THE RÔLE OF SOME COMMON ANOPHELINE MOSQUITOES OF PANAMA IN THE TRANSMISSION OF MALARIA

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INTRODUCTION

The Panama Canal Zone is often referred to as the region in which was carried out one of the most successful malaria control campaigns in tropical countries. Darling (1) concluded from his infection experiments that Anopheles albimanus was the dangerous vector, and most of the anti-mosquito measures were directed against this species. At the time that Darling performed his experiments, eleven species of Anopheles were listed from Panama, but as A. gorgasi, A. malefactor, and A. franciscanus were later shown to be synonymous with A. tarsimaculatus, A. punctimaculata, and A. pseudopunctipennis respectively, actually only eight species were known. These were A. argyritarsis R.-D., A. tarsimaculatus Goeldi, A. albimanus W., A. neivai H.D. & K., (2) A. apicimaculata D. & K., A. punctimaculata D. & K., A. eiseni Coq., and A. pseudopunctipennis Theo.

Conditions in Panama have changed since Darling completed his experiments. The formation of Gatun Lake has created new problems, and more careful systematic work has been done, with the result that today no less than 17 species of Anophelini are known to be present in Panama. Most of these are rare, limited in their distribution, or breed only during certain seasons of the year, so that they are of no importance in malaria transmission. The recently discovered species that might be dangerous are those belonging to the subgenus Nyssorhynchus; they are A. albitarsis L.-A., A. bachmanni Petr., A. strodei Root, and A. oswaldoi Peryassú.

Anopheles oswaldoi breeds in fresh water in the depths of the
jungle, particularly in the heavily wooded areas of the Mojinga Swamp near the mouth of the Chagres River. A few specimens have been taken from shaded pools in a partially dried creek, during the dry season, on the Pacific slope of the Isthmus, near the town of Juan Diaz. Anyone staying overnight in the Mojinga Swamp will be bitten by *A. oswaldoi*, but because this mosquito is abundant only in unpopulated districts, under existing conditions in Panama it is of no importance in malaria transmission. Curry (3) recorded the capture of only one adult female in Colon, and stated, “... it apparently is not prone to seek out human habitations, or else its flight range is limited.”

*Anopheles strodei* breeds in fairly large numbers in restricted locations during the early part of the dry season, but does not seem to be prevalent enough, even during its short breeding season, to be of much importance. Judging from the reluctance of the females to suck human blood in several attempts to infect them with malaria parasites, it seems that *A. strodei* does not feed readily on man.

This leaves *A. bachmanni* and *A. albirtarsis* as possible vectors, and the work of Simmons (4, 5, 6) shows that *A. punctimacula* must also be taken into consideration. This paper deals with the rôle of these three mosquitoes as malaria vectors in Panama.

**Experimental Infections**

*A. bachmanni*. The writer (7) has published results of experiments dealing with the relative susceptibility of *A. bachmanni* and *A. albimanus* to *Plasmodium vivax* and *P. falciparum*. Although the number of infected mosquitoes was small, it was shown that *P. vivax* can develop to the sporozoite stage in *A. bachmanni*.

*A. albirtarsis*. Table 1 summarizes the results of several experiments in which the infection of *A. albirtarsis* with *P. falciparum* was attempted. In each experiment batches of *A. albirtarsis*, with *A. albimanus* controls, were fed at the same time on the gametocyte carrier. Unfortunately, almost every one of the carriers were infants, and their struggles made it impossible for more than a few mosquitoes to become engorged. In experiments 5, 7, 8A, and SB, the gametocyte carriers were not good infectors, and the results obtained are of no particular significance.
<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Type of Malaria</th>
<th>Intensity of Infection</th>
<th>Species of Mosquito</th>
<th>Number of Mosquitoes Dissected</th>
<th>Number of Mosquitoes Feeding</th>
<th>Number of Mosquitoes Fecund</th>
<th>Percentage of Infected Mosquitoes</th>
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</table>

All experiments | EA       | A. albitaris | 100 | 4 | 0 | 4.0
|                 |          | A. albimanus* | 113 | 37 | 0 | 32.7

* Control.

when considered by themselves, but perhaps mean more when they are compared with those of experiments 11, 12, and 13. Experiment 11 was the most successful; 24 A. albimanus controls
were dissected, and oöcysts were found in 21, or 87.5 per cent, while of 14 *A. albitarsis* only 4, or 28.6 per cent were infected. There was also a great difference in the intensity of infection in the two species; 8 *albimanus* had approximately 100 or more oöcysts, while the rest had from 3 to 75. The first positive *albitarsis* was dissected on the fifth day after the infective meal, and 17 small oöcysts were found. The rest of the *albitarsis* were dissected from 8 to 15 days after the infective meal, and a positive mosquito was found on the eighth day, another on the ninth day, and one on the fifteenth day. Each of these mosquitoes had one small oöcyst apiece, and all had negative glands.

