LABORATORY OVIPOSITION, FECUNDITY AND EGG HATCHING ABILITY OF COLONIZED *ANOPHELES ALBIMANUS* FROM SOUTHWESTERN MEXICO[1]

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ABSTRACT. Fecundity, oviposition patterns and egg hatching characteristics were studied in two colonies of *Anopheles albimanus* isolated from the Pacific coast of southern Mexico. Fecundity was inversely proportional to the cage space available to the female and was influenced by the bloodmeal source, feeding method and previous feeding history. The length of the gonotrophic cycle decreased with succeeding experience from a mean 6.6 in the first to 2.6 days for the fifth cycle. Oviposition timing was also dependent on availability of oviposition substrate. Hatching success of eggs increased significantly when the oviposition site was witheld until 48 hr post-bloodmeal.

INTRODUCTION

Anopheles albimanus Wiedemann is a major vector of malaria on the Pacific Coast of Central America from Mexico in the north to Colombia in South America. Isolates of the species from Colombia (Carillo et al. 1981), Panama (Collins et al. 1977) and El Salvador (Breeland et al. 1970) have been colonized and studied in an effect to elucidate life cycle characteristics, population dynamics and to test potential control measures. Variations have been observed in pupal (Warren et al. 1977) and adult behavioral characteristics (Bown, unpublished data) despite the lack of chromosomal polymorphism (Hobbs 1962) and failure to find any evidence of sibling speciation. These variations may reflect the extensive range of the species and may exert a considerable influence on its vectorial capacity with respect to malaria transmission.

Two populations of *An. albimanus* have been colonized from the Suchiate region of the State of Chiapas, southern Mexico. The first colony was isolated from animal bait collections in 1983 and is designated CIP/AnA1. The second was colonized from human bait-collected mosquitoes in 1986 and is designated CIP/AnA2. We describe here details of the gonotrophic cycle, fecundity and rates of egg hatching of both colonies, as well as rearing procedures different from those used with other isolates of the species. These observations are discussed in relation to other geographic isolates and the population's vectorial capacity.

MATERIALS AND METHODS

Colony maintenance procedures. Adult mosquitoes of stock colonies were maintained on a diet of 10% sucrose solution in 90 cm^3 cages, at 25°C, with 60–90% variable humidity. Approximately 11,000 mosquitoes were maintained in these cages by a weekly addition of 600 males and 600 females. Females were allowed to take a bloodmeal from rabbits once every 7 days. Oviposition sites consisted of 10 cm diam glass bowls with 3 cm water depth and lined with white filter paper (covering sides and bottom). Eggs were recovered at 24 hr intervals by filtration of the oviposition bowl contents using a funnel and vacuum pump. The eggs were washed with fresh water to remove excess debris and/or microbial contamination, then transferred for hatching as batches of 1,000–2,000 in similar bowls with filter paper along the sides extending above the air interface in order to maintain a moist substrate for stranded eggs. At 24 hr intervals following initiation of hatch, new larvae were singly transferred and counted to determine the hatching success rate. After the hatching period, remaining eggs were collected by filtration and the numbers unhatched were counted. The numbers of new larvae were counted over the hatching period as they were transferred to rearing pans (34 cm wide × 41 cm long × 4 cm deep) and immediately fed with finely ground poult chow (Alpesur) as a surface dust. First and second instars were fed daily with approximately 38 mg/pan; older larvae were fed with approximately 118 mg/pan. Photoperiodicity of the insectary was 11 hr light and 13 hr darkness.

Oviposition methodology. Fecundity, oviposition dynamics and egg hatching success were assessed by placing oviposition bowls in colony cages either 24 or 48 hr following a bloodmeal. Bloodmeals were offered to mosquitoes between 1000 and 1200 hr on the day of feeding. Bowls were replaced daily in the morning and eggs counted for the next 7 days.

The number of eggs oviposited per mosquito was measured using individual females main-

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tained in two types of cages. AnA2 females were given either one human or one rabbit bloodmeal using an artificial feeder or the natural bait. The mosquitoes were maintained individually in 50 cm^3 vials containing water for oviposition following isolation from males and other females 48 hrs. post-bloodmeal (4 days post-emergence). The time required to complete the gonotrophic cycle and oviposit, and the number of eggs oviposited per female were recorded for individual mosquitoes.

