A HISTORICAL REVIEW OF THE F-1 STRAIN OF ANOPHELES FREEBORNII AS A HOST AND VECTOR FOR STUDIES OF MALARIA

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ABSTRACT. A review was made of the use of a specific strain of Anopheles freeborni from California (F-1) that has been used extensively in experimental investigations of malaria for more than 50 years. The F-1 strain of An. freeborni has been shown to be a suitable experimental host and vector for different species of Plasmodium that cause malaria in humans and nonhuman primates for biologic, immunologic, and chemotherapeutic studies. Eleven species of Plasmodium fully completed sporogonic development; development of sporozoites within mature oocysts occurred in an additional 7 species. Transmission through An. freeborni from human to human, monkey to human, or monkey to monkey has been demonstrated for 9 species of Plasmodium.

KEY WORDS Anopheles freeborni, Plasmodium, malaria, mosquitoes

INTRODUCTION

Over the last half century, several species of anopheline mosquitoes have served as laboratory hosts for experimental studies on malaria. Among these are Anopheles quadrimaculatus Say, An. atroparvus Thiel, An stevensi Liston, An. albimanus Wiedemann, and An. freeborni Aitken. Here we summarize some of the studies that have established An. freeborni as one of the most useful and suitable hosts for a variety of investigations of malaria.

The F-1 strain of An. (An.) freeborni Aiken was established from eggs laid by wild-caught females collected near Marysville, CA, on March 3, 1943. The colony was supplemented by an additional 800 wild-caught females on March 15 and 250 to 400 females collected from the same location on April 30, 1943. On August 21, 1944, eggs were shipped from the U.S. Public Health Service laboratory at Letterman General Hospital, San Francisco, CA, to the U.S. Public Health Service laboratory in Columbia, SC. This was part of the wartime “Imported Malaria Studies” effort to determine the potential of native anopheles to transmit malaria parasites from infected returning servicemen. Since its establishment, this strain of An. freeborni has been maintained continuously in various laboratories where it has served as the standard host and vector for comparative studies with many species of Plasmodium causing human and nonhuman malaria.

A suitable experimental host and vector should be able to be reared in relatively large numbers using standard insectary techniques, should readily feed on animal or human hosts, should support sporogonic development to the production of infectious sporozoites, and should be capable of transmitting the infection by feeding on a susceptible host. Preferably, a laboratory vector will be susceptible to infection with and be capable of transmitting a number of different species of Plasmodium. A review of the use of this mosquito for studies with human and nonhuman primate malaria parasites indicates that this is a remarkably useful vector for biologic, immunologic, and chemotherapeutic studies.

HUMAN MALARIA PARASITES

The earliest studies with the F-1 strain involved the feeding of colony-reared mosquitoes on Plasmodium vivax-infected soldiers returning from the South Pacific and the Mediterranean during World War II. Young et al. (1945) reported that 1,358 of 2,581 mosquitoes that fed on these patients became infected; several infections with P. vivax originating from the Pacific were experimentally transmitted by An. freeborni. Moore et al. (1945) reported on the feeding of 64 lots of An. freeborni on relapsing P. vivax infections in soldiers. Mosquitoes became infected and 8 infections were transmitted to other patients by this vector. Oocyst counts as high as 800 per gut were found, although the usual oocyst counts ranged from 1 to 24. Hardman (1947) summarized the procedures being used at that time for the laboratory rearing of these mosquitoes. These early studies indicated that An. freeborni had high potential for more extensive investigations. Of early interest was the relative efficiency of different laboratory-reared anopheline vectors in transmitting different species and strains of human malaria parasites. Using simultaneous feedings, Young and Burgess (1948) reported on the comparative susceptibility of An. quadrimaculatus from the southeastern United States and An. freeborni from California to foreign vivax malaria; their studies indicated that the latter was more susceptible (more oocysts per gut and higher percentage of infection). Young et al. (1948) reported the infection of An. freeborni with P. vivax from different geographic origins, including the
Table 1. Studies on the infection and transmission of *Plasmodium vivax*, *P. falciparum*, *P. malariae*, and *P. ovale* by *Anopheles freeborni*.

<table>
<thead>
<tr>
<th>Species of <em>Plasmodium</em></th>
<th>Strains (geographic origin)</th>
<th>Primate hosts</th>
<th>References</th>
</tr>
</thead>
</table>
transmission to humans ceased. With the subsequent establishment of *P. vivax* in New World monkeys, a large number of different isolates and strains of *P. vivax* were now available for study in *An. freeborni* (Table 1).