The gametocyte carrier in experiment 12 was not a good infector, but 2 of the 5 *albimanus* controls became infected, one with 1 oöcyst and the other with 2, while none of the 6 *albitarsis* that took full meals was positive. The dissections were made 8 and 9 days after the infective feed.

In experiment 13, eight *albimanus* controls were examined 8 days after the infective feed, and 6 of these had oöcysts. Two had only 3 oöcysts apiece, but one had 13, another 14, the fifth had approximately 60, and the sixth had over 100. Seven *albitarsis* were able to fill up with blood; two of these died before their blood meals had been digested, but none of the remaining five, dissected on the eighth day, was infected.

In these seven experiments, a total of 100 *albitarsis* were fed on *P. falciparum* gametocyte carriers, and only 4, or 4.0 per cent, became infected, while of a total of 113 *albimanus* controls fed at the same time with the *albitarsis* on the same gametocyte carriers, 37, or 32.7 per cent, became infected.

*A. punctimacula*. Simmons (6) proved that *A. punctimacula* can be readily infected with both *P. vivax* and *P. falciparum*.

**HABITS OF ADULT MOSQUITOES**

Observations were made on the habits of adult *A. albimanus*, *A. bachmanni*, and *A. punctimacula* in the neighborhood of two unsanitized Chagres River villages, situated several miles above the town of Gamboa. Such a study is dependent upon accurate identification of the adult females caught in nature, but unfor-
fortunately, *A. albimanus* and *A. bachmannii* both belong to the *tarsimaculatus* series of the *Nyssorhynchus* group. The mosquitoes in this series vary so much that identification based on adult characters is uncertain. To overcome this difficulty, a study was made of the eggs of the *Nyssorhynchus* group of mosquitoes in Panama, and it was found that identification was possible by examination of the eggs (8). The location selected for the study of *A. albimanus* and *A. bachmannii* was particularly favorable, for not only were these two species abundant, but the other members of the *Nyssorhynchus* group were either absent from this region or were very scarce. *A. oswaldoi*, the only mosquito whose eggs are liable to be mistaken for those of *A. bachmannii*, is absent. The eggs of *A. tarsimaculatus* and *A. albimanus* might be confused, but the former species is also absent. *A. albitarsis* is absent, and *A. strodei* and *A. argyritarsis* are both very rare. The problem of identification of the females, therefore, involved only the separation of *A. albimanus* from *A. bachmannii*, and this could be accomplished easily by examining the frill of the mature eggs in the ovaries. The eggs, before deposition, are entirely colorless, so that it is difficult to make out the structure of the floats. But the frill can be seen with ease; in *bachmannii* eggs it can be seen lying for its entire length on the dorsal surface of the egg, while in *albimanus* eggs part of the frill dips down below the tips of the egg.

The precipitin test could not be used in determining the blood preferences of *A. bachmannii* and *A. albimanus*, as the blood meals were digested before the ovaries became sufficiently mature to make identification possible by means of egg characters.