Using a second method, 50 to 100 female mosquitoes (with the same number of males) were allowed to emerge directly into cages providing approximately 135 cm^3/mosquito. Females were allowed their first bloodmeal 2 to 4 days following emergence and subsequent meals at weekly intervals. AnA1 mosquitoes were fed rabbit blood, while AnA2 females were given either only human or only rabbit blood from the host on each occasion. Following the bloodmeal, any unfed females were removed from the cages and discarded. Female insemination rates were measured by dissecting spermathecae beginning 2 days following emergence until 9 days of age (or the time of the second bloodmeal). The resulting insemination rate was used to correct for the number of eggs oviposited per female.

Experimental egg treatment. For comparison of egg viability after exposure to drying conditions, batches of eggs were: a) left on wet filter paper in a humid chamber at 10°C, b) dried on filter paper with a cool air flow then set to hatch or, c) dried on filter paper and maintained in plastic eppendorf tubes in bulk at 10°C. Control eggs were washed, and transferred immediately to hatching bowls containing only filtered tap water. After 1, 2 or 3 days, aliquots of dried eggs were allowed to hatch in bowls at 25 or 30°C.

For hatching success experiments, both experimental and control eggs were floated on the surface of either filtered water or water containing a 1% (w/v) solution of feed suspension. Hatching bowls were then placed for an initial 24 hour period at either 25 or 30°C constant temperature, followed by either 25 or 30°C for an additional 72 hour period to determine the effect of higher temperature on hatch success rate.

RESULTS

Individual females from the AnA2 colony studied for their first gonotrophic cycle were maintained following bloodmeal and insemination in 50 cm^3 cages. The portion of females successfully ovipositing following the first single bloodmeal, eggs oviposited/female, and the mean number of days for oviposition are reported in Table 1. Significantly fewer females succeeded in producing eggs and ovipositing if the human blood was offered artificially rather than from the natural host. These females oviposited, however, similar numbers of eggs and the feed to oviposition period was the same between the two. When mosquitoes were given a natural rabbit bloodmeal, oviposition success was similar to that of females given a natural human bloodmeal. Egg production by these females was significantly higher as compared to the numbers of eggs produced from a natural human bloodmeal.

Table 1. Effect of blood type and feeding method on oviposition of single *Anopheles albimanus* females in their first gonotrophic cycle. The artificial feed was given by a blood feeder, while the natural directly from the host.

Type of bloodmeal	Percent females ovipositing	Eggs oviposited/ female ± SD	GMD*
Human-artificial (n = 17)	49	36 ± 22	6.6
Human-natural (n = 98)	98	54 ± 18	6.7
Rabbit-natural (n = 112)	89	83 ± 23	5.9

* GMD Geometric mean days for oviposition.

Groups of 100 females were maintained along with equal numbers of males following emergence in cages providing more space per mosquito than the previous (135 cm^3/mosquito). Following an initial bloodmeal between 2 and 4 days post-emergence, 60% of the females had been naturally inseminated. This rate rose to 90% by 4 days post-bloodmeal. Mean numbers of eggs oviposited per fertilized bloodfed female and the geometric mean number of days until peak oviposition are reported in Table 2. Data reported for all ovipositions have been corrected for 60% insemination 24 hr following the first bloodmeal, and 90% insemination for all succeeding ovipositions. Quadruplicate batches of AnA1 mosquitoes were fed on rabbit blood at weekly intervals for up to 4 gonotrophic cycles. These mosquitoes oviposited a mean of 28 eggs/female for all 4 feeds combined. Highest fecundity per female (34 eggs/mosquito) was observed in the first gonotrophic cycle. The geometric mean peak oviposition occurred at 6.0, 3.9, 4.1 and 2.6 days postfeed for gonotrophic cycles 1–4, respectively.

One group of AnA2 mosquitoes was given rabbit blood for 5 consecutive gonotrophic cycles. These mosquitoes produced an overall mean of 23 eggs/female. The highest egg production per female occurred after the second gonotrophic cycle (34 eggs/mosquito). The periods for peak oviposition fluctuated between 3.7

Table 2. Mean numbers of eggs per female and geometric mean numbers of days (GMD) for peak oviposition of successive gonotrophic cycles from AnA1 and AnA2 *Anopheles albimanus* with either human or rabbit blood.

