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Abstract approved: 

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This study describes and quantifies some microprocesses of malaria transmission including blood vessel location by *Anopheles stephensi* and sporozoites delivery of *An. stephensi* infected by *Plasmodium berghei*. The study models the effects of malaria parasite-induced changes in probing behavior and mosquito mortality on disease transmission. Finally, host, vector, and parasitological aspects of malaria optimal control are explored, concluding that classical entomological parameters are insufficient; this unexpected conclusion may lead to revisions of control strategies.
Epidemiological Implications of Sporozoite Aggregation in Malaria Vectors

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The most important effect of mosquitoes on human beings is as vectors of diseases, rather than as pests. Many parasites pathological to humans are thus transmitted. The best known, malaria, infects about 1 out of 15 people on earth. Because of its pathology, it has been suggested that malaria seriously impacts more people than any other single disease. The transmission cycle of malaria was first described by Ronald Ross late last century in India, for which he received the first Nobel Prize in Medicine in 1902. Since then, extensive research has been done on the parasite and on the interactions between parasite, vector and host. Nevertheless, numerous basic processes of transmission still remain unclear.
Review of Literature

Malaria species

Malaria parasites are protozoa belonging to the Order Coccidiidae, Suborder Haemosporidiidea, Family Plasmodiidae, which consists of various parasites found in the blood of reptiles, birds and mammals and undergo two types of asexual multiplication, namely, schizogony in the vertebrate host (two separates events in the case of mammals, namely, in the liver cells and in the red blood cells) and sporogony in the vector (on the basal gut wall in Anophelines).

There are about 100 species of Plasmodium, including 4 found exclusively in humans (the most common is P. vivax=benign tertian malaria, the most lethal P. falciparum=malignant tertian, P. malariae=quartan, and the rarest P. ovale=ovale), 50 of birds or reptiles, at least 22 different species of monkeys and apes and others are found in rodent, buffalo, deer, fruit-bat and many other species. Animal malaria parasites do not naturally infect human hosts and vice-versa, although a recent study of evolutionary relationships by DNA sequencing suggests that P. falciparum is more closely related to bird malaria that simian malaria, unlike other human species (Waters et al. 1991).

Mosquito species

As is the case with all mammalian malariae, the four human malaria species are only transmitted by mosquitoes of the genus Anopheles; other non-mammalian malaria species are transmitted by culicine mosquitoes. The vectors of human malaria belong to the order of Diptera, family Culicidae, sub-family Anophelinae and genus Anopheles, of which there are several sub-genera. In addition to transmitting malaria, anophelines also carry lymphatic filariases and a few viral diseases, but culicine species are more important vectors of the two latter groups of agents. There are about 400 species of Anopheles mosquitoes
have been recorded in the world, but only 60 species, many of them cryptic, are important in malaria transmission under natural conditions (Bruce-Chwatt, 1985).

**Malaria life cycle**

A mosquito taking blood from a malaria-infected human can become infected only if this person's blood contains the stages of the parasite called micro- and macrogametocytes. These are stages of the parasite that have differentiated by meiosis into sexual individuals, male and female, respectively. These organisms are still within a red blood cell, but, once inside the vector's gut and in response to specific cues therein, quickly rupture through the membrane, and are then referred to as micro- and macrogametes. During the process, the microgametocyte exflagellates, releasing numerous active and motile gametes. Macrogametocytes contain a single individual. A microgamete will join with a macrogamete and form a zygote, called an ookinete, which is capable of slow movement. It will travel through the blood meal, pass through the gut wall, which is one cell thick, and settle on the basal side, lodged between the cell layer and the basal lamina. There is nothing particular about this site, for if ookinetes are injected directly into the hemolymph, they can continue development. Further differentiation occurs, and the stage is now called an oocyst. Within it, asexual reproduction by a specialized form of mitosis takes place and after a period of 10 to 14 days, depending on species and temperature, thousands of sporozoites burst out of both the oocyst and the basal lamina into the hemolymph. Sporozoites then enter the salivary glands where they will reside until the vector injects them into another host via the saliva. Incidentally, recent reports suggest that sporozoites are haploid, suggesting that meiosis occurs in the vector prior to sporogony (Krotoski et al.)
1982). Sporozoites are apparently haploid, indicating that meiosis occurred during sporogony, but this remains to be elucidated.

Once inside a suitable host, a successful sporozoite eventually is carried by the blood flow to the liver, where it passes through the reticulo-endothelial cell layer and settles inside a hepatocyte. The sporozoite now transforms from its elongated from into a spheroid shape and is now called a merozoite. Asexual reproduction proceeds through a form of mitosis termed schizogenesis. This intracellular stage is called a schizont. The products of this process are again merozoites, which, once released, proceed to the bloodstream and invade red blood cells, or erythrocytes. The merozoite may proceed along two paths of differentiation. One will lead to an ameboid trophozoite and erythrocytic schizogony and the eventual release of merozoites into the blood, thus repeating the erythrocytic cycle; it is this synchronous event that elicits the periodic fevers and chills so characteristic of malaria. Alternatively, a certain number of merozoites will become gametocytes. This stage will not develop any further unless it is taken up by a suitable mosquito.

It is, at first encounter, a complicated life cycle. Even though Ronald Ross is credited with elucidating it, it must be realized that he concluded that the life cycle of malaria was completed in a mosquito vector based on the presence of sporozoites in the salivary glands of a culicine mosquito species following its feeding on an infected bird (his pet canaries, legend has it). Having previously observed oocysts in mosquitoes having fed on a malarial patient, he could strongly suggest that human malaria was vector-borne as well. Subsequent confirmation of the human cycle and discovery of the various stages was done by Italian workers under the guidance of Grassi as well as by various English workers, notably Garnham and Shute. Ronald Ross received the first Nobel prize in Medicine, was knighted and spent most of the remainder of his
life discrediting the contributions of others. The history of the discovery of malaria and subsequent events is eloquently related in Harrison's *Mosquitoes, Malaria and Man* (1978).

So intrinsically difficult is work on human parasites that this last decade still yielded a major discovery in the field. An anomaly had existed in that *P. vivax* has a number of geographic strains which are relapsing, especially in northern regions. Incidentally, the perception of malaria as a tropical disease is a 20th century one and quite false; only falciparum malaria is exclusively tropical. An episode may be followed by a number of others although no subsequent reinfection occurs. In human malaria, the phenomenon is specific to vivax malaria. Previous workers postulated that it was unique in that merozoites released from hepatic schizonts could reinvade hepatocytes, rather than only erythrocytes as other malaria species all do. Recently, researchers (Krotoski *et al.* 1982) have identified a dormant phase, which they called a hypnozoite, occurring after invasion of hepatocytes by sporozoites of vivax-like malaria in primates and of vivax and ovale in human beings. This discovery presents a parsimonious life cycle and thus brings relapsing malaria in line with other species.

**Mosquito probing and feeding behavior**

In order to obtain a blood meal, a female (males cannot blood-feed) mosquito has to locate and lacerate a blood vessel, either directly tapping the vessel or causing a hematoma. Mosquitoes salivate before ingesting blood (Griffiths and Gordon 1952) and some pathogens, malaria included, are transmitted into the host with the vector saliva; a few parasites, such as filaria, do not depend on salivation. Mellink (1982) reported that successful virus infection of a host seems to correlate with duration of probing rather than
duration of ingestion. Similarly, it was reported that malaria was transmitted by mosquito that have not yet begun to ingest blood (Boyd and Stratman-Thomas 1934). Mosquito probing behavior is obviously related to pathogen transmission.

A mathematical model of mosquito blood location behavior has been described that accurately simulates blood location (Ribeiro et al. 1985). The model is valuable because it uses only biological parameters such as vessel distribution, apyrase level, probability of blood location, desistance, and host switching. This model, however, has one major drawback in that it relies on a single species, the yellow fever mosquito, *Aedes aegypti* (L.), a vector of avian malaria. Although the salivary gland structures of culicine and anopheline mosquitoes are somewhat similar (Wright 1969; Janzen & Wright 1971), and salivary apyrase has been identified in anopheline vectors of human malaria (Ribeiro et al. 1985), the behavioral parameters of blood location by anophelines have not been well described. In addition, the model mentioned above does not explain the phenomenon that probability of feeding success increases rapidly at first and then decreases.

Once a mosquito successfully locates and lacerates the blood vessel of a host, it must overcome the host's hemostatic apparatus in order to maintain blood availability during feeding (Ribeiro et al. 1984, 1985). Because platelet aggregation in vertebrates largely determines hemostasis (Mustard and Packham 1977; Vargaftig et al. 1981) and adenosine tri- and diphosphate (ATP and ADP) are one of the most important component for this activity, ADP-degrading enzymes might serve as a common salivary component of blood feeders. Experiments have shown that apyrase is an enzyme that posses such function (Ribeiro et al. 1984; Ribeiro et al. 1985). More interestingly, it was found that malaria parasites could reduce the apyrase level in mosquito salivary glands.
(Rossignol et al. 1984) and result in increased intradermal probing time (Rossignol et al. 1984; Ribeiro et al. 1985; Rossignol et al. 1986).

Increased intradermal probing time might not only increase the size of the sporozoite inoculum to the host that the mosquito feeds on, but also increase the number of hosts that the mosquito contacted if the mosquito’s probing is often interrupted by host reaction. Monte Carlo simulations were developed to assess the potential impact of parasite pathology on vector salivary function as well as of host hemostasis on transmission by Rossignol and Rossignol (1988), and reported that an exponential relationship exists between parasite load and transmission. Burkot (1988) reviewed certain aspects about non-random host selection by anopheline mosquitoes, and presented a summary of factors influencing host selection by malaria vectors. The effects of parasite-mediated behavioral changes on malaria dynamics were discussed in it.

However, the fact that pathogen is transmitted during vector probing period (before blood-sucking) was ignored in the discussion, therefore some arguments are not very convincing. Dye and Hasibeder (1986) studied the consequence of non-uniform exposure on disease persistence and equilibrium level, and reported that the basic reproduction rate and vectorial capacity are always greater than or equal to their values under completely homogeneous mixing. In addition, Kingsolver (1987) studied the non-random host choice of mosquitoes caused by hosts attraction and no parasite mediated mosquito behavior changes were considered in this work.