As is shown on the accompanying map, one of the two towns, Las Guacas, is almost surrounded by water. North and west are extensive lagoons, choked with decaying trees and various water plants. Here *A. bachmannii* breeds abundantly in large patches of water lettuce, *Pistia stratiotes*. Some *albimanus* breeding takes place in these lagoons. South of the village is a small, well-shaded creek, which furnishes many excellent breeding places for *A. punctimacula*. East of Las Guacas is a slough, which becomes a swamp during the rainy months. *A. albimanus*
breeds neither in the creek nor in the swamp. The second town, Santa Rosa, is situated on the western bank of the Chagres River, where the swiftness of the river current prevents mosquito breeding. On the eastern bank, opposite Santa Rosa, is a lagoon in which both *A. albimanus* and *bachmanni* breed, although not in great numbers. Back of the town are some small creeks in which *A. punctimacula* may be found occasionally. About a mile southeast of Santa Rosa is an extensive lagoon which the natives call Aguardiente, and in it *A. albimanus* often breeds in enormous numbers.
No flight experiments with stained mosquitoes were performed, but it seems evident that most of the *albimanus* that attack the inhabitants of the villages originate in Aguardiente. As Santa Rosa is situated between Aguardiente and Las Guacas, one would expect that most of the *albimanus* females would stop in Santa Rosa for their blood meals rather than continue their flight across the river to Las Guacas. Adult catches in the villages indicate that this actually takes place. During a three month period, from the middle of May to the middle of August, 1937, adult *Anopheles* were collected in the huts of the two villages. In 17 collecting trips, a total of 122 houses was visited in Santa Rosa, and 758 Anophelines were taken, while 356 Anophelines were captured in a total of 90 houses in Las Guacas during the same number of trips. Usually the collections were made in both towns on the same day, although in a few cases they were made on different days. About 50 of the mosquitoes found in Santa Rosa were *A. punctimacula*, leaving a total of approximately 700 *A. albimanus* collected in 122 houses, an average of almost 6 *albimanus* per house. Over 100 of the *Anopheles* captured in Las Guacas houses were *A. punctimacula*, so that approximately 250 *albimanus* were taken in 90 houses, an average of almost 3 *albimanus* per house.

At this point, a word of explanation is necessary concerning the discrepancies between the numbers of mosquitoes mentioned above and those in table 2. *A. albimanus* and *A. bachmanni* could be distinguished from one another with certainty only by microscopical examination of the eggs in the mature ovarian follicles. Actually over 1000 *albimanus* were collected in houses, although only 472 are included in table 2. The remainder either died before dissections were possible, or had ovaries that were too immature for positive identification, and so were discarded. The 103 *punctimacula* included in table 2 do not represent the total number taken in houses, as some of these died also before dissections were possible; however, no specimen of *punctimacula* had to be discarded because of undeveloped ovaries. The figures given in the above paragraph are not exact—no identifications were attempted at the time of capture, and the total number of
punctimacula taken is not known. Those that died before dissection were not counted, and the number of A. albimanus was determined by subtracting the number of punctimacula from the total catch. However, the number of uncounted punctimacula was not large, and so the above figures are accurate enough to show that the density of albimanus in Santa Rosa houses was almost twice that in Las Guacas houses. Actually, the average number of mosquitoes in the Santa Rosa houses should be higher. A. albimanus females do not as a rule remain long in houses after sunrise unless they are trapped in bed nets or behind screens. In the collecting trips, the usual procedure was to visit the Las Guacas houses early in the morning, and then cross the river to Santa Rosa. This alone would insure a larger catch in the Las

<table>
<thead>
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<th>TABLE 2</th>
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<td>SPECIES OF MOSQUITO</td>
<td>IN HOUSES</td>
</tr>
<tr>
<td></td>
<td>Santa Rosa</td>
</tr>
<tr>
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<td>355</td>
</tr>
<tr>
<td>A. bachmanni</td>
<td>1</td>
</tr>
<tr>
<td>A. punctimacula</td>
<td>28</td>
</tr>
<tr>
<td>All species</td>
<td>384</td>
</tr>
</tbody>
</table>

Guacas houses, but in addition to this, the inhabitants of Santa Rosa, upon learning that a mosquito collection was in progress, had the annoying habit of shaking out their bed nets before the arrival of the collectors. Had collecting conditions been equal in the two towns, the average number of A. albimanus in the Santa Rosa houses would have been higher than that given.

Table 2 shows that of 578 Anopheles collected in houses, 472, or 81.7 per cent, were A. albimanus; 103, or 17.8 per cent, were A. punctimacula, while only 3, or 0.5 per cent, were A. bachmanni. Near Las Guacas there are a few small pig pens, from which Anophelines were collected during the day, or after dark while they were feeding on the pigs. Of 349 Anopheles collected from pigs, 320, or 91.7 per cent, were A. bachmanni; 25, or 7.2 per cent, were A. punctimacula; while only 4, or 1.1 per cent, were A.
_albimanus_. This demonstrates that although _A. bachmanni_ breeds in great numbers in the immediate vicinity of the villages, it does not care enough about human blood to enter man's houses in search of it. _A. albimanus_ prefers human blood to that of pigs. _A. punctimacula_ visits man's habitations frequently, but also feeds readily on pigs.