	AnA2 colony				AnA1 colony	
	Human blood		Rabbit blood		Rabbit blood	
Gonotrophic cycle	Eggs/female (1)	GMD	Eggs/female (2)	GMD	Eggs/female (3)	GMD
1	14 ± 10	6.4	26	ND	34 ± 8	6.0
2	19 ± 15	4.5	34	4.8	26 ± 15	3.9
3	19 ± 3	3.2	19	3.7	23 ± 1	4.1
4	ND	ND	23	5.4	24 ± 7	2.6
5	ND	ND	14	3.9	ND	ND
Combined mean	17 ± 9	4.7	23	4.7	28 ± 4	4.8

ND = Not done.
(1): mean ± SD 3 replicates; (2): 1 exp.; (3): mean ± SD 4 replicates.

and 5.4 days, with an overall mean of 4.7 days. Triplicate batches of AnA2 mosquitoes fed on human blood for 3 consecutive gonotrophic cycles produced an average of 17 eggs/female. Again, highest egg production occurred following the second cycle (19 eggs/female). A consistent decrease of mean peak oviposition time (5.4, 4.5 and 3.2 days, respectively) occurred from the first to the third gonotrophic cycle. Overall mean oviposition occurred at 4.7 days post-bloodmeal, equivalent to the other groups fed on rabbit blood. The difference in numbers of eggs oviposited per fertile female between human and rabbit blood was significant ($P = 0.03$).

Figure 1 summarizes oviposition at successive time intervals post-bloodmeal from the stock colonies. When oviposition bowls were provided at 24 hours post-bloodfeed (24/AnA1 and 24/AnA2), peak oviposition occurred at 72–96 hr. However, when the bowls were placed in cages at 48 hr, there was a significant shift by the AnA1 colony to peak oviposition at 48–72 hr, whereas the peak for AnA2 remained at 72–96 hr.

Mosquitoes from the colony breeding cages (providing approximately 365 cm^3/mosquito) were given a rabbit bloodmeal and oviposition bowls provided 24 hours later. When these wet eggs were left to hatch on filtered water at 25°C, 60.9% and 63.9% hatched from AnA1 and AnA2 colonies, respectively (Table 3). However, when the eggs from AnA1 were allowed to hatch on a feed suspension, or when the eggs were dried and then set to hatch, only 27.2% and 51.3%, respectively, hatched. Only 52.5% of eggs from AnA1 and 56.9% from AnA2 hatched if they were dried and stored for 2 days at 10°C.

Hatching rates increased by 13.6–18.5% when eggs from both colonies were placed on filtered water at 30°C for 24 hours as opposed to those left to hatch at 25°C for up to 72 hr incubation

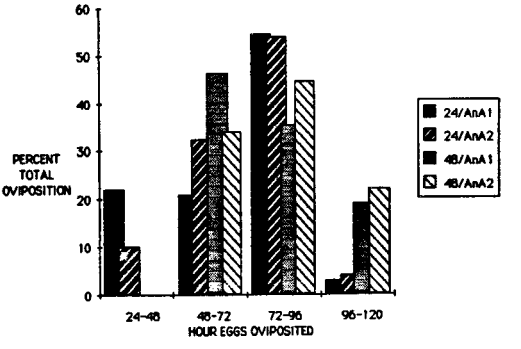

Fig. 1. Oviposition dynamics of AnA1 and AnA2 *Anopheles albimanus* colonies when oviposition bowls were provided at 24 or 48 hr post-bloodfeed.

period (Table 3). Dried eggs showed only slightly increased hatch capacity (1.8–4.1%) when treated in the same procedures at 30°C. Eggs oviposited at different intervals of time post-bloodfeed demonstrated differential hatching characteristics (Table 4). Although eggs oviposited at 72–96 hr had a higher hatch capacity when placed wet as opposed to dried for 2–4 days, those oviposited at 48–72 hr had significantly lower hatch capacity when left wet as opposed to being maintained dry for the same period prior to hatching. In all but one case, AnA2 eggs were less viable than AnA1 eggs (Table 4). When eggs from both oviposition intervals were dried for 2, 3, or 4 days, there was a progressive decline in hatching capacity with the storage period after drying.