**Sporozoites delivery**

It is important to learn how many sporozoites are injected into a host when a infective mosquito probing on a host because experiments have shown that inoculum may determine survivability of parasites in the host or the course
of the parasite development later and severity of the resultant disease (James et al. 1966). Indirect field observations also revealed dose effects on malaria transmission (Greenwood et al. 1991).

Some researchers have explored the nature of sporozoites delivery. Rosenberg et al. (1990) used An. stephensi mosquitoes infected with P. falciparum and made them produce time-dependent series of saliva droplets in mineral oil. The relative volume of each droplet and the number of sporozoites each contained were determined microscopically. They concluded that most sporozoites were injected within the first few 'drop' of saliva in the mineral oil. They inferred that infected mosquitoes depleted their available sporozoites in the first few probes. This study, however, did not take into account delivery into oil may be pathogenic in itself (many mosquitoes were unable to salivate after a few minutes) nor did it determine whether mosquitoes could deliver sporozoites after probing on a host. Beier et al. (1991a) used similar apparatus, but filled capillaries with human blood or sucrose to test sporozoites delivery, and found that transmitting Anopheles contained significant more salivary glands sporozoites than non-transmitters. Mosquitoes were still passively injecting saliva in this process. Ponnudurai et al. (1991) let infected mosquitoes probe through mouse skin membranes on 200 ul of continuously-stirred blood to count the number of sporozoites injected. Using mouse skin as probing medium for mosquitoes might be more natural than other materials previously used. However, when they correlated the number of probes and the total number of sporozoites injected into skin, the degree of the mosquitoes' infection was not accounted; nor did they consider the probing time when they correlate the number of sporozoites injected and sporozoite load. Habluetzel et al. (1992) induced individual mosquitoes to salivate on coverslips, and sporozoites deposited on the glass surface were visualized by Giemsa staining. The
advantage of this method is that after testing, mosquitoes are still unaffected, though the number of sporozoites observed is a relative number.

It has been recognized that knowledge of the biology of the parasite's life cycle cannot lead to a complete understanding of what causes the radical differences in malaria distribution patterns in different parts of the world (Koella 1991). Such understanding can only be reached by considering the various dynamic factors that affect malaria transmission. Analytical mathematical modelling is one of the effective approach for that.

**Basic reproduction rate and malaria control strategy**

1. *General assumption for basic reproduction*

   The earliest quantitative method used to describe the dynamics of malaria was developed by Ross (1911), Lotka (1923) and Macdonald (1952, 1957). The formula for basic reproduction rate (number of secondary cases arising from a primary case over the period of infectivity) might be the most important result in these studies. Although the basic reproduction rate captures the basic feature of the dynamics of malaria, some strict assumptions should be borne in mind. The major assumptions are that, (1) the vector is fully effective in acquiring parasite and infecting host, (2) vectors die at a constant rate, independent of age, (3) vector longevity is unaffected by the parasite, (4) vector feeding is random, that is, the probability of feeding on infected and noninfected, human host and other hosts are same. Based on these assumptions Ross and Macdonald developed a model to describe malaria dynamics.

2. *Two derivation approach to the basic reproductive rate*

   Ross (1911) used differential method to set up a deterministic formula of the population dynamics of malaria. The variables used in the model are listed as follows:
\[ t : \text{ time} \]
\[ n : \text{ total number of humans at a given time} \]
\[ n' : \text{ total number of mosquitoes at given time} \]
\[ y : \text{ total number of infected humans} \]
\[ y' : \text{ total number of infected mosquitoes} \]
\[ f : \text{ proportion of infected humans who are also infectious} \]
\[ f' : \text{ proportion of infected mosquitoes which are also infectious} \]
\[ r : \text{ recovery rate of human hosts} \]
\[ r' : \text{ recovery rate of mosquitoes} \]
\[ u : \text{ birth-rate of human host} \]
\[ u' : \text{ birth rate of mosquitoes} \]
\[ v : \text{ death rate of human host} \]
\[ v' : \text{ death rate of mosquitoes} \]
\[ b' : \text{ man-biting rate of mosquito} \]

If mosquitoes and human hosts are homogeneously distributed, then in time \( \Delta t \) infected mosquitoes make \( b'f'y'\Delta t \) infectious bites, of which a proportion \( (n-y)/n \) are on susceptible humans. If we assume that there is no superinfection or the proportion of infected human host is very low, then the number of new human infections in \( \Delta t \) is \( b'f'y'(n-y)\Delta t/n \). The number of recovered and dead hosts from infected part within \( \Delta t \) are \( ry\Delta t \) and \( vy\Delta t \). We obtain the differential equation describing the rate of the human infected population is,

\[
\frac{dy}{dt} = \frac{b'f'y'(n-y)}{n} - (r + v) y
\]

The vector's infection is caused by biting an infectious hosts. Thus, similarly but not symmetrically the infected mosquitoes are calculated as follows. Within time \( t \), \( n'-y' \) non-infected mosquitoes make \( b'(n'-y')\Delta t \) bites,
of which $yf/n$ are infectious. Therefore, the number of new infected mosquitoes are $b'y(n' - y')\Delta t/n$. Recovered and dead mosquitoes in time $\Delta t$ are $r'y'$ and $v'y'$, respectively. The differential equation describing the rate of mosquito population infection is:

$$\frac{dy'}{dt} = \frac{b'y(n' - y')}{n} - (r' + v') y'$$

If we neglect the death of infected human hosts and recovery of infected mosquitoes, and assume that the birth and death rates of mosquito and human populations are exactly balanced with each other, $v'$ could be replaced by $u'$ and then the above equations could be rewritten as follows:

$$\frac{dy}{dt} = \frac{b'y(n - y)}{n} - r y$$

$$\frac{dy'}{dt} = \frac{b'y(n' - y')}{n} - u' y'$$

If we let $m = y/n$, $w = y'/n$ and $a = n'/n$ then above equations become,

$$\frac{dm}{dt} = b'f w (1 - m) - rm$$

$$\frac{dm}{dt} = b'f m (a - w) - u' w$$

One nontrivial steady state solution of the nonlinear equations is,

$$m = \frac{ab'^2ff' - ru'}{b'f(r + ab'f') }$$

$$w = \frac{ab'^2ff' - ru'}{b'f (u' + b'f)}$$

and the nominator of either solution is the basic reproduction rate,

$$Z_0 = ab'^2ff' - ru'$$

If $Z_0 < 1$, that is $ab'^2ff'/ru' < 1$, then malaria will eventually disappear; if $Z_0 > 1$ that is $ab'^2ff'/ru' > 1$, then malaria will increase.
Macdonald (1952, 1957) used algebraic methods and obtained the basic reproduction rate formula. The symbols used for this derivation are:

- \(a\): number of bites per host per mosquito per day; so called *man-biting habit*
- \(b\): the proportion of mosquito with infective sporozoites
- \(m\): relative density of mosquitoes (vectors per host)
- \(ma\): total number of bites per host per day; so called *man-biting rate*
- \(n\): the length of extrinsic incubation period
- \(p\): the probability of a mosquito survive through one day
- \(r\): recovery rate of host
- \(x\): the proportion of hosts infected

If the probability of a mosquito surviving through one day is \(p\), then the expectation of life is \(1/-\ln p\) (\( \ln \) being the 'natural', or base \(e\), log). If the average number of infective bites taken on one day is \(ax\), the probability of not doing so is \(e^{-ax}\). Thus the expectation of non-infected life mosquitoes is \(1/(ax-\ln p)\). Therefore, over the course of infectivity \((1/r)\), a case will be bitten each day by \(ma\) mosquitoes of which the proportion not yet infected will be \((1-ax/(ax-\ln p))\). The proportion of these surviving for \(n\) days is \(p^n\), and their subsequent expectation of life is \(1/-\ln p\). During this time they will bite \(a\) times each day, and the proportion \(b\) of these bites will be infective, therefore

\[
Z_0 = \frac{(1-ax/(ax-\ln p))ma^2bp^n}{-r \ln p}
\]

Based on a general assumption (proportion of infected human host is very low)

*let \(x\) close to zero, then*

\[
Z_0 = \frac{ma^2bp^n}{-r \ln p}
\]
3. System analysis and the application of basic reproduction rate

3.1 System stability analysis

We first analyze the stability conditions of the malaria dynamics system. The following equations describe them.

\[
\frac{dx}{dt} = abm(1-x) - rx
\]

\[
\frac{dy}{dt} = ax(1-y) - \mu x
\]

The details of this model are available in Chapter V. Two equilibria exist,

\((x_{e0}, y_{e0}) = (0, 0)\)

\((x_{e1}, y_{e1}) = \left( \frac{a2bm - ru}{a2bm + ra'}, \frac{a2bm - ru}{abm(a + u)} \right)\)

Firstly, we analyze the stability of the system around \((0, 0)\). The Jacobian matrix of the system at \((x, y) = (0, 0)\) is:

\[
J = \begin{pmatrix}
-r & -amb \\
ar & -u
\end{pmatrix}
\]

The symbol \(\lambda\) representing eigenvalues, then the system may be represented with the matrix,

\[
\begin{pmatrix}
-r-\lambda & -amb \\
ar & -u-\lambda
\end{pmatrix}
\]

from the determinant of which the characteristic polynomial is,

\[
\lambda^2 + (r + u)\lambda + ru - a^2bm = 0
\]

According to Routh-Hurwitz criteria, if \(ru - a^2bm > 0\), then the equilibrium \((0, 0)\) is stable.

Secondly, we analyze the stability of the system around non-zero equilibrium mentioned above. The Jacobian matrix of the system at \((x_{e1}, y_{e1})\) is:
Similarly, it can be shown that if $ru < a^2bm$ then the system is stable.

3.2 Control strategies based on the basic reproduction rate analysis

The difference of basic reproduction rate $Z_0$ derived by Ross and Macdonald is that former did not consider the mosquito incubation period the later did. If we multiply Ross's basic reproduction rate by $p^n$ then it will be exactly identical to the $Z_0$ derived by Macdonald. Obviously, it is more reasonable to consider the mosquito incubation period. The biological meaning of $Z_0$ is that if $Z_0 < 1$ then malaria can not maintain itself and will go extinct; if $Z_0 > 1$ then malaria will increase.