Burgess and Young (1946) were the 1st to report the experimental transmission by *An. freeborni* of *Plasmodium falciparum* (McLendon strain) to 2 patients; prepatent periods were 15 and 29 days. *Anopheles freeborni* then became one of the standard hosts and vectors for transmission studies with this parasite. Jeffery et al. (1963) reported on studies with a multidrug-resistant strain of *P. falciparum* from Thailand in which 6 patients were infected via the bites of *An. freeborni*. In a comparative susceptibility study based on oocyst densities, Collins et al. (1964a) demonstrated that, with a strain of *P. falciparum* from Panama, *An. freeborni* was more heavily infected than was *An. quadrimaculatus* or even a coindigenous strain of *An. albimanus*. However, the adaptation of various strains of *P. falciparum* to New World monkeys opened up a new field of study where *An. freeborni* was exposed to isolates and strains of the parasite from distant geographic areas (Table 1).

*Plasmodium malariae* was first shown to be transmissible by *An. freeborni* by Young and Burgess (1947). Little further success in the transmission of this parasite was obtained until Contacos and Collins (1969) reported the transmission of *P. malariae* from an infected night monkey (*Aotus* sp.) to humans. Although subsequent studies indicated monkeys and chimpanzees would support the development of *P. malariae* gametocytes infective to *An. freeborni*, transmission via sporozoites to other monkeys and chimpanzees was not successful.

Chin et al. (1966) reported the transmission of a West African strain of *Plasmodium ovale* from human to human using *An. freeborni*. Successful experimental studies with *P. ovale* and *An. freeborni* have also been conducted in chimpanzees. Different strains of this parasite readily infect *An. freeborni*, and exoerythrocytic (liver stages) of the parasite have been demonstrated in squirrel monkeys (*Saimiri* spp.). However, no erythrocytic-stage infections have been established in New World monkeys.

The ready infectivity of many different strains of human malaria parasites from diverse geographic areas to *An. freeborni* suggested that this mosquito could serve as a universal host for a variety of chemotherapeutic and immunologic studies. In fact, this has become the case in several laboratories in the United States.

### NONHUMAN PRIMATE MALARIA PARASITES

Monkeys have long been used as models for chemotherapeutic and immunologic studies with malaria parasites, and both Old World and New World monkeys have been infected with various species of *Plasmodium*. For almost 40 years, we have used *An. freeborni* as a laboratory vector for a variety of studies on malaria in nonhuman primates (Table 2). It early became apparent that, although in most instances *An. freeborni* was highly susceptible to the development of the oocyst stage, some species of *Plasmodium* failed to develop to the point of sporozoites being present in the salivary glands. With all but a few species, oocyst densities in *An. freeborni* were high; oocyst counts could be used as an indicator of gametocyte infectivity. However, transmission via sporozoites could only be obtained if oocysts containing mature sporozoites were crushed and then injected into the primate. *Plasmodium cynomolgi*, *P. gonderi*, *P. simium*, *P. fragile*, and *P. brasilianum* parasites of monkeys, developed fully, including the presence of sporozoites in the

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**Table 1.** Continued.

<table>
<thead>
<tr>
<th>Species of <em>Plasmodium</em></th>
<th>Strains (geographic origin)</th>
<th>Primate hosts</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. malariae</em></td>
<td>USPHS (U.S.A.), Philippines, Uganda, China/ICDC</td>
<td>Humans, night monkeys, squirrel monkeys, chimpanzees</td>
<td>Young and Burgess 1961; Collins and Contacos 1969; Contacos and Collins 1969; Coatney et al. 1971; Collins et al. 1973a, 1975a, 1984a, 1989a, 1990d, 1994b, 1997a; Millet et al. 1988a; Nagasawa et al. 1988; Chin et al. 1966; Collins et al. 1969c, 1987c; Coatney et al. 1971; Mazier et al. 1987; Nagasawa et al. 1987; Millet et al. 1994; Morris et al. 1996</td>
</tr>
<tr>
<td><em>P. ovale</em></td>
<td>Donaldson (South Pacific), West Africa, Nigerian</td>
<td>Humans, chimpanzees</td>
<td>Chin et al. 1966; Collins et al. 1969c, 1987c; Coatney et al. 1971; Mazier et al. 1987; Nagasawa et al. 1987; Millet et al. 1994; Morris et al. 1996</td>
</tr>
</tbody>
</table>
### Table 2. Studies on the infection and transmission of nonhuman primate-infecting species of *Plasmodium* by *Anopheles freeborni.*

