (1990) characterized the larval abundance of *Anopheles* by site and season in Suriname. He went on and presented an explanation for the seasonal dynamics of larvae in relation to river level and rainfall. Breeding sites were classified into 4 types; small shaded forest streams, deep water mats of water hyacinth and water fern at river edges, pools in or near river beds, and flooded forest. The first 3 disappeared when the rains came, but as the dry season approached they again became important. He concluded that time and abundance of breeding at these sites, as the dry season continued and sites dry up, depend on the local geomorphology; therefore, influencing the adult abundance and possibly the malaria transmission. This again emphasizes the importance of landscape in vector population dynamics.

To my knowledege at present in the Americas there are two projects which are looking more closely at larval ecology of Anophelines to better understand the influence of abiotic and biotic factors on the dynamics of larval populations over time and space. They are in Venezuela on *An. nuneztovari* (Scorza, pers. comm.) and in Mexico on *An. albimanus* (Bown, pers. comm.). Both studies take as their premise that habitat structure determines the presence or absence of a species and this structure can be ecologically mapped (Gabinaud, 1987). During the first year of the *An. nuneztovari* project it was found that in the premontane area, geomorphology determined presence and duration of breeding sites by directly affecting water drainage and flow.

The Mexico project is using remote sensing and ground truthing to characterize breeding sites. This project evolved from a recent study, on *An. freeborni* in California rice fields where it was determined that rice fields that matured early had higher mosquito populations than those that matured later. One could predict fields of high population densities more than 2 months beforehand using remote sensing. In Mexico the initial stages have classified breeding sites of *Anopheles* and related these sites to abiotic and biotic factors. Preliminary results show positive association between *An. albimanus,* altitude, and planktonic algae (sunlit, productive pools). *Anopheles, psuedopunctipennis* was correlated with altitude, *Heteranthera,* and filamentous algae (shallow floodplain pools and along rivers). Further analysis is being made to predict the presence of breeding sites. It remains to be seen if these methods will benefit regional control programs.

A field which has recently become a major area of research in Latin America is biological control of *Anopheles* and its role in an integrated control program. Several small scale projects have been conducted in Latin America (Zimmerman & Ranjel, 1990). The main emphasis is placed on testing presently available products and searching for new strains of biological control agents in the Americas. New strains have been found and a network among interested institutions is being formed to conduct more research in this field. This will mandate a parallel commitment to the study of the ecology of Anopheline larvae and their relation to the aquatic environment. For example, after 3 years of research on the ecology of immature and mature *Anopheles* in eastern Venezuela it was determined that there were areas of high vivax malaria transmission where indoor house spraying was not working (the vector *An. aquasalis* is exophilic and exophagic). The immature breeding sites were defined by location and season and they were accessible. Based on well-defined ecological research, indoor house spraying was suspended, and larval sites treated with *Bacillus thurengiensis israelensis.* Focal outdoor ULV spraying is used when warranted. The field persistence of BTI against *An. aquasalis* is 10 days and along with the development time of immatures (7 days) there is a window of 2 weeks between application. We anxiously await the results.

It is hoped that with all the present interest and research on biological control, new formulations of BTI will be made to persist longer in the environment, and other pathological strains or agents will become available.

Taxonomy – Though I will not discuss taxonomy *per se* in this paper there are several new biochemical methods being used for identifying Anophelines including cuticular hydrocarbons, isoenzymes (Rosa-Freitas, 1989) and DNA probes (Conn, pers. comm.). These techniques have opened new avenues of research and new questions are being asked concerning species identification and evolution of *Anopheles.*

DISCUSSION ON RECENT RESEARCH AND FUTURE DIRECTION

I have mainly discussed two important components of *Anopheles* biology, biting activity over time and space, and larval population dynamics. From the control perspective these are the most crucial topics as we continue to use adulticiding to control malaria vectors and use larvacides in integrated control programs.

The majority of the recent research projects use immunoassay tests, not salivary gland dissection, to determine the presence of sporozoites. The main reason for this change is the ease with which the tests can be performed when one is dealing with a malaria vector shown to have a low infection rate. Also, one can determine the *Plasmodium* species. The main argument against its use is that a positive test for sporozoites does not mean that salivary glands are infected. Comparative studies are presently recommended. This cautious approach is partially due to the fact that several other Anopheline species have been found infected and until we understand their role in malaria transmission we may be implicating vector species which are not vectors. In addition, the discovery of a second strain of vivax malaria (Rosenberg et al., 1989) means that an immunoassay for this strain need also be included on the immunoassay test.

In several of the above studies what is striking is the number of different species of Culicidae collected biting man and animals and those collected in larval studies. Our knowledge of community structure and diversity and how it relates to Anopheline biology is close to zero. Is a species that is abundant (or rare) now, rare (or abundant) later? What effect does human disturbance have on diversity, community structure and malaria vectors?

To answer these questions a new ecological approach needs to be taken. Anopheline biologists need to examine their research objectives and results from an ecological perspective. We need to be aware of new concepts in ecology such as the theory of environment proposed by Andrewartha & Birch (1984) and landscape ecology which relates pattern and process. We need to be creative when using remote sensing and geographical information systems as we work with other scientists involved in the field of tropical diseases.

Wilson (1989) in a stimulating article predicts a "pluralization of biology". His premise is that there will be a return of the expert naturalist to a position of leadership in biological research. There will be a shift from horizontal research as in level of organization analysis (e.g. molecular, cell, or ecosystem biology) to a more 45-degree tilt where biologists cross from molecular to population levels and use the tools of each to study groups of organisms. The biologist will be at ease whether discussing DNA probes or community structure.

His prediction is already occurring in the field of *Anopheles* biology. The coming of pluralization can be seen in systematics with the comparison of morphological, biochemical, chromosomal, and behavior characteristics of species (Rosa-Freita, et al., 1990; Rubio, 1991), and in research on control methodologies which use new experimental techniques and take into account the biology of the species (Bown et al., 1991). Klein and colleagues studied biting rhythm, vector competency, established laboratory colonies and ran hybridization studies as well as collected data on non-anopheline Culicidae in order to better understand the local vectors. In addition, there is a return to research in the field of landscape epidemiology and in the new field of landscape-ecology (e.g. remote sensing projects, California, Mexico and larval ecology research in Venezuela).

This pluralization approach must be expanded to include other parameters related to the ecology of malaria transmission (e.g. epidemiology, sociology). This amplification and the results it yields will be the basis for the design of future control programs in the Americas.

ACKNOWLEDGEMENTS

To D. Bown, and M. Nelson of the Pan American Health Organization for their help in clarifying information on regional vectors. To R. Barrera, J. Berti, J. Conn, F. Lopez-Antunano, C. Machado-Allison, J. Need, T. Klein, M. Klowden, M. Quinones, Y. Rangel, D. Roberts, Y. Rubio, and the participants of the I. Simposio Latinoamericano sobre biologia y control de vectores de enfermedades tropicales, Mexico, 1989, for providing recent research results for this review. To P. Lounibos who brought to my attention the concept of pluralization in biology.

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