MAINTENANCE OF *ANOPHELES ALBIMANUS* ON FROZEN BLOOD

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The maintenance of anautogenous mosquito colonies usually involves the use of live animal hosts as a blood source, such as snakes, chicks, pigs and guinea pigs (Hubert et al. 1954), as well as human forearms exposed to feeding adults (McDonald et al. 1977). However, Bailey et al. (1978) described techniques for maintaining *Anopheles albimanus* Wiedemann on preserved blood fed through a natural membrane (condom). This technique presently requires 16–24 liters of preserved bovine blood per week to maintain stock colonies of six species of ca. 30,000 mosquitoes each at the USDA Insects Affecting Man and Animals Research Laboratory in Gainesville, Florida. This necessitates weekly trips to a slaughterhouse located ca. 55 km from the laboratory, since defibrinated blood can be stored for only one week under refrigeration at ca. 5°C. An inexpensive method for preserving blood for long periods of time would be advantageous. Such a storage procedure could reduce the need for frequent trips to a slaughterhouse, since large quantities of blood could be collected at one time, defibrinated, and stored for extended periods of time. This would not only save time and travel expenses, but also provide better utilization of laboratory personnel. It would also provide a backup system for times when fresh blood is not available, or when other factors, such as personnel shortages and assignments, make it inconvenient for the weekly procurement of fresh blood. When live animals were used as a blood source the capital cost of mass-rearing large numbers of *Culex pipiens fatigans* Wiedemann [-*Culex quinquefasciatus* Say] for research was quite high (Singh and Razdan 1977). A significant cost reduction can be realized if live animals are not required for maintaining adult mosquitoes.

Therefore, experiments were conducted to determine the feasibility of using defibrinated blood frozen for extended periods of time for maintaining adults of *An. albimanus* in the laboratory. *Anopheles albimanus* adult females fed on frozen bovine blood were compared with those reared using standard colony procedures as described by Bailey et al. (1978). Parameters used to determine blood suitability were the percentage of females taking blood, the number of eggs per ovipositing female and the percent of eggs hatching.

Fresh blood was collected weekly from a slaughterhouse as cattle were being processed. The blood was transferred to 8-liter plastic jugs that had been previously sterilized with a 1% sodium hypochlorite solution and defibrinated as described by Bailey et al. (1978); the blood was then taken to the laboratory and refrigerated at 4°C. A sample of the fresh blood was offered to *An. albimanus* adults, 4–5 days old, to establish a base line of the previously described parameters to which future testing of frozen samples could be compared. (Throughout the experiment adult feeding was determined by visual inspection, and only those females observed to be fully engorged were considered as blood-fed.) The remaining blood was divided into 200-ml samples in small plastic bags and placed in a freezer at a temperature of ca. -6°C.

Prior to use, a frozen sample was removed from the freezer and allowed to thaw in tap water (ca. 26°C). The blood sample was then heated in a hot water bath to ca. 44°C, poured into a membrane and offered to adult mosquitoes from the standard colony, which has been maintained by membrane feeding for ca. six years. Each week thereafter, two separate cages (46 × 46 × 46 cm) were stocked with ca. 1,000 pupae each. Feeding response, egg production, and percentage egg hatch were evaluated after offering 4-5-day-old adults fresh blood in one cage and frozen blood in the other. The females were removed from the cage and counted, and those having taken a complete blood meal were determined. All of the females were then transferred to smaller holding cages (15 × 25 × 25 cm). Two days after the blood meal, 50 gravid females were selected at random and placed into capped 4-dram plastic vials containing rearing water as an oviposition medium. After 72 hr the number of eggs laid per female and the percentage hatch were recorded. This procedure was repeated each week for 12 weeks, then every fourth week thereafter through week 24.

The test for significance throughout was the student-*t* distribution test for small sample size.