From the formula of $Z_0$, we can see that mosquito daily survival rate has large effect on $Z_0$. In practice, therefore, adulticiding will more effective than larviciding for malaria control. The second largest effect on $Z_0$ is mosquito daily biting habit, $a$.

We should point out that the basic reproduction rate demonstrates the relationships between biological parameters and malaria development to a certain degree. However, we can qualitatively infer some malaria control strategies based on $Z_0$ instead of quantitatively. We will further discuss the question in Chapter V.

4. Parameter estimation

4.1 Parameter bias

It is important to accurately estimate the parameters in the models in order to make the models effective. Experiments have shown that parasites could greatly affect their insect hosts, and therefore result in differences
between infected and noninfected vectors in the magnitude of many biological parameters. Dye (1990) points out that vectorial capacity is inestimable in practice and cannot be calculated with confidence because estimates of most its components are biased (besides being imprecise). Man-biting rate and survival rate of mosquitoes are the most important parameters in basic reproduction rate, but they are also the most difficult parameters to estimate. Numerous experiments have shown that mosquito daily survival rate is not a constant, but significantly affected not only over time (age of mosquitoes) but also by parasites. These facts have shaken the prerequisite assumptions of Macdonald's model. Therefore more experiment should be done on these aspects, especially on survival analysis.

4.2 Survival analysis

From the above discussion, we noticed that it is important to estimate accurately the survival rate of mosquitoes in malaria dynamics. Because some misconceptions on animal survival rate estimation exist in some research work (such as in Dye 1990, Dye 1992), we feel it is necessary to clarify the problem here. This may not only dismiss some further abuse of survival rate data but may also provide a useful direction for further experimental designs. We might began to address the problem with the basic derivation. Let, 

\[ P(t) \] 
be probability of survival to time \( t \) of a vector and \( F(t) \) probability of not surviving the interval. Then, 

\[ P(t) + F(t) = 1. \]

If we know the probability distribution of death \( f(t) \), we can derive the mortality curve by continuous integration,

\[ F(t) = \int_{0}^{t} f(t) \, dt \]
On the other hand, if the mortality curve is known, we can find the probability distribution function by differentiation,

\[ f(t) = \frac{dF(t)}{dt} \]

Let \( Z(t) \) be the death rate per unit time, then by definition (Bompas-Smith 1973),

\[ Z(t) = \frac{\int_{0}^{t+1} f(t) \, dt}{F(t)} = \frac{\frac{d}{dt} F(t)}{P(t)} = \frac{F(t)}{F(0)} \left[ -\ln (1-F(t)) \right] \]

Now we can get relationship between \( P(t) \) and \( Z(t) \),

\[ \int_{0}^{t} Z(t) \, dt = \int_{0}^{t} \frac{dF(t)}{P(t)} \, dt = \frac{F(t)}{1-F(t)} \int_{F(0)}^{F(t)} \frac{dF(t)}{F(0)} \]

\[ = \left[ -\ln P(t) \right]_{0}^{t} = -\ln P(t). \]

Therefore,

\[ P(t) = e^{-\int_{0}^{t} Z(t) \, dt} \]

If we assume that a insect population has a constant mortality rate \( q \), then we have,

\[ Z(t) = q, \text{ and } P(t) = e^{-qt} \]

(2)

From (1) we have \( f(t) = Z(t) P(t) \). If \( Z(t) = q \), then \( f(t) = q e^{-qt} \).

We then can use maximum likelihood method to estimate parameter \( q \).

The likelihood function is:

\[ \ln L(q,t) = n \ln(q) - q \sum_{i=1}^{n} (t_i) \]
Let,

\[ \frac{d}{dt} \ln L(q, t) = \frac{n}{q} - \sum_{i=1}^{n} (t_i) = 0 \]

From which

\[ q = \frac{n}{\sum_{i=1}^{n} (t_i)} = \frac{1}{t} \]

(3)

From equation (3) we have average life span \( t = \frac{1}{q} \).

From equation (2), \(-q = \ln P(t)\), \( t = \frac{-\ln P(t)}{q} \).

If \( t = 1 \), then \( q = -\ln P(t) \). Therefore, \( t = \frac{1}{q} = \frac{1}{-\ln P} \).

From which it can be seen that only when \( t = 1 \), does \( \frac{1}{q} = \frac{1}{-\ln P} \).

Experimental design for measuring vector mortality should depend on specific insect life cycle.

In some studies the death rate \( q \) obtained by the researchers is far larger than 0.1; \( e.g. \ q = 0.4 \); this would create a large difference between \( 1/q \) and \( 1/-\ln P \). This is because the death rate is high, and the unit time \( t \) at which the observations were made is too wide. For example, if \( q = 0.4 \) then \( t = \frac{-\ln(t)}{q} = \frac{-1}{0.4} = 2.511 \). This means that data observation intervals should be narrowed (increase observation frequencies) by 1.3 times. As another example, if we set to calculate a certain beetle's longevity, because the beetles usually can live for a few months, every two or three days as our observation unit time will be good enough to keep \(-\ln P/q\) approaching 1. If however we calculate a mayfly's longevity, it may only last 2 days as an adult. If our time unit is one day, then the daily death rate is very high and \(-\ln P/q\) will not approach 1. This means that the observation time unit is too large and a substantial error may
result. If we observe the population every two hours then \(-\ln P/q\) will approach 1. The calculation based on this data would well describe a mayfly's life expectancy. We therefore can see that it is very important to determine the observation time unit for a specific research object; \(-\ln P/q\) should be close to 1. If not, it means that the time unit is too large. Arbitrarily using a high death rate \(q\) (roughly say \(q > 0.2\)) will introduce error.

In the above discussion, we assumed that a population has a constant death rate \((Z(t) = q)\). However, many studies have shown that the mortality rate increases with age (Kershaw et al., 1954; Clements and Paterson 1981). This could be well described by a classical probability model, the Gompertz function.

In this model the death rate is no longer a constant but a variable as a function of time \(t\).

\[ Z(t) = \alpha e^{\beta t}. \]

Then the survival function becomes:

\[ P(t) = P_0 e^{-\int_0^t \alpha e^{\beta t} dt} = P_0 e^{-\frac{1}{\beta} \int_0^t e^{\beta t} d(\beta t)} = e^{\frac{\alpha}{\beta} (e^{\beta t} - 1)} \]

The later analysis is similar to that of constant mortality rate.

Overall, the Ross-Macdonald model provides us with some insight on the malaria dynamics, based on certain assumptions. However, some assumptions in the model are too strict and a few factors such as immunity, mosquito population dynamics, host age have not been considered.
Review of malaria transmission models

Modelling approaches have been widely used in infectious disease studies (Bailey 1975; Bailey 1977; Anderson 1982). More recently, nonlinear transmission has been extensively studied (Hadeler and Dietz 1983, 1984; Kretzschmar 1989, 1989; Hethcote and van den Driessche 1991; Hochberg 1991). Population biology theory of infectious diseases has been experimentally tested by some researchers and reviewed by Anderson and May (1979).

Many models have been developed to describe malaria dynamics more accurately since the recognition of the Ross-Macdonald model. Nedelman (1985) mainly reviewed the Ross-Macdonald model and gave a detailed interpretation and estimation of some parameters. Aron and May (1982), based on the Ross-Macdonald model, created a few concise models that accommodated more factors such as incubation period in mosquito, variable mosquito density, superinfection and immunity. Nasell (1985) reformulated the Ross-Macdonald model with stochastic transitions for infection and recovery in humans, and infection and death in mosquitoes. The mean values of the infection rates were used to replace stochastical infection rates; the model is called a hybrid model. The results from this model showed that it is essentially an extension of the Ross-Macdonald model. Bailey (1982) gave an extensive review on malaria dynamics model. In this work he not only evaluated different malaria models, but also elaborated applications of control theory and sensitivity theory in infectious disease study. Dietz (1988) surveyed and developed five models of malaria transmission, in which age structure of human hosts population, superinfection and heterogeneous contact rate were considered. Age dependent mortality in the vector, models with temporary states of complete resistance and a 'Garki' (named after a WHO control program in an African region of this name) model were also evaluated in this work.
Effects of immunity on malaria transmission have received a certain attention. A mathematical model in which the effect of naturally induced transmission blocking immunity was accounted for has been developed (Arjuna et al. 1988) based on field data to assess the immunity effect on malaria transmission. Two simulation studies (Struchiner et al. 1989; Halloran et al. 1989) analyzed the possible population effects of mass vaccination programs against malaria. In addition, a simulation study (Struchiner et al. 1990) compared the behavior of common measures of association derived from case-control study in the context of a malaria vaccine programme. De Zoysa et al. (1991) developed a multi-state mathematical model to estimate the impact of transmission-blocking immunity on malaria transmission in an endemic area by using field data.

We have to be cautious, in that more detailed or complex models do not necessarily result in better prediction. In fact, the results of more complex models may be less reliable than those of simple ones (Lee 1973; O'Neill 1973). Generally speaking, these malaria models are too complex to analytically study them with control theory.

**Malaria optimal control**

Once we have developed models that could describe a system with reasonable accuracy, then we could think, based on the models, how to manage it to a particular state. To drive a system from any state to a desired state with the least cost or expense is the major problem that optimal control theory deals with. The theory has been widely used in economics, ecology and medicine in addition to engineering. A wide range of applications of the theory to the control of the pests and infectious disease have been reviewed by Wickwier (1977). The first main work studying malaria optimal control was done by
Dietz (1975) in which discrete optimal control method was used. This work showed that a drug administration program can be specified by an accessible proportion of population, a proportion of drug administered population, a rate of loss of protection and length of interval of drug administration. Dietz especially studied the control strategies for high or low initial basic reproduction rate $Z_0$ and found that for high $Z_0$ drug administration alone may be relatively ineffective, but if the rate is substantially reduced by spraying, eradication of the parasite may be achievable. Gonzalez-Guzman (1980), based on Ross' single equation model gave an theoretical study of the problem of controlling a parasitic disease using a time-continuous optimization method. This study concluded that vector reduction and drug application programs have greater long-term effects than the sum of the effects for each program carried out separately; and insecticides and drug applications should be applied as extensively as possible. The two works mentioned above both used Ross' single model which have been proved that some potential shortcomings exist when compare it with Ross' two equations model (Lotka 1923). To use Ross' two equations model to study malaria control problem might be worthwhile. I delineate such an attempt in Chapter V.