Since egg hatch rates were significantly lower than reported from other colonies of the same species under similar conditions, an attempt was made to increase egg viability by making females retain them for a longer period prior to oviposition. Egg hatching dynamics for the two colo-

Table 3. Effect of initial incubation temperature and drying on percent hatching of eggs oviposited at 96 hr, when bowls were provided at 24 hr post-bloodmeal (weighted mean ± SD of 4 replicates except * one test).

Treatment prehatch	Hatching substrate	25°C, 72 hr		30°C, 24 hr then 25°C, 48 hr	
		AnA1	AnA2	AnA1	AnA2
None	Filtered water	60.9 ± 0.7%	63.9 ± 14.4%	80.8 ± 18.3%	82.3 ± 17.4%
None	Feed suspension	27.2 ± 4.9%	43.2 ± 12.3%	ND	ND
Dry 2 hr	Filtered water	51.3 ± 13.3%	55.5 ± 8.5%	ND	46.1%*
Dry 2 days	Filtered water	52.5 ± 4.3%	56.9 ± 2.3%	54.1 ± 5.5%	61.1 ± 6.7%

ND = Not done.

Table 4. Effect on percent egg hatching of drying and storing eggs at 10°C on AnA1 (4 replicates except * one test) and AnA2 (6 replicates) colonies, oviposited 48–96 hr post-feed; Oviposition bowls provided at 24 hr post-feed and eggs incubated for 24 hr at 30°C. (Weighted mean ± SD).

	Post-bloodmeal oviposition period (hr)			
	48–72		72–96	
Treatment	AnA1	AnA2	AnA1	AnA2
Humid	44.6 ± 6.7%	42.4 ± 7.7%	79.5 ± 15.2%	78.9 ± 15.3%
Dry 2 days	70.5 ± 16.3%	66.9 ± 8.9%	54.1 ± 5.5%	61.1 ± 6.7%
Dry 3 days	64.4%*	50.3 ± 17.5%	52.9%*	42.7 ± 8.7%
Dry 4 days	41.4%*	28.6 ± 9.1%	49.7%*	27.4 ± 5.3%

Table 5. Daily percent hatch of eggs oviposited 72–96 hr in bowls provided at 24 (2 replicates) or 48 hr (4 replicates) incubated wet at 25 or 30°C for the first 24 hr, followed by 25°C for a succeeding 48 hours.

	AnA1				AnA2			
Hatch time	24/25°C	48/25°C	24/30°C	48/30°C	24/25°C	48/25°C	24/30°C	48/30°C
0–24 hr	0.0%	0.0%	57.3%	73.9%	0.0%	0.0%	25.9%	80.1%
24–48 hr	53.1%	73.0%	17.3%	22.0%	27.3%	67.8%	41.2%	13.2%
48–72 hr	1.8%	1.0%	0.9%	0.1%	21.4%	1.1%	0.8%	0.7%
72–96 hr	0.4%	0.0%	1.0%	0.0%	1.1%	0.0%	0.0%	0.0%
Total	55.3%	74.0%	76.5%	96.0%	49.8%	68.9%	67.9%	94.0%

nies were compared when egg bowls were first provided 24 or 48 hr post-bloodfeed, and when wet eggs were incubated on water at 25 or 30°C for the first 24 hr followed by 25°C until 72 hr (Table 5). When eggs from both colonies were left at 25°C, no hatching took place within 24 hr, whereas 25.9–80.1% of eggs hatched within 24 hr when incubated at 30°C. The overall hatching capacity of AnA1 eggs was lower at 25°C than at 30°C (54.9% as opposed to 75.5% when bowls were provided at 24 hr). Whether eggs were allowed to hatch at 25 or 30°C, hatch success was higher by 19.1–26.1% for both colonies when the egg bowls were provided at 48 hr post-bloodfeed.