Figure 1 shows the results of these tests. As can be seen in Fig. 1A, there was a significant decrease in the percentage of females taking frozen blood with an increase in time the blood was frozen, when compared with those feeding on fresh blood. This decrease could have been due either to a gradual loss of attractiveness of the frozen blood or a change in the physical characteristics of the blood which prevented consumption after the females had penetrated the membrane. The number of eggs produced by females that successfully engorged on frozen

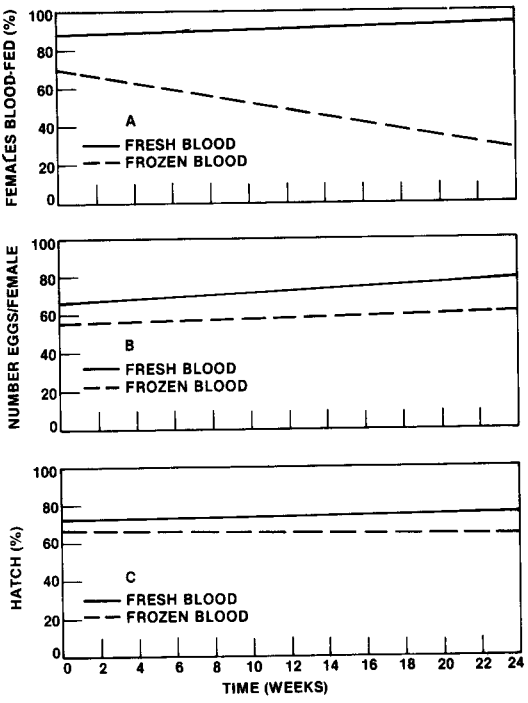

Fig. 1. Computer-fitted lines of data showing the percentage of females taking blood (A), number of eggs produced per blooded female (B), and percentage of egg hatch (C) when female *Anopheles albimanus* were offered fresh or frozen blood during a 24-week period.

blood and the percentage hatch of those eggs were not significantly different from those laid by females fed fresh blood (Figs. 1B and 1C).

In order to compare *An. albimanus* mosquitoes maintained on frozen blood for several generations with those fed on fresh blood, 2 cages (46 × 46 × 46 cm) were each stocked with ca. 1,000 pupae from the standard colony and held at 27 ± 2°C and 70 ± 5% RH. With each succeeding generation one cage was stocked with pupae from the standard colony of *An. albimanus* and the adults were fed fresh blood, and the other was stocked with pupae from the test strain and the adults were fed on frozen blood. The blood used in this test had been frozen for 1–12 weeks, with the average time frozen being ca. 8 weeks. The mosquitoes were otherwise maintained as described by Bailey et al. (1978). Each stock was offered its respective blood for three consecutive days and then a single oviposition pan for three days beginning on the third day after their first blood meal. The eggs from each collection were allowed to hatch, and then reared to the pupal stage for stocking cages for the next generation. In the 17th generation the percentage hatch of eggs from both groups was recorded. Trays were set with 0.06 ml of eggs and reared to the pupal stage. The number of pupae per ml and the total number of pupae per tray were recorded. The test was replicated three times.

Results of this test are shown in Table 1. The average volume of eggs obtained from the first gonotrophic cycle during the 17th generation was 0.49 ml and 0.33 ml for fresh and frozen blood, respectively, which represented a significant difference ($P = 0.05$). This reduction probably reflects a reduction in the number of females that successfully fed. There was no significant difference in the percentage hatch of eggs collected from the two strains. The total number of pupae produced per tray and pupal size also showed no significant differences.

Table 1. Fecundity and production of pupae from eggs collected from *Anopheles albimanus* females fed fresh or frozen blood for 17 generations (average of 3 replicates during 17th generation).

Parameter	Fresh blood	Frozen blood
Milliliters eggs/cage	0.49	0.33
% Egg hatch	91	90
No. pupae/ml	209	220
No. pupae/tray	2424	2662

These data suggest that *An. albimanus* can be successfully maintained on frozen blood even though a reduction of ca. 33% in egg production was observed. In most mosquito production facilities the ease of using frozen blood rather than live host animals would more than compensate for the reduced production. A similar reduction in egg production (42%) was observed by Bailey et al. (1978) when comparing *An. albimanus* fed on bovine blood through similar membranes with those fed on live rabbits. However, they also found that egg production increased to the previous level after several generations of selection in an actual mass rearing facility in El Salvador, Central America. Since the reduction in eggs in the current study was probably a result of the failure of some individual females to feed, similar improvements might possibly be expected through long-term selection of mosquitoes on frozen blood. However, studies were not conducted beyond the 17th generation to determine if this occurred in our rearing system.