Effect of parasite induced mortality and parasites distribution patterns on malaria dynamics

Many researchers have reviewed the interactions between parasites and hosts from different point of views (Ewald 1983; Dobson 1988; Johnson 1986; Scott and Dobson 1989; Anderson 1991; Read and Schrag 1991; Medley 1992). Anderson and May (1982) studied the coevolution of hosts and parasites based on prevalence models and found that the coexistence of different strains depends not only on their virulence but also on factors influencing their overall
transmissibility, and a 'well-balanced' host-parasite association is not necessarily one in which the parasite does little harm to its host. Levin and Pimentel (1981) presented a simple mathematical model to demonstrate how easily group selection can theoretically stabilize a parasite-host system. The distribution pattern of parasites in their host is of potential effects on the interaction processes.

Parasite distribution patterns have been proven to play important roles in parasites hosts interaction (Anderson and May 1981; Dobson 1988). Under the assumption that the net rate of parasite-caused host mortality is related to the average parasite burden of the members of a host population, and therefore related to the statistical distribution of the parasite within a host population, Anderson and May (1978) studied the general effects of parasites distribution patterns on the host-parasite interactions. It was demonstrated that when the parasites are randomly distributed within host population, a linear relationship between host death rate and parasite burden gives rise to the pathological condition of neutral stability. If however, the rate of parasite-induced host mortality is an increasing function of parasite burden, random distributions of parasite may lead to globally stable equilibria where the parasite is effectively regulating the growth of its host population. The factors which create various patterns of distribution of parasites within their host populations has been examined by using Monte Carlo simulation method (Anderson and Gordon 1982).

Many field and laboratory studies have shown that malaria parasites are not evenly distributed in infected vector or host populations, but seem highly aggregated. Such distribution patterns might result in density dependent transmission of malaria, parasite density dependent mortality of vectors or hosts and heterogeneous immune reaction of host.
The aggregated distribution patterns of malaria parasites have been revealed in many field and laboratory observations (Collins et al. 1984; Rosenberg et al. 1990; Sattabongkot et al. 1991; Beier et al. 1992). Numerous studies have shown density dependent transmission effects. Janse et al. (1985) reported that there is a positive relationship between gametocytes and ookinetes. The ultimately number of sporozoites are positively correlated to gametocyte density in the vertebrate host (Boyd 1949; Eyles 1952; Carter and Graves 1988). The density of first generation asexual parasites in the blood might reflect the dose of sporozoites received by the host (McGregor 1965; Vanderberg 1977).

Density dependent mortality of mosquitoes have been observed in some experiments. Klein et al. (1986) reported that a correlation between mortality and oocyst number may exist with P. cynomolgi in A. dirus. This mortality was most evident at high loads. Cox (1966) reported that death rate of laboratory mice infected by P. vinckei is nonlinear and positively correlated to parasite load. When infected vector or host die, the parasites will be lost and parasites not only effect vector and host populations but also effects itself. It can bee seen, therefore, that there are potential effects of parasite aggregation on the population dynamics of malaria.
Objectives of the Study

When a malaria-infected mosquito acquires a blood meal, sporozoites may be transmitted to the host during the mosquito's probing and feeding activity. Many complex actions and interactions occur in this transmission process. Initially, a mosquito attempts to locate a vessel, namely, an arteriole or a venule. She may need many probes to be successful in this blood location. When she lacerates a blood vessel during this process, platelet aggregation would normally inhibit the subsequent hematoma. However, a salivary enzyme, apyrase, inhibits platelet aggregation (Ribeiro et al. 1984; James and Rossignol 1991). Malaria sporozoites manipulate this system and damage mosquito salivary glands, thus reducing apyrase levels and prolonging probing time (Rossignol et al. 1984). Throughout probing, saliva delivers sporozoites into host tissues. The number of sporozoites delivered or the number of gametocytes ingested could impact on infection (Christophers 1924; Sinton 1926; Boyd 1949; Gamage-Mendis et al. 1993) and may modify dynamic behavior of malaria transmission.

This study therefore focused on following aspects:

A. It determined whether or not an anopheline mosquito, *Anopheles stephensi*, a well-known vector of human malaria, has similar probing parameters as the only previously studied vector, the yellow fever mosquito, *Aedes aegypti*.

B. It modelled the potential transmission effects of parasite-induced changes on mosquito probing behavior.
C. It determined whether or not infected mosquitoes deliver sporozoites continuously. The effects of observed sporozoite aggregation was modeled in an attempt to understand the evolution of this phenomenon.

D. Optimal control theory was applied to the two equations model of malaria transmission in order to compare different objectives and priorities in future campaigns.

E. The evidence of sporozoite aggregation and pathology in the vector was used to derive a model analyzing epidemiological implications.
CHAPTER II
BLOOD VESSEL LOCATION TIME BY ANOPHELES STEPHENSI
(DIPTERA: CULICIDAE)

Introduction

To bloodfeed successfully, a female mosquito must first locate blood in the form of a venule or arteriole, either by tapping it directly or lacerating it and causing a substantial enough hematoma at the site of the bite (Gordon & Lumsden 1939, Ribeiro et al. 1984, Ribeiro et al. 1985a). This action can be accomplished more efficiently if one of the host's hemostatic mechanisms, namely platelet aggregation, is impaired. Mosquitoes that have been prevented surgically from salivating have difficulty locating blood (Ribeiro et al. 1984). A salivary enzyme, apyrase, specifically inhibits platelet aggregation (Ribeiro et al. 1984). The role of salivation in bloodfeeding has been reviewed by James and Rossignol (1991) and Ribeiro (1987).

Based on these findings, a mathematical model of mosquito blood location behavior has been described that accurately simulates blood location (Ribeiro et al. 1985a). The model may be used to make potentially important predictions on parasite manipulation, such as a bias towards infected hosts and enhanced rate of contact by infected mosquitoes (Rossignol & Rossignol 1988). The model is also valuable both because it uses only biological parameters, namely vessel distribution, apyrase levels, probability of blood location, desistance and host switching and because malaria parasites have been shown, in one model tested so far, to decrease apyrase levels (Rossignol et al. 1984) and host platelet function (Rossignol et al. 1985), thus possibly impacting on transmission. This model, however, has one major drawback in that it relies on
a single species, the yellow fever mosquito, *Aedes aegypti* (L.). Although salivary gland structure is somewhat similar between culicine and anopheline mosquitoes (Wright 1969, Janzen & Wright 1971) and salivary apyrase has been identified in anopheline vectors of human malaria (Ribeiro et al. 1985b), the behavioral parameters of blood location by anophelines have not been described. We therefore analyzed the probing, or blood vessel location behavior, of *Anopheles stephensi* Liston and determined whether or not epidemiological implications derived from *Ae. aegypti* work can be considered valid for this anopheline vector of human malaria.
Materials and Methods

Mosquitoes used in this study were *Anopheles stephensi* (India strain). Larvae were fed finely powdered Tetramin (R) fish food. Adults were fed sucrose and given free access to water. Mosquitoes were held at room temperature (70°F ±2°) with a 12:12 hr L:D cycle. Mosquitoes used in the experiments were 3 to 6 days old and not exposed to males.

A probe is defined as the insertion and complete withdrawal of the stylets. The duration of probing is defined as the length of time that the stylets are within tissue before blood location or do not probe further and is therefore the sum of the duration of the individual probes; time that the stylets are not in tissue is not included (see Ribeiro et al. 1984, 1985a). Data on duration of probing was recorded and entered directly into a computer through a BASIC program (available from X. L. upon request).

White female laboratory rats (Sprague-Dawley strain) were anesthetized with Nembutal and had their backs shaved. Rats were the only vertebrate host used in all trials. As stated in the objectives, we wished to assess the validity of the model in a human malaria system. We therefore used rats as hosts because they have identical skin blood volume as human hosts (Hansard et al. 1970) and are thus the most appropriate models. Furthermore, the low vasculature of the back of rats maximized the number of probes and duration of probing.

Multiple range analysis and function plotting were performed using STATGRAPHICS 3.0.
Results and Discussion

The duration of probing on the shaved back of anesthetized rats by 230 female *An. stephensi* differed between the first and subsequent probes, similar to *Ae. aegypti* (Ribeiro et al. 1985a). On average, *An. stephensi* spent more time on the first probe compared to subsequent ones (Table II.1. & Fig. II.1.), which also was similar to previous observations made on *Ae. aegypti*. Only the first five probes were compared in Table 1 because samples were too small in further probes.

This difference between the first and subsequent probes may be an adaptation to host irritation due to the immune reaction set off by saliva (Gillett 1967). The results differ from *Ae. aegypti*, however, in that *An. stephensi* probes for a longer period initially, almost 20 seconds more for the first probe. Although a few (3) *An. stephensi* females probed more than 25 times, this is not normally the case on a human host (Reisen & Emory 1976) and is due to the low vasculature of the back of rats.

In *Ae. aegypti*, the probability of blood location rises within the first 40 seconds and then drops gradually (Ribeiro et al. 1985a). In *An. stephensi*, the pattern is similar, but the peak is reached in about 20 seconds; 20 seconds earlier than in *Ae. aegypti* and lower by half (Fig. II.2.). This rise and fall has been attributed to the probability of host-seeking female selecting an area where vessels are abundant or located favorably (Ribeiro et al. 1984). The difference in height of the curve is attributable to differences in apyrase which are much lower in *An. stephensi* (Ribeiro et al. 1985a) and possibly to the different host used. We have no clear explanation why it is shifted to the left slightly.