The dynamics of egg hatching differed according to whether bowls were provided 24 or 48 hr postfeed (Fig. 2). At 25°C, when the bowls were provided at 48 hr, almost 98% eclosion occurred within 48 hr for AnA2 eggs, while only 56% eclosed in the same period when bowls were provided at 24 hr. Similarly, at 30°C when bowls were provided at 48 hr, 85% of eggs eclosed within the first 24 hr but only 38% eclosed in the same time interval when bowls were provided at 24 hr. In contrast, the dynamics of AnA1 egg hatching were similar at 25 or 30°C regardless of whether bowls were placed at 24 or 48 hr post-bloodmeal.

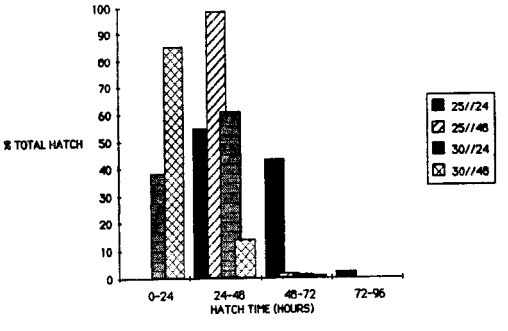

Fig. 2. Hatching time of AnA2 *Anopheles albimanus* eggs incubated at 25°C or 30°C, when oviposition bowls were placed in cages at 24 or 48 hr following bloodfeed.

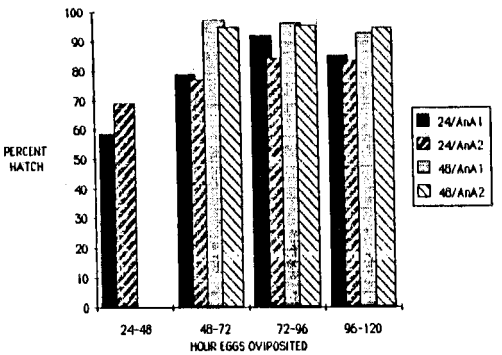

Fig. 3. Hatching capacity of *Anopheles albimanus* eggs oviposited daily post-bloodmeal when bowls were provided at 24 or 48 hr postfeed.

Egg viability for both colonies was consistently greater when the bowls were placed at 48 hr as compared with 24 hr post-bloodmeal. The most significant increase in egg hatch capacity (15%) was for those oviposited 24–48 hr post-bloodmeal (Fig. 3).

DISCUSSION

The Suchiate populations described herein have been colonized for differing periods. However, both colonies were maintained "outbred," eggs from field collected individuals were included at least 3 times per annum into the colonies since their initiation. The purpose of the "outbred" colonies was specifically to maintain the populations as similar as possible to the field gene pool during malaria susceptibility studies, and while maintaining a production of over 10,000 newly-emerged individuals per week. In order to succeed with both goals, breeding and maintenance procedures had to be adapted from those already established for other isolates of the same species.

The majority of these studies whether breeding individual or colony stock females, provided the opportunity for mosquitoes to feed 3 times per day (Bailey et al. 1979a, Dame et al. 1978, Dame et al. 1974, Ford and Green 1972, Rabbani et al. 1976, Savage et al. 1980). These previous studies reported that the majority of eggs were oviposited within 24 hr of bloodfeed, a not unlikely event since stock mosquitoes were given a constant blood source. This was not the case with the colonies reported in the present study where mosquitoes were allowed only one bloodmeal/week.

Oviposition of stock females (mixed ages) over time post-bloodfeed was dependent on the type of colony and on the time at which oviposition bowls were placed in the cages. When bowls were placed at 24 hr post-bloodfeed, peak oviposition for both colonies occurred between 3 and 4 days post-bloodmeal. However, if females were forced to retain eggs longer by placing the egg bowls at 48 hr, the AnA1 colony alone shifted to a peak oviposition at 2–3 days.

If individual females or groups of females of the same age instead of stock populations were followed to measure oviposition rates and gonotrophic period, those rates were higher due to the restricted space provided, and the gonotrophic period was found to be longer and dependent on the number of previous bloodfeeds.

The number of eggs oviposited per mosquito was related in part to the space allowed to them, as previously reported by Rabbani et al. (1976). Both AnA1 and AnA2 mosquitoes oviposited less than half the number of eggs/fertilized female with approximately 3 times the space. Egg production was additionally dependent on the type and method of blood provided. Rabbit blood stimulated significantly higher oviposition rates as compared to human blood irrespective of the space allowed. There was a significantly higher number of eggs oviposited/female if the mosquito fed from the natural host as opposed to a membrane feeder.