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ABSENCE OF EASTERN EQUINE ENCEPHALITIS (EEE) VIRUS IN IMMATURE *COQUILLETTIDIA PERTURBANS* ASSOCIATED WITH EQUINE CASES OF EEE[1]

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During the summer of 1983, 5 fatal equine cases of eastern equine encephalitis (EEE) were confirmed in New Jersey (Crans, unpublished data). These cases were conspicuous by their occurrence at sites, ca. 30 km from the nearest salt marsh, in Ocean and Sussex counties. Historically, equine cases of EEE have been repeatedly diagnosed from several inland localities in New Jersey (Goldfield and Sussman 1968). Hayes et al. (1962) indicated that the distance from coastal marshes made it unlikely that the salt marsh mosquito, *Aedes sollicitans* (Walker), served as the vector for EEE virus in these areas.

Four of the 1983 cases occurred at a distinct focus in Jackson Township, Ocean County. After the initial case with an onset of July 8, mosquito surveillance was initiated and concentrated on 3 Jackson Township farms within a 5-km radius of each other. Subsequent cases from this area had onsets of July 23, August 12 and August 26. The dominant mosquito species collected at these farms was *Coquillettidia perturbans* (Walker), ranging from 77% of the total in July to 44% in September. From the 2,158 mosquitoes assayed from this area by the New Jersey State Department of Health, 2 isolates of EEE virus were obtained from 1,225 *Cq. perturbans*. These viral isolates, from collections made on July 25 and September 7, reflected a minimum field infection rate (MFIR) in this area of 1:612 for this species. Three additional EEE virus isolates were obtained from 263 *Culiseta melanura* (Coquillett) (MFIR = 1:88).

The first reported isolation of EEE virus from an arthropod was from *Cq. perturbans* collected in Georgia in 1948 (Howitt et al. 1949) and EEE virus has since been isolated from this species in New York (Morris and Srihongse 1978) and New Jersey (Veazey et al. 1980). *Coquillettidia perturbans* was likely responsible for transmission of EEE virus to equines and humans in southwestern Michigan in 1980 (Francy 1982) and has a high vector potential for this virus in Massachusetts (Nasci and Edman 1981). Field studies of the blood-feeding pattern for this species have linked it closely to large mammals, especially equines (Nasci and Edman 1981).

In laboratory studies with *Cq. perturbans* and EEE virus, Chamberlain et al. (1954) demonstrated virus transmission by this species and gave it a "good vector potential rating." Schaeffer and Arnold (1954) found that EEE virus, ". . . could be demonstrated in eggs laid by infected (*Mansonia* [*Coquillettidia*] *perturbans*." Following oral infection of *Cq. perturbans* with Venezuelan equine encephalitis (VEE) virus, Chamberlain et al. (1956) recovered VEE virus from 7 of 16 egg rafts. In a similar study, they found that 2 of 19 egg rafts laid by *Cq. perturbans*, after ingesting an infectious blood meal, contained EEE virus. In an attempt to preclude transovum viral contamination, Chamberlain and coworkers rinsed the surface of the eggs they assayed with a serum-saline solution. The difficulty associated with laboratory colonization of *Cq. perturbans* has prevented any definitive studies of its role as an overwintering host for arboviruses.

The association of EEE virus with equine fatalities in New Jersey during 1983 suggested a more important role for *Cq. perturbans* in the ecology and transmission of this virus. The maintenance of the virus by larval mosquitoes has been proposed as an overwintering mechanism during interepidemic periods. Therefore, we viewed this as an opportunity to explore the hypothesis that EEE virus was maintained through the winter in *Cq. perturbans* larvae.

From October 1983 through March 1984, immature *Cq. perturbans* were collected in areas adjacent to the sites where the 4 Ocean County

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