Finally, we determined the probability of blood location over the series of probing sequences. The pattern would tell us whether or not blood location
is strictly deterministic, i.e., whether based on a probability of blood location fixed for a particular vessel distribution, or whether mosquitoes are capable of actually detecting where the vessels are located. The results indicate a deterministic exponential-like decay, well simulated by a probability model assigning a particular value to a feeding site (Fig. II.3.). This assumption can replicate the rise and fall that we observed in the previous analysis. If $P$ is the probability of locating blood at a site during a probe and $i$ the number of probes required before success, then the probability of locating blood on the $i+1$ probe is $P_{i+1} = P(1-P)^i$. In *An. stephensi*, $P=0.195$ in contrast to $P=0.315$ for *Ae. aegypti* on the back of guinea pigs (Ribeiro et al. 1985a).

In conclusion, *An. stephensi* probing behavior during blood location is qualitatively similar to *Ae. aegypti* but quantitatively different. The difference may relate to different levels in salivary apyrase (Ribeiro et al. 1985b) as well as to vertebrate hosts vascularization and response to probing. Thus, epidemiological implications derived for the *Ae. aegypti* paradigm also may be applicable to human malaria vectors.
Table II. 1. Multiple range analysis of probing time between first and subsequent probes. The first probe is significantly longer than subsequent probes (P < 0.001).

<table>
<thead>
<tr>
<th>Probe sequence</th>
<th>Sample size</th>
<th>Mean</th>
<th>Homogeneous groups</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>185</td>
<td>66</td>
<td>A</td>
<td>2.6</td>
</tr>
<tr>
<td>2</td>
<td>158</td>
<td>45</td>
<td>B</td>
<td>2.9</td>
</tr>
<tr>
<td>3</td>
<td>140</td>
<td>43</td>
<td>B</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>119</td>
<td>38</td>
<td>B</td>
<td>3.3</td>
</tr>
<tr>
<td>5</td>
<td>108</td>
<td>35</td>
<td>B</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Only samples where mosquitoes did not locate blood during the particular interval were used, which is the reason that the means differ slightly from Fig. II.1.
Figure II. 1. Mean duration of successive probes (sec) by *Anopheles stephensi* on the back of rats.
Figure II. 2. Probability of blood vessel location as a function of probing time (sec).
Figure II.3 Probability of blood vessel location as a function of probing sequence (solid line, observed data; dotted line, $P_{i+1} = P(1-P)^i$, where $P = 0.195$)
LITERATURE CITED


CHAPTER III
EPIDEMIOLOGIC IMPLICATIONS OF MALARIA-INDUCED SALIVARY MICROPROCESSES

Introduction

Numerous vector-borne parasites induce vector or host lesions that modify, often positively, transmission. Many of these changes occur at the level of the salivary glands, which is not too surprising since saliva is the one medium that is exchanged between vector and host and by which parasites are most often transmitted. The general principle that parasite induce modifications with epidemiologic implications was demonstrated first in plague-infected fleas, and the specific targeting of the salivary microprocess, especially centering on platelet aggregation, has been reported in numerous host-parasite relationships since then (see reviews by Molyneux and Jefferies, 1986; Rossignol, 1987).

Complex functions may thus relate parasite-induced changes and basic reproduction rate of some parasites. However, in most reported cases, changes have only been described qualitatively, simply positive or negative. If the function between the two parameters is limiting, then one might expect the particular pattern of parasite-induced microprocess to be reflected at the epidemiologic level. Such a relationship has been suggested in various filaria-diptera relationships, where 'facilitation' or 'limitation' of passage at the vector midgut level may result in either heterogeneous or homogeneous distribution in the vertebrate host (Pichon et al., 1974), although even this example remains disputed (Jerwood et al., 1984).

In order to anticipate experimental and epidemiologic questions arising from such modifications in malaria, we therefore undertook to anticipate some potential transmission patterns that might arise if sporozoite-induced changes in
salivary apyrase levels of vector mosquitoes followed certain theoretical relationships, either linear or non-linear. Using a Monte Carlo simulation designed to accurately predict mosquito blood locating behavior (Ribeiro et al., 1985a; Rossignol and Rossignol, 1988), we carried out a factor analysis on generated data. The model introduced parameters to describe and predict mosquito probing behavior in the presence or absence of a parasite. Three important factors that we wish to consider are: 1) vector gland function, the inherent ability of the saliva in enhancing blood location associated mostly with the level of apyrase, an anti-platelet aggregation inhibitor, 2) vessel geometry, the inherent geometry of blood vessel orientation and its effect on the probability of locating blood and 3) host switch, the tendency of a mosquito to desist in its search for blood on a host and try on another host. We analyzed the relative importance of these three parameters under three conditions of biological relevance, namely, that vector gland function varies with parasite load in either a linear, exponential or logarithmic fashion. Experimental work has established that parasites strongly influence this parameter, but the functional response has not been determined yet. Step-wise method of polynomial fit elucidated the relationships of the parameters on transmission under these three conditions, suggesting that complexity increases from logarithmic to exponential, from two variables containing only one parameter to nine variables containing all three parameters. Factor analysis identified the most important parameters.

Having elucidated the relative importance of vector gland function, we then developed a probability analysis, under specific and fixed vessel geometry and host switch, to assess potential impact of the biases that this parameter introduces in heterogeneous (coexisting infected and non-infected individuals) populations of hosts and vectors.
Factor Analysis

In order to evaluate the impact of linear and non-linear interactions of parasites on vector or host, we generated three runs of 125 points each using the Monte-Carlo model of mosquito probing behavior. From each set of data, we derived a set of polynomial equations. (Symbols are listed in App. A and equations in App. B). The combinations of variables in the equations were chosen by the back-forward stepwise method from 35 possible combinations of \( X_1, X_2 \) and \( X_3 \), with a maximum of power 4.

In the case of a logarithmic relationship, the equation is simple and only one important parameter affects \( Y \). When parasite load and behavioral changes are linearly related, five variables influence the number of hosts contacted (\( Y \)), while nine variables must be taken into account if the relationship is exponential. From the equations, it is hard to determine the major variables. We therefore used factor analysis to attempt to identify the important ones.

In the case of both \( Y_1 \) and \( Y_2 \), the first two rotated factors have good representation for original information (Tables III.1. & III.2.). We therefore chose the major variables based on their loading values on the rotated factors. Vessel geometry and switch host factor are the dominant components of the major variables of \( Y_1 \) and gland function and switch host factor are the overwhelming components of the major variables of \( Y_2 \). We therefore conclude that the major parameters that effect the number of hosts contacted are those dominant components mentioned above, namely vessel geometry and switch host factor for \( Y_1 \) and gland function and switch host for \( Y_2 \).
Probability Model of Modified Behavior

Infected mosquitoes contact more hosts than non-infected mosquitoes

A mosquito has to probe $J_i$ times in order to successfully obtain blood with a probability $P_i$ (see appendix C).

$$J_i > \frac{\log(1-P_i)}{\log(1-P_j)}$$

For $P_1 < P_2$ we get that $J_1 > J_2$, indicating that infected mosquitoes require more attempts than noninfected ones to locate blood and therefore contact more hosts than noninfected mosquitoes, independent of attraction. The relationship between $P_i$ and $J_i$ is illustrated in Fig. III.1.

Infected hosts receive more bites from mosquitoes than noninfected hosts.

Let us consider a mixed population system (containing infected mosquitoes and hosts as well as noninfected mosquitoes and hosts). We assume that every mosquito lands on a host 'randomly', that is, independent of attraction. If a mosquito can get blood on its first host, then it will not bite again in this time unit. If it cannot locate blood, then an attempt is made, possibly on another host, and if so again at random.

The total (from infected and noninfected mosquitoes) number of bites which result in successful engorgement on infected and noninfected hosts (see Appendix D), $S'_1$ and $S'_2$, respectively, are:

$$S'_1 = \frac{Em_1a_1\{[E(1-\alpha_1)+F(1-\beta_1)]h_1 - 1\}}{\{E(1-\alpha_1)+F(1-\beta_1)\} - 1} + \frac{Em_2a_2\{[E(1-\alpha_2)+F(1-\beta_2)]h_2 - 1\}}{\{E(1-\alpha_2)+F(1-\beta_2)\} - 1}$$

$$= S'_{11} + S'_{12}$$

$$S'_2 = \frac{Fm_1b_1\{[E(1-\alpha_1)+F(1-\beta_1)]h_1 - 1\}}{\{E(1-\alpha_1)+F(1-\beta_1)\} - 1} + \frac{Fm_2b_2\{[E(1-\alpha_2)+F(1-\beta_2)]h_2 - 1\}}{\{E(1-\alpha_2)+F(1-\beta_2)\} - 1}$$
= \sum_{k=w}^{s_2} \binom{s_2}{k} \left( \frac{1}{N_2} \right)^k \left( 1 - \frac{1}{N_2} \right)^{s_2-k}

Because \( \alpha > \beta \), then, if \( E = F \) and \( m_1 = m_2 \), then \( S'_{11} > S'_{22} \), meaning that the infective population of hosts always receives more bites.

On the other hand, the total number of effectively infective bites \( S''_1 \) and \( S''_2 \), either to or from vector respectively (Appendix E), are:

\[
S''_1 = \frac{Em_2\alpha_2 \{[E(1-\alpha_2)+F(1-\beta_2)] \} - 1}{\{E(1-\alpha_2)+F(1-\beta_2)] - 1\}} = S'_{12}
\]

\[
S''_2 = \frac{Fm_1 \{[E(1-\alpha_1)+F(1-\beta_1)] \} - 1}{\{E(1-\alpha_1)+F(1-\beta_1)] - 1\}}
\]

from which it is apparent that \( S''_1 > S'_{22} \) and \( S''_2 > S'_{11} \).

We can relate gland function (reflected by \( \alpha \) and \( \beta \)) to the Ross-Macdonald model (Bailey, 1982) as follows,

\[ a = \frac{S''_2}{Tm_1}, \]

where \( a \) is the 'man-biting habit'.