In Panama, adult Anophelines do not congregate in daytime resting places where they can easily be found, and the writer has not been able to discover any type of shelter that might be considered a natural resting place. Tree holes, cavities between exposed roots of trees and under overhanging creek banks, crab holes, crevices in rock piles, brush piles, bushes, clumps of grass, and other similar situations were examined carefully. The cool and damp atmosphere in most of these shelters would seem to be ideal for mosquitoes, but although Culicines were plentiful, a total of only 12 Anophelines was collected. Two _A. albimanus_ females were found, one in a tree hole and the second on the damp, shady side of a piece of corrugated iron set up on end against a fence. Two _A. bachmanni_ females were found on wet fallen leaves beneath trees with dense foliage, and another in a dark stable; one male _A. bachmanni_ was captured in an iron culvert, and another at the base of a buttressed root of a tree. Three male and one female _A. punctimacula_ were taken on the piece of corrugated iron mentioned above, and one _A. neomaculipalpus_ female was found on the wet, grassy bank of a pool.

**NATURAL INFECTION RATES**

The three months in which adult mosquito collections were made in Santa Rosa and Las Guacas were not favorable for determining the natural infection rate among the mosquitoes, as the malaria rates in the natives of the villages were low, particularly in Las Guacas. None of the 117 _A. albimanus_ from Las Guacas was infected. In Santa Rosa the malaria rates were higher, and of the 355 _A. albimanus_ captured in houses, 4, or 1.1 per cent, had _oöcysts_ on the stomach wall, and one, or 0.3 per cent, had sporozoites in the salivary glands. None of the 323 _A. bachmanni_ collected from pigs and houses was infected. The
28 A. punctimacula taken in Santa Rosa houses were all negative, but a single oöcyst, containing fully-developed sporozoites, was found on the stomach wall of one of the 75 A. punctimacula collected in Las Guacas houses.

**DISCUSSION**

*Anopheles albitarsis*, because of its reluctance to attack man (9), is of little importance in Panama as a malaria vector, and the infection experiments recorded in this paper furnish additional evidence of this mosquito's harmlessness. *A. albitarsis* can be infected with *P. falciparum*, as 4 specimens in Experiment 11 had oöcysts on the stomach wall, but the fact that 3 mosquitoes, dissected on the eighth, ninth, and fifteenth days following the infective feed, had one small oöcyst apiece, indicates that although *A. albitarsis* can be infected under conditions as favorable as they were in Experiment 11, the parasites are not able to develop to maturity. It is not impossible that in another experiment the results of Experiment 11 might have been reversed, with a heavy infection in the *albitarsis* and a light one in the *albimanus* controls. However, seven different batches of *albitarsis* and *albimanus* were fed on seven different gametocyte carriers, and in each case the *albimanus* controls became infected, while the *albitarsis*, with the exception of the four individuals in Experiment 11, remained negative. If the susceptibility of *albitarsis* were comparable to that of *albimanus*, one would expect to find more than 4 lightly infected specimens among the 100 *albitarsis* used in the seven experiments.

It was not until 1932 that *A. albitarsis* was found in Panama (3), and it represents one of the problems that has arisen since the formation of Gatun Lake. It is possible that it was carried to the Isthmus by migratory birds or by ships; the more likely possibility is that it was always present in Panama, breeding in secluded pools or creeks where it missed observation, and that the biological changes taking place with the formation of Gatun Lake so favored this species that it was able to breed in tremendous numbers in the Lake. Whether indigenous to Panama or
not, the *A. albitarsis* of Panama is different from the dangerous *albitarsis* of Brazil (10), and the refractoriness of the former to *P. falciparum* may be part of its physiological make-up. On the other hand, if the mosquito has been recently introduced into Panama, its resistance to infection with the Panama strain of *P. falciparum* may be a phenomenon similar to that exhibited by *A. albimanus*, which is highly susceptible to the strain of *P. vivax* and *P. falciparum* from its own regions, but refractory to Nearctic strains of these parasites (11).