<table>
<thead>
<tr>
<th>Species of <em>Plasmodium</em></th>
<th>Strains (geographic origin)</th>
<th>Primate hosts</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. brasilianum</em></td>
<td>S (Colombia), AT (Panama) Peruvian I-III</td>
<td>Night monkeys, squirrel monkeys, spider monkeys, humans</td>
<td>Collins et al. 1969b, 1985d, 1990b, 1993; Sodeman et al. 1969; Coatsney et al. 1971</td>
</tr>
<tr>
<td><em>P. coatneyi</em></td>
<td>Hackeri (Malaysia)</td>
<td>Macaques</td>
<td>Held and Contacos 1967; Coatsney et al. 1971; Collins et al. 1992a</td>
</tr>
<tr>
<td><em>P. fieldi</em></td>
<td>Hackeri, N-3, ABI, Malaysian</td>
<td>Macaques</td>
<td>Coatsney et al. 1971; Collins et al. 1984b, 1992a</td>
</tr>
<tr>
<td><em>P. fragile</em></td>
<td>Sri Lanka, Nilgiri (India)</td>
<td>Macaques, Night monkeys, squirrel monkeys</td>
<td>Collins et al. 1967, 1974b, 1975c, 1990a, 1992a; Coatsney et al. 1971</td>
</tr>
<tr>
<td><em>P. gonderi</em></td>
<td>Mandrill (West Africa)</td>
<td>Macaques</td>
<td>Collins et al. 1964b, 1992a; Coatsney et al. 1971; Collins and Contacos 1980</td>
</tr>
<tr>
<td><em>P. hylobati</em></td>
<td>WAK (Indonesia)</td>
<td>Gibbons</td>
<td>Coatsney et al. 1971; Collins et al. 1972d</td>
</tr>
<tr>
<td><em>P. inui</em></td>
<td>Taiwan I and 2 (Taiwan), N-34, Perlis, Perak, Leaf Monkey I and II, Leucosphyrus, Mulligan, and others (Malaysia), OS (India)</td>
<td>Macaques, Night monkeys, squirrel monkeys</td>
<td>Collins et al. 1968a, 1981b; Held et al. 1968; Coatsney et al. 1971; Nguyen-Dinh et al. 1980; Collins and Warren 1998</td>
</tr>
<tr>
<td><em>P. jefferyi</em></td>
<td>Malaysian</td>
<td>Gibbons</td>
<td>Collins and Orihel 1969; Coatsney et al. 1971; Rodenberg 1985; Millet et al. 1988b</td>
</tr>
<tr>
<td><em>P. knowlesi</em></td>
<td>H, Malaysian, Hackeri (Malaysia)</td>
<td>Macaques, humans, Night monkeys, squirrel monkeys</td>
<td>Coatsney et al. 1971</td>
</tr>
<tr>
<td><em>P. reichenowi</em></td>
<td>Chimpanzee (West Africa)</td>
<td>Chimpanzees</td>
<td>Coatsney et al. 1971; Collins et al. 1986b</td>
</tr>
<tr>
<td><em>P. schwetzi</em></td>
<td>Chimpanzee (West Africa)</td>
<td>Chimpanzees, humans</td>
<td>Collins et al. 1969c; Contacos et al. 1970; Coatsney et al. 1971</td>
</tr>
<tr>
<td><em>P. simiovale</em></td>
<td>Sri Lanka</td>
<td>Macaques</td>
<td>Coatsney et al. 1971; Collins et al. 1972a, 1992a; Collins and Contacos 1979</td>
</tr>
<tr>
<td><em>P. simium</em></td>
<td>Howler (Brazil)</td>
<td>Night monkeys, squirrel monkeys</td>
<td>Collins et al. 1969a, 1973b, 1974a, 1979b, 1987a; Coatsney et al. 1971</td>
</tr>
</tbody>
</table>