**Parasite modification on the malaria transmission process**

For every noninfected host, the probability \( W \) of receiving \( w \) brood of sporozoites or more in time \( T \) is:

\[
W = \sum_{k=w}^{s_2} \binom{s_2}{k} \left( \frac{1}{N_2} \right)^k \left( 1 - \frac{1}{N_2} \right)^{s_2-k}
\]
If $\delta_k$ is the probability of a noninfected host becoming infected when it receives $w$ broods or more from infective mosquitoes and $k$ is the number of bites received, then the probability of a noninfected host becoming infected in the population is:

$$W_{\text{new}} = W\delta_k = \sum_{k=w}^{\infty} \delta_k \left( \frac{S''}{k} \right) \left( \frac{1}{N_2} \right)^k \left( 1 - \frac{1}{N_2} \right)^{s_{2-k}}$$

where,$$
\delta_k = 1 - e^{-c(k-d)^2}
$$

Where $c$ and $d$ are appropriate constants, based on actual observations.

The probability of a host incurring $t$ superinfections is:

$$H_t = \left( \frac{S'}{t} \right) \left( \frac{1}{N_1} \right)^t \left( 1 - \frac{1}{N_1} \right)^{S'_{11} - t}$$

$t = 0, 1, 2, ..., k$

The probability of a host acquiring one or more superinfections $H_s$ is:

$$H_s = \sum_{k=1}^{\infty} S'_{11} \left( \frac{1}{N_1} \right)^k \left( 1 - \frac{1}{N_1} \right)^{S'_{11} - k} = 1 - \left( \frac{1}{N_1} \right)^{S'_{11}}$$
Discussion

In the first part of the work, we used a Monte Carlo model to simulate the effects of three parameters that determine the blood-location success of a mosquito, namely, gland function which is related either to vector apyrase levels or host platelet level, geometric factor which describes the pattern of blood vessel distribution in the patch of host skin that the vector is attacking, and host switch factor. It has previously been demonstrated that a parasite such as malaria may modify gland function, C, in such a way as to promote or inhibit the vector's ability to locate blood (Rossignol et al., 1985; Rossignol et al., 1986).

Based on this, we postulated that this interference might be either linear, exponential or logarithmic. We modified the model to generate data points for each one of the three conditions and fitted equations to each by optimal regression. The variables in these equations were analysed by factor analysis. We conclude that if the relationship is linear, the most important variables determining the number of hosts contacted and ultimately infected are the geometric factor and host switching. If the relationship is exponential, then the important variables are gland function and switch host factor; geometric factor takes effect only in combination with these two variables. Finally, when the relationship is logarithmic, then switch host predominates and gland function and geometric factor have no direct effect on number of hosts contacted, and ultimately on transmission. If such effects occur in nature, the model predicts that not only are consequences different but that mechanisms differ as well. The type of density dependent effect can dramatically impact on transmission pattern.
In the second part of the work, we examined a more general epidemiologic pattern towards which the three parameters lead. Assuming that there are no differences in attraction between infected and non-infected hosts and that some mosquitoes do randomly switch host following failure on one host, we demonstrate that the ratio of successful blood meals on a mixed host population will be inevitably biased towards infected hosts. These results formalize a conclusion empirically achieved previously (Rossignol & Rossignol 1988). In addition, we demonstrate the converse, namely that the number of bites delivered by infected vectors will be greater than that of noninfected vectors. Finally, the effect of parasite modifications on malaria transmission is described with a probability model.

In a recent and important theoretical model addressing transmission from host to vector, Kingsolver (1987) concludes that 'non-random' infected host preference might increase stability, although possibly at a reduced equilibrium level. Other workers (Dye and Hasibeder, 1986; Hasibeder and Dye, 1988) found that if a mosquito population were to exhibit biting preferences, the basic reproduction rate of the parasite might be increased under certain circumstances. The deduction is based on the idea that host patch fidelity stays coupled with non-uniform host selection and the authors look at 'infected vector-non-infected host' coupling and vice-versa, but not at intercrossings between the two, which we show can have impact on transmission. Introducing this type of heterogeneity provides our analysis with slightly more realism than both of these studies, although additional parameters are introduced. Dobson (1988) investigates the effect of increased biting rate and its effect on equilibrium, which is increased but possibly at the expense of stability. We are concerned with a more basic level, that is with quantifying the relationship between gland function and biting rate. Dye (1990) has recently shown how bias, of whatever
cause, can be handled in the standard Ross-Macdonald model to predict the direction that bias imparts to transmission. We describe a type of bias induced by parasite changes on gland function, resulting in a mathematical preference of the vector for an infected host, rather than the more intuitive one of attraction.

Over the last decade, we have come to realize that vector-host-parasite interactions introduce important biases in the Ross-Macdonald model of vector-borne parasites. Although such biases can result from numerous nonparasitic factors (Burkot 1988; Davies, 1990), many of them can be reduced to probing behavior and salivary components (Molyneux and Jefferies, 1986; Rossignol, 1987). This is not too surprising since the role of saliva appears to be primarily to shorten probing time and since saliva is the vehicle of sporozoite delivery. In essence, all three organisms collide during these few seconds, and parasites have exploited, so to speak, this opportunity. We should point out however that not all mosquito species have identical level of apyrase (Ribeiro et al., 1985b). Consequently, the impact of sporozoite or parasite-induced thrombocytopenia would not be uniform or universal. For example, if a vector has low apyrase levels, sporozoite infection does not result in an increased number of bites (Li, Sina and Rossignol, submitted for publication), although such a species would probably be strongly biased to infected hosts; the reverse may be expected for a species with high levels of apyrase. Discussion of the implications of these two potential extremes must await experimental confirmation.

Indeed, much remains to be investigated. For example, the increased number of hosts contacted by infected mosquitoes will be relevant only if these hosts become infected. Recently, infected mosquitoes have been shown to lose sporozoites following an initial burst of salivation (Rosenberg et al., 1990). If such mosquitoes do not recover infectivity quickly, then the importance of that aspect of our model might be lessened. The period of recovery is unknown,
however, as are parameters of host switching and correlation between sporozoite load and apyrase levels. Improvement may need be made on estimating parameters which having been commonly used in mathematical simulations of malaria transmission as well as introducing some new parameters.
Table III. 1. Loading values of factor analysis for linear relationship.

<table>
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<th>Rotated factors</th>
<th>Variables</th>
<th>Eigen value (%)</th>
<th>Cum. %</th>
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Table III. 2. Loading values of factor analysis for exponential relationship.

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<th>Cum. %</th>
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<td>Factor 2</td>
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Table III. 3. Parameters associated with vector-host contacts.

<table>
<thead>
<tr>
<th>Host</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>infected</td>
<td>$\alpha_1$</td>
</tr>
<tr>
<td>noninfected</td>
<td>$\beta_1$</td>
</tr>
</tbody>
</table>
Figure III. 1. Number of attempts (equivalent to J in text) required by mosquitoes with different relative salivary functions (equivalent to $P_i$ in text) to locate blood at a probability of .99. For example, a mosquito with salivary function such that it had a .3 probability of locating blood would require 12 probes to have a .99 probability of locating blood.
LITERATURE CITED


CHAPTER IV

PROBING BEHAVIOUR AND SPOROZOITE DELIVERY BY ANOPHELES STEPHENSI INFECTED WITH PLASMODIUM BERGHEI


with Dr. Barbara Sina, Dept Entomology, Univ. Maryland, College Park, MD

Introduction

Mosquitoes must probe a host to obtain blood, and this period of contact is obviously crucial in parasite delivery and uptake (James & Rossignol, 1991; Rossignol, 1987). Many parasites can modify this contact time between vector and its host (Molyneux and Jefferies, 1986). In particular, sporozoites can cause lesions in the salivary glands that impair probing behaviour, which, linked to host switching, could lead to an increase in the number of hosts contacted and infected (Rossignol et al., 1985).

Potential epidemiologic implications of such modifications have been explored (Rossignol and Rossignol, 1988), but relied on the assumption that infected mosquitoes are indeed infective beyond the 'normal' probing period. A recent study reported, based on observations of infected mosquitoes salivating into mineral oil, that most sporozoites were ejected within the first 'drop' of saliva in the mineral oil (Rosenberg et al., 1990). It was inferred that infected mosquitoes deplete their available sporozoites in the first few probes and that, therefore, longer probing time might not increase potential of disease transmission. That study, however, did not take into account that delivery into oil may be pathogenic in itself (many of their mosquitoes were unable to
salivate after a few minutes) nor did it determine whether mosquitoes could deliver sporozoites after probing a host.

We first tested *Plasmodium berghei*-infected *Anopheles stephensi* to determine whether or not there is any effect of sporozoite infection on the probing time, as reported for *Aedes aegypti* (Rossignol et al., 1984). Second, we compared the number of sporozoites delivered by mosquitoes into mineral oil before and after probing a host. Third, a mathematical analysis of sporozoite delivery was developed which provides a clue as to the value of intermittent sporozoite delivery. Possible epidemiologic implications are discussed.
Materials and Methods

Mosquito rearing and infection. Anopheles stephensi (India strain) mosquitoes were infected with gametocyte producing ANKA strain of Plasmodium berghei maintained in outbred mice, essentially as described in Vanderberg and Gwadz (1980). Four to five days after emergence, female mosquitoes were allowed to bite anesthetised P. berghei-infected mice with a 5-10% parasitemia. After infection, mosquitoes were maintained on 10% glucose solution at 20°C. Twenty one days after feeding, salivary glands were dissected and examined microscopically for presence of sporozoites.

Probing behaviour. Mosquitoes were allowed to probe the back of anesthetised rats and measurements were taken as in Ribeiro et al. (1984) and Rossignol et al. (1984). Briefly, duration of probing is defined as the time mosquito stylets remain in the skin until appearance of blood in the midgut under 5X magnification. When mosquitoes withdrew their mouthparts before taking blood, timing was stopped and begun anew following subsequent penetration. Each insect was used only once and checked for sporozoite infection. The results were ranked and plotted cumulatively.

Sporozoite delivery. Sporozoites were collected in oil-filled capillary tubes (Rosenberg et al., 1990) and counted microscopically under a 40X oil objective (Rossignol and Spielman, 1982). The process consisted of rapidly plucking off wings and legs of a mosquito, separating the sheath of the proboscis and inserting the stylets into an optically clear oil-filled (immersion oil) rectangular tube (Microslides #5005, VitroDynamics, Rockaway, NJ, USA).
Results

Probing behaviour and sporozoite output

We first determined whether or not any difference exists in blood location, or probing, time between *P. berghei*-infected and non-infected *An. stephensi* on rats. Contrary to observations with *Ae. aegypti*, we noted no statistically significant difference between the two groups (Fig. IV.1.). The ranked groups were not different, as confirmed by a contingency table analysis of the results arranged in rank order and grouped in 100 sec. intervals (Ribeiro *et al.*, 1984, 1985; Rossignol *et al.*, 1984, 1985) that gave a $\chi^2 = 1.4$ (degrees of freedom = 2; $P = .49$; n=22 and 21, respectively). Using this standard approach, *An. stephensi* appears not to be impaired.

We then compared the number of sporozoites delivered into mineral oil by two treatments of infected mosquitoes, one having probed to blood location or inherent desistance and another not having done so. No difference between probing and non-probing mosquitoes was noted either in number of sporozoites ejected over three minutes (mean =35.9 vs 31.7, respectively; $P = .90$; rank-sum test) or proportion of mosquitoes delivering sporozoites (60% vs 50%, respectively; n=10 for each group; $P = .99$; two-tailed Fisher exact test).

Mathematical analysis

To better understand the dynamics and potential importance of sporozoite delivery, we mathematically described both sporozoite delivery and depletion as well as explored the phenomenon of sporozoite clumping in the salivary glands and its possible 'advantage' to the parasite.

We first assumed that when saliva is driven through the stylet and into the host during probing, active components such as apyrase are probably depleted to some degree, but that the 'watery carrier' is continuously
replenished, since otherwise the glands would be rapidly exhausted (Rossignol and Spielman, 1982). Let us assume that \( Q \) = volume of salivary lumen, \( v \) = salivary flow and \( m(t) \) = number of sporozoites or number of clumps of sporozoites left in the glands at time interval \( t \). Then, sporozoites, whether individuals or clumps, in the gland lumen would be lost at a rate,

\[
\frac{dm(t)}{dt} = -\frac{m(t) v}{Q}
\]

from which,

\[
m(t) = \frac{m(0)}{e^{\frac{vt}{Q}}}
\]

However, sporozoites delivery may not best be described as continuous. In fact, it is obviously discrete and not a direct function of saliva flow, although we make such an assumption above. The above analysis is useful nevertheless to describe the overall process. We noted, as have previous workers (Rosenberg et al., 1990), that sporozoites appear to be delivered in a quantum fashion, that is, few most of the time and many occasionally; some aggregation process seems to be occurring. Upon observing infected salivary glands, we found that sporozoites are not lying in the salivary duct lumen in a pell-mell fashion, but rather in 'clumps'. This observation was previously reported and photographed through transmission electron microscopy of \( P. berghei \)-infected \( An. stephensi \) salivary glands (Sterling et al., 1973). A histological study indicates that \( P. falciparum \) sporozoites injected by mosquitoes into host tissue are clumped as well (Ponnudurai et al., 1991). Furthermore, Beier et al. (1991a) observed that, in \( P. falciparum \), "...transmitted sporozoites were observed frequently on microslides to be clumped together in a viscous-like substance."
In order to make the model more realistic, therefore, we introduced a quantum or discrete aspect of delivery. We assume that clumps are evenly distributed in the salivary lumen, that is, satisfy a binomial distribution, thus,

\[ \binom{M}{\gamma} P^\gamma (1-P)^{M-\gamma} \]  

(3)

where \( \gamma = 0, 1, 2, \ldots, M \) clumps of sporozoites, and \( P \) is the probability of delivery of clumps and is estimated by \( \frac{V_t}{Q} \).

Were a sporozoite threshold to exist, then a mosquito would be infective only if a minimum number of sporozoites were effectively delivered into a host. The accumulated probability of infection would be,

\[ \sum_{\gamma=\delta}^{M} \binom{M}{\gamma} P^\gamma (1-P)^{M-\gamma} \]  

(4)

where \( \delta = \frac{\text{threshold}}{\text{mean no. sporozoites per clump}} \).

Using the Demoivre-Laplace theorem, we have,

\[ \sum_{\gamma=\delta}^{M} \binom{M}{\gamma} P^\gamma (1-P)^{M-\gamma} = \int_{\alpha}^{\beta} \frac{1}{\sqrt{2\pi}} e^{-\frac{x^2}{2}} dx \]

\[ = 1 - \int_{0}^{\alpha} \frac{1}{\sqrt{2\pi}} e^{-\frac{x^2}{2}} dx \]  

(5)

where \( \alpha = \frac{\delta-MP}{\sqrt{MP}(1-P)} \) and \( \beta = \frac{M-MP}{\sqrt{MP}(1-P)} \).

When the threshold is less than the mean number of sporozoites per clump, then the probability of infection is greater if sporozoites are not clumped, whereas when the threshold is more than that number, then sporozoite clumping will result in more efficient transmission. It is now possible to establish a relationship between sporozoite delivery, saliva flow and transmission efficiency (Fig. IV.2.). It can be seen that the presence of a non-zero threshold, as analyzed in equations 3-5, would make clumping
advantageous to the parasite in terms of number of hosts potentially infected because sporozoites would be unlikely to be delivered in a dose smaller than the threshold. Sporozoite delivered into a host in numbers below the threshold might be of little importance in transmission if no clumping occurred. With clumping, although an individual host would be less likely to receive a dose of sporozoites, it would be much more likely to become infected from that dose of sporozoites.
Discussion

From our results, we conclude that first, probing time of *An. stephensi* is not affected by sporozoite invasion, and second, this vector is as efficient at sporozoite delivery before as after extensive probing activity. Our results suggest that mosquitoes still keep the ability to transmit disease after a long probing session. The fact that this species of mosquito appears not to be impaired by sporozoite invasion is probably due to its inherently low apyrase levels. Apyrase is an anti-platelet aggregation constituent of mosquito saliva whose level is strongly and inversely correlated to duration of probing in anopheline mosquitoes (Ribeiro *et al.*, 1984). In the case of three species of *Anopheles*, these workers reported that *An. stephensi* has a much lower apyrase level than *Ae. aegypti* or *An. freeborni*, although higher than the autogenous *An. salbairi*. It is therefore unlikely that such a mosquito, already quite inefficient at locating blood, would be significantly impaired by sporozoite-induced damage. Quantitative aspects of saliva may thus determine epidemiologic consequences of parasite-vector relationships. As a corollary, we would predict, from theoretical analyses (Rossignol and Rossignol, 1988), that a mosquito such as *An. stephensi* would be greatly facilitated by feeding on an infected host that has thrombocytopenia. Although this facilitation appears to occur in *Ae. aegypti* (Rossignol *et al.*, 1985), this remains to be tested for *An. stephensi*.

Mosquitoes inefficient at locating blood, either inherently or due to parasite modification, may be more efficient at transmission (Ribeiro *et al.*, 1985; Rossignol and Rossignol, 1988). This hypothesis depends heavily on the assumption that such vectors could indeed deliver parasites to subsequent hosts. Preliminary work had suggested that this assumption was not correct (Rosenberg *et al.*, 1990). We demonstrate here, however, that no difference could be
detected in mosquitoes tested after extensive probing. We suggest that probing has little impact on sporozoite delivery and that the technique of observation, namely, delivery into mineral oil is itself injurious and unreliable in making observation on long-term salivary processes. Indeed, we observed as did other workers (Rosenberg et al., 1990) that many mosquitoes were unable to salivate more than a few minutes into oil. Our observations confirm anecdotal reports that infected mosquitoes can infect successive hosts in a short period of time (Boyd, 1949). In addition, a recent study demonstrates that *P. falciparum*-infected mosquitoes deliver equal numbers of sporozoites whether feeding is interrupted or not (Ponnudurai et al., 1991).

Modelling of sporozoite delivery may explain the scattered aspect of sporozoite delivery and contribute to future direction of sporozoite studies. Quantum of infection, or number of sporozoites, is an important determinant of infection rate, incubation period and of disease severity (James et al., 1936; Boyd and Kitchen, 1937; Boyd, 1949). In fact, early malariologists had come to the conclusion, by comparing infection rates on human volunteers by sporozoite-infected anophelines, that there was a "minimal effective dosage" required for successful inoculation (Boyd and Stratman-Thomas, 1933). A model of salivary flow is potentially valuable in malariology because sporozoite delivery is linked, at least from an intuitive level, directly to salivation rate and duration. A model may allow us to identify important parameters of the system.

Our mathematical analysis also provides a possible adaptive explanation for the observed clumping of sporozoites in the salivary gland lumen, namely, that it maximises success of sporozoites by ensuring delivery above a required threshold. If such a threshold of infection exists, it is a strong selection factor in the evolution of a vector-borne parasite such as malaria; clumping is one
possible solution to the threshold problem because fewer sporozoites would be 'wasted' where interruption of the blood-feeding process is common and because it may also ensure that even mosquitoes with very low sporozoites numbers would have some capability of infecting a host. Clumping would also explain some long-standing observations where even heavily infected mosquitoes do not consistently infect a host, which was attributed to host heterogeneity (Boyd, 1949). Possibly, some mosquitoes were not delivering a clump in such hosts.

Recent studies reported that *P. falciparum*-infected mosquitoes deliver sporozoites in an unpredictable fashion, sometimes not at all (Ponnudurai *et al.*, 1991) and transmit inconsistently (Rickman *et al.*, 1990). Workers also visually confirmed the presence of 'clusters' present in host tissue, rather than into vessels (Ponnudurai *et al.*, 1991); clumping of transmitted sporozoites and uneven delivery was reported *in vitro* by other workers (Beier *et al.*, 1991a, b). These aspects of malaria and vector biology, namely clumping, dose threshold and site of delivery, deserve continued scrutiny, especially in view of current sporozoite vaccine work.
Figure IV. 1. Duration of probing by infected (triangles) and non-infected (squares) *An. stephensi* mosquitoes exposed to anesthetised rats. Each point represents a single mosquito and each line ranks the points in order of increasing duration of probing.
Figure IV. 2. Graph of equation (2) of the sporozoite reserve as a function of the number of hosts contacted under different relative rates of flow (A = .001; B = .01; C = .1; D = .2; E = .4). The solid line represents a theoretical threshold below which there are not enough sporozoites to cause an infection as explored in equations (3-5). None of the data is empirical.


Boyd, M. F. and S. F. Kitchen. 1938. The clinical reaction in vivax malaria as influenced by the consecutive employment of infectious mosquitoes. American Journal of Tropical Medicine, 18, 723-728.


CHAPTER V

OPTIMAL CONTROL OF MALARIA: POTENTIAL CONFLICTS BETWEEN DIFFERENT OBJECTIVES AND PRIORITIES

With Dr. Ronald R. Mohler and Radoslaw R. Zakrzewski
(Dept. Electrical and Computer Engineering, OSU)

Introduction

Based on Ross' two equations model of transmission (Aron & May, Ross), we demonstrate that malaria is theoretically only controllable locally, mathematically speaking, if measures are bounded, that is, insecticide application, vaccine availability or drug use is realistically limited. In addition, the system is not observable from measurements of the basic reproduction rate, commonly used to assess malaria interventions; information on prevalence of infection in both host and vector populations is required. We analyse four optimal control strategies, optimising either cost or time for eradication or establishing a tolerance level. We identify paradoxical conditions under which the need for intervention and host prevalence may be inversely related.
Analyses and Results

The equations for the dynamic system of malaria may be expressed as follows [Aron & May, 1986],

\[
\frac{dx}{dt} = a b m y (1 - x) - r x \tag{1}
\]

\[
\frac{dy}{dt} = a x (1 - y) - u y \tag{2}
\]

where \( x \) is the infected host population and \( y \) infected vector population, and constants are \( a \) biting habit, \( b \) vector efficiency, \( m \) relative number of vectors, \( r \) host recovery rate, \( \mu \) vector mortality. There are two equilibrium points, \( (x_e, y_e) \) and the origin \( (x_0 = 0, y_0 = 0) \). For equations (1) and (2), the region, \( Z \), in which the equations represent the malaria dynamics is given by constraints \( 0 < x < 1, 0 < y < 1 \). When \( a^2 b m > r \mu \), then a stable equilibrium exists at

\[
x_e = \frac{a^2 b m - r \mu}{a^2 b m + r a} \tag{3}
\]

\[
y_e = \frac{a^2 b m - r \mu}{a b m (a + \mu)} \tag{4}
\]

and an unstable one at the origin. When \( a^2 b m < r \mu \), then the origin is stable and \( (x_e, y_e) \) is unstable.

Intervention can be through both vector mortality and host infectivity (\( \mu \) and \( r \), respectively) from any point to a desired or prespecified target point \( (x_f, y_f) \). Then successful intervention conditions required for \( \mu \) and \( r \) are

\[
\mu = \frac{a x_f (1 - y_f)}{y_f} \tag{5}
\]

\[
r = \frac{a b m (1 - x_f) y_f}{x_f} \tag{6}
\]
Given that $0 < y_f, x_f < 1$, and that $\mu$ and $r$ are not bounded, then the set of attainable equilibria is the whole set of $x$ and $y$. If $\mu$ and $r$ are bounded, the set of attainable equilibria is constrained and may not include the whole area of interest. The particular shape of the set of attainable equilibria will depend on values of parameters $a, b, m$, as well as on ranges of admissible controls $r$ and $\mu$.

If intervention is through only one of these two variables, that is, either vector mortality or host infectivity, then the set of attainable equilibria is a one dimensional set, a curve parametised by the value of the control signal (either $r$ or $\mu$). In such a case, a choice of final desired value of $x_f$ uniquely determines the final value of $y_f$, and vice versa. For example, with only $r$ available for control, the value necessary to obtain steady state host prevalence $x_f$, is given by,

$$ r = \frac{a^2 bm (1 - x_f)}{ax_f + \mu} \quad (7) $$

If only $\mu$ is being manipulated, the necessary steady state is given by,

$$ \mu = \frac{a^2 bm - x_f (a^2 bm + ra)}{r} \quad (8) $$

Overall then, with unbounded control measures, the system is stabilisable around any chosen equilibrium. With bounded control measures, the set of equilibria around which the system is stabilisable may be smaller. Then in region $Z$, with only one control available, it is possible to chose a steady state $x_f$ or $y_f$, but not both.

We then studied the optimal control problem of malaria dynamics described by equations (1) and (2), to which were added the control measures, through host infectivity or vector mortality, respectively, thus

$$ \frac{dx}{dt} = a \ b \ m \ y \ (1 - x) - x \ (r + h, k) \quad (9) $$
\[
\frac{dy}{dt} = ax(1 - y) - y(\mu + h_2k_2)
\]  

(10)

where \( h_i \) is the amount of intervention material and \( k_i \) is its efficiency; \( i = 1,2 \).

Let us assume an objective of specifically reducing the proportion of infected hosts from \( x \) to \( x_f \) with a minimal intervention effort (as defined below), but where the final proportion of infected vectors, \( y_f \), is not targeted. This is so because we assume that only one of the control measures will be used, and as discussed above, this does not allow to set both \( x_f \) and \( y_f \) at will. This effort will be measured either as time or as cost. That is, to minimise the objective function, \( J \),

\[
J = \int_{0}^{t_f} h_i \, dt, \text{ for 'least cost' considerations}
\]

(11)

\[
J = \int_{0}^{t_f} 1 \, dt, \text{ for 'least time' considerations}
\]

(12)

where \( i = 1 \) or 2 and the final constraints \( x(t_f) = x_f, y(t_f) = y_f \); which defines \( h_{i \, eq} \), the control signal required to maintain equilibrium. The equilibrium, corresponding to the desired value \( x_f \) and final time \( t_f \), is free. The minimisation is performed with respect to control signal \( h_i(t) \), constrained by \( 0 \leq h_{i \, eq} \leq h_i(t) \leq h_{i \, max} \). For the system to be kept at final state, we must have \( h_i(t) \) equal to a value specified by equations (7) or (8). For analysis of the problem, we used the Pontryagin maximum principle (reference from R. Z.).

From equations (1) and (2), the Hamiltonian function is, for least cost control measures aimed at reducing the proportion of infected hosts (as with an anti-malarial drug),

\[
H = h_i + \lambda_1 \left\{ a \, bm \, y \, (1 - x) - x \, (r + h_1k_1) \right\} + \lambda_2 \left\{ a \, x \, (1 - y) - \mu \, y \right\}
\]

(13a)
where $\lambda$ is the adjoint variable. For least cost control measures reducing proportion of infected vectors (as with insecticide or anti-gamete vaccine),

$$H = h_2 + \lambda_1 [a b m y (1 - x) - r x] + \lambda_2 [a x (1 - y) - y (\mu + h_2 k_2)]$$  \hspace{1cm} (13b)

For least time control measures reducing proportion of infected hosts,

$$H = 1 + \lambda_1 [a b m y (1 - x) - r x] + \lambda_2 [a x (1 - y) - y (\mu y + h_2 k_2)]$$  \hspace{1cm} (14a)

$$H = 1 + \lambda_1 [a b m y (1 - x) - r x] + \lambda_2 [a x (1 - y) - y (\mu y + h_2 k_2)]$$  \hspace{1cm} (14b)

The costate equations for (13a) are,

$$\frac{d\lambda_1}{dt} = \frac{-\partial H}{\partial x} = a b m y \lambda_1 - r \lambda_1 - h_1 k_1 \lambda_2 + (1 - y) \lambda_2$$  \hspace{1cm} (15)

$$\frac{d\lambda_2}{dt} = \frac{-\partial H}{\partial y} = a b m (1 - x) \lambda_1 - a x \lambda_2 - \mu \lambda_2$$  \hspace{1cm} (16)

Similar equations can be derived for equations (13b) and (14a, b).

Given the system described by equations (1) and (2), the objective of control is to drive the system from the initial state $(x, y)$ to a final target state with the least cost or duration, by intervention through drug, insecticide, vaccine, etc. We assume that cost or duration will be limited by a maximum and will be equal to or greater than zero. The problem is therefore to minimise $H$. Because the amounts of the intervention tools, $h_1$ and/or $h_2$, have a linear relationships with $H$, the control problem becomes what is called an on-off switch control or a so-called 'bang-bang' measure. From the Pontryagin maximum principle,

$1 - \lambda_1 k_1 x < 0 \implies h_1(t) = h_{1,\max}$ and $1 - \lambda_1 k_1 x > 0 \implies h_1(t) = 0$

or for equation (13b),

$1 - \lambda_2 k_2 y < 0 \implies h_2(t) = h_{2,\max}$ and $1 - \lambda_2 k_2 y > 0 \implies h_2(t) = 0$
or equation (14a),
\[ \lambda_1 k_1 x < 0 \implies h_1(t) = 0 \quad \text{and} \quad \lambda_1 k_1 x > 0 \implies h_1(t) = h_{1\ max} \]
and for equation (14b)
\[ \lambda_2 k_2 y < 0 \implies h_2(t) = 0 \quad \text{and} \quad \lambda_2 k_2 y > 0 \implies h_2(t) = h_{2\ max} \]

For time optimal control, it could be proven that for the system, it is impossible that \( \lambda_1(t) = 0 \) or \( \lambda_2(t) = 0 \) on finite interval of time. For cost optimal control, our simulation results suggest that no singular control (such as \( I - \lambda_1 k_1 x = 0 \)) exists.

Because equations (1) and (2) could not be solved analytically, we carried out numerical solutions on a computer. We present the results of the consequences of control measures that would result in an increase in the host recovery rate, \( r \), as with an antimalarial drug. Conclusions based on an intervention through an increase in vector mortality, \( \mu \), are similar and are not shown. Using the decision criteria above, we describe the consequences of the two realistic control priorities, one aiming at minimizing cost and the other at minimizing duration for two final objectives, eradication and a non-zero tolerance threshold. Based on our results, we conclude that a cost priority campaign aiming at a tolerance threshold will have an area where intervention is not recommended despite very high host and vector infection levels, an area where intervention is recommended despite low host infection level due to high vector infection, and another where it is not recommended despite host infection levels above the desired goal due to low vector infection (Fig. V.1.). The goal of eradication with least cost presents fewer options but still leads to the difficult decision of sometimes not intervening at high vector infection, a possibility that exists to some degree at any level of host infection (Fig. V.