During the past few years, so many species of *Anopheles* have been experimentally infected with the *Plasmodia* causing human malaria that one is led to believe that under proper conditions almost any Anopheline can be infected with the parasites. For example, in Panama, Simmons (6, 12) not only infected *A. punctimacula*, but *A. neomaculipalpus*, *A. apicimacula*, and *A. eiseni* as well. In the United States, *A. atropos* is as susceptible to *P. vivax* as is *A. quadrimaculatus* (13); *A. walkeri* is an efficient host for *P. vivax* (14), although less so for *P. falciparum* (15); and *P. vivax* has been transmitted from a malaria patient to an uninfected person by means of *A. barberi* (16). This demonstrates the truth of the statement made by Barber, Komp, and Hayne (17): “Certainly the fact that a species may be infected under laboratory conditions does not prove that it is of sanitary importance. Probably any species of *Anopheles* could be infected if one made trials enough with good gametocyte carriers.”

In Venezuela, epidemiological evidence indicated that *A. bachmanni* may be a malaria vector (18), and precipitin tests showed that although it prefers animal blood, it might be a vector in regions where its density is high (19). The density of *A. bachmanni* is certainly high in the immediate vicinity of Las Guacas. If it were a malaria carrier, one would expect the malaria rates among the natives of this village to be much higher than in Santa Rosa, but the monthly rates, as Komp and Clark (20) have pointed out, have been consistently lower in Las Guacas than in Santa Rosa. This may be due to the presence of immune families in Las Guacas (20), but another factor may be that Las
Guacas is almost surrounded by *bachmanni* breeding places, while Santa Rosa is nearer the *albimanus* breeding areas in Aguardiente, and has a higher *albimanus* density.

On a number of occasions, the writer has observed *A. bachmanni* to feed on man during the daytime, and as the malaria parasites can develop in this mosquito, it is possible that once in awhile *A. bachmanni* transmits malaria. If this actually does occur, it must be very unusual. The writer has also observed huge numbers of *A. bachmanni* attacking pigs after dark, and it is evident that pigs, and perhaps other animals as well, are its normal hosts. If present alone, *A. bachmanni* would probably be unable to keep malaria going in the native population.

The infection rates among the mosquitoes caught in nature furnish additional evidence that *A. bachmanni* is not a malaria vector.

*A. punctimacula* can become infected with the human *Plasmodia*, and so it too could transmit malaria; the infected specimen captured in a Las Guacas house shows that this may take place occasionally. This mosquito was found at a time when the malaria rate in Las Guacas was zero, but it does not seem likely that the oöcyst belonged to a species other than one of the *Plasmodia* causing human malaria. Monkeys are exceedingly rare in this region, and Anopheline mosquitoes are not supposed to be susceptible to the parasites of bird malaria, although Mayne (21) infected *A. subpictus* with bird *Plasmodia*. However, *A. punctimacula* is relatively scarce, and furthermore, its willingness to feed on animals as well as on man, makes it doubtful that it is of great importance as a malaria vector. The author wishes to point out that this conclusion is based on his observations on *A. punctimacula* in the Chagres River villages, and is not to be interpreted as an attempt to discredit Simmons’ (6) conclusion that *punctimacula* is an important factor in malaria transmission among the military forces on the Atlantic side of the Canal Zone.

**Summary and Conclusions**

1. In seven experiments, a total of 100 *A. albitarsis* and 113 *A. albimanus* controls were fed on *P. falciparum* gametocyte car-
riers; 4, or 4.0 per cent, of the *albitarsis* became lightly infected, while 37, or 32.7 per cent, of the *albimanus* controls became infected, many of them heavily, indicating that *A. albitarsis* in Panama is quite refractory to infection with *P. falciparum*, as compared with *A. albimanus*.

2. *A. bachmani* can be infected with human malaria parasites, but its preference for animal blood renders it harmless so far as malaria transmission in Panama is concerned.

3. *A. punctimacula* frequently seeks blood in man's habitations, but it also feeds on animals. It is too scarce to be of importance in malaria transmission in the Chagres River villages.

4. The long list of Anophelines known to be present in Panama is an imposing one; however, as most of the species are rare, limited in their distribution, seasonal in their breeding habits, or are animal feeders, malaria control in Panama is still dependent upon the control of the only really dangerous Anopheline present in this region: *Anopheles albimanus*.

REFERENCES