Some parasites failed to develop beyond the presence of mature sporozoites in oocysts and transmission could be obtained only through the injection of mechanically harvested sporozoites. Those parasites requiring mechanical harvesting included *Plasmodium knowlesi,* *P. simiovale,* *P. coatneyi,* *P. fieldi,* and *P. inui.*

*Anopheles freeborni* has been shown to support the complete sporogonic development of many salivary glands of *An. freeborni. Plasmodium schwetzi* and *P. reichenowi,* parasites of chimpanzees and gorillas, and *P. hylobati,* a parasite of gibbons, also developed to maturity in this vector.
different species of Plasmodium, but fails to do so for other parasites (Fig. 1). This mosquito readily supports full development, to the presence of sporozoites in the salivary glands, of P. vivax and P. ovale and the related nonhuman tertian parasites P. cynomolgi, P. gonderi, P. schwezti, and P. simium; P. falciparum and the related parasite P. reichenowi; P. malariae and the related quartan parasite P. brasilianum; and the tertian parasite P. hylobati. Anopheles freeborni supports to a lesser extent the sporogonic development of the tertian parasites P. fragile, P. jefferyi, P. fieldi, P. simiovale, and P. coatneyi; the quartan parasite P. inui; and the quotidian parasite P. knowlesi. In these latter species, infective sporozoites are present in mature oocysts, but are rarely found in the salivary glands. Infectivity of these sporozoites has been demonstrated by the injection of susceptible hosts with sporozoites mechanically released from mature oocysts. In our studies with monkeys (Table 3), transmission via bite or the injection of sporozoites dissected from An. freeborni has been demonstrated with 9 species of Plasmodium. Be-

**Table 3.** Transmission of 9 species of Plasmodium from monkey to monkey by Anopheles freeborni mosquitoes.

<table>
<thead>
<tr>
<th>Species of Plasmodium</th>
<th>Donor</th>
<th>Recipient</th>
<th>Transmissions</th>
<th>Prepatent period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bite</td>
<td>Injection1</td>
</tr>
<tr>
<td>P. brasiliannum</td>
<td>Saimiri</td>
<td>Saimiri</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ateles</td>
<td>Ateles</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>P. coatneyi</td>
<td>Macaca mulatta</td>
<td>M. mulatta</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>P. cynomolggi</td>
<td>M. mulatta</td>
<td>M. mulatta</td>
<td>32</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>—</td>
<td>27</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>Aotus</td>
<td>Aotus</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>44</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>P. fieldi</td>
<td>M. mulatta</td>
<td>M. mulatta</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>P. gonderi</td>
<td>M. mulatta</td>
<td>M. mulatta</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>P. inui</td>
<td>M. mulatta</td>
<td>M. mulatta</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>P. knowlesi</td>
<td>M. mulatta</td>
<td>M. mulatta</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>P. vivax</td>
<td>Aotus</td>
<td>Aotus</td>
<td>37</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Saimiri</td>
<td>Saimiri</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Samiri</td>
<td>Samiri</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

1 Intravenous or intrahepatic injection of sporozoites dissected from oocysts or infected salivary glands.

Fig. 1. Mean oocyst diameter at 8, 10, 12, and 14 days and days when sporozoites are present in salivary glands or mature oocysts in Anopheles freeborni mosquitoes infected with 18 species of Plasmodium when incubated at 25 ± 1°C.
cause of the invariable success in all species tested, there is no reason to believe that sporozoites from salivary glands or mature oocysts of the other species of human and nonhuman primate malaria parasites produced in An. freeborni could not also be infective to susceptible primate hosts.

Anopheles freeborni, after long-term establishment in the laboratory, has been shown to be highly susceptible to many species of Plasmodium from widely separated geographic areas. In addition, the susceptibility to many different strains of the parasites from widely separated geographic areas allows us to use this mosquito as a standard for all studies with human malaria parasites and most of the nonhuman-infecting species.

Many questions remain concerning the relationship between this mosquito host and the different species of human and nonhuman primate malaria parasites. The reasons why one species of Plasmodium develops completely, whereas another does not, deserves investigation. Why are oocyst densities higher in An. freeborni than in other mosquitoes when simultaneously fed on certain strains of Plasmodium? Why is the reverse true with other species and strains of anophelines simultaneously feed on the same animal? The extensive data available from studies on the F-I strain of An. freeborni generate more questions than answers on the vector-parasite relationships for this extremely important group of human pathogens.

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