Previous studies on laboratory colonies of this species have used various but mainly bovine blood as a source for egg production. Colonies with a longer period in the laboratory have been reported to produce 145 eggs/female (Bailey et al. 1979a) and approximately 70 eggs/female (Thomas et al. 1985). Recent laboratory isolates of the species are reported to produce less, 74.9 eggs/female (Bailey et al. 1980). The isolates reported herein produced between 54 and 83 eggs/female when space was restricted as in the latter study. The isolates from the Suchiate region do not, therefore, appear to vary significantly regarding egg production from those isolated from the Pacific Coast of El Salvador. Independent of blood or colony type, highest egg production occurred with the second gonotrophic cycle for the AnA2 colony, but the first for the AnA1 colony.

Mosquitoes of both AnA1 and AnA2 colonies oviposited sooner with each successive gonotrophic cycle. In the most extreme case (AnA1 mosquitoes given rabbit blood), peak oviposition decreased from 6.0 to 2.6 days following the first through fourth gonotrophic cycles. These data indicate that physiological "preparedness" of the females influences the duration of the gonotrophic cycle, and probably the interval between feeds. If the oviposition interval decreases in successive cycles, mosquito age calculation by either Detinova's or Povolodova's techniques must be adjusted in order to reflect an accurate estimate of longevity, and vectorial capacity.

The geometric mean oviposition time was

slightly longer when mosquitoes were given human as opposed to rabbit blood. In the first gonotrophic cycle, the difference was half a day (6.6 as opposed to 6.0 days). If the geometric mean oviposition time for all feeds independent of the source of blood are combined, the mean is 4.7 or 4.8 days. Correlative field mark-recapture studies currently being conducted on wild populations in the same Suchiate region suggest a feeding interval for animal bait collected mosquitoes of 5.2 days (Bown, unpublished data). Data from the laboratory support field studies and therefore suggest a longer gonotrophic cycle than previously reported for *An. albimanus* (Breeland 1972).

Various methods have been used to obtain maximum hatch of *An. albimanus* eggs. Early hatching studies with this species were carried out using wet eggs placed directly on water (Ford and Green 1972). However, later studies demonstrated a capacity for eggs to withstand drying (Bailey et al. 1979b, Dame et al. 1974, 1978) and this technique was subsequently used to mass produce and synchronize pupal production. These studies utilized several isolates of *An. albimanus* from El Salvador. Without an initial 24 hr incubation period of eggs prior to drying, the data indicate that neither drying nor the use of a feed suspension during hatching are an improvement over hatching of wet eggs in water.

The hatching success of all eggs increased when they were retained longer by the females (oviposition site provided at 48 hr post-bloodmeal). The increase is most significant in those eggs oviposited first. These data may have implications for naturally occurring situations. If a suitable oviposition site is not found before 48 hr post-bloodmeal, it may not detract from the viability of eggs oviposited. In fact, if the female has an oviposition site readily available post-feed, the eggs will have on average 21% decreased viability.

Eggs oviposited by both colonies were very sensitive to hatching temperature. At 25°C, no eggs hatched from either colony within 24 hr. Overall, there was an average of 23% increase in total hatch success between 25° and 30°C. These data correspond with the observation that in the Suchiate region, *An. albimanus* breeding sites are sunlit ponds with grassy vegetation.

The two *An. albimanus* colonies described here were isolated within a 10 km area in the coastal Suchiate region of Chiapas. As previously stated, AnA1 was initiated from animal bait collection almost 3 years prior to that of AnA2. AnA2 was initiated from human bait collections and data presented here were collected within 6 months of its initiation in the laboratory. On 3 occasions within that period new females collected from the field were introduced into each of the 2 colonies. Differences observed between the 2 colonies are limited to their oviposition characteristics and the timed hatching of eggs. Egg viability was slightly lower for AnA2 under the same conditions. These differences may be a result of the time of adaptation to the laboratory despite their "outbred" maintenance, however, and may not represent real differences between the two populations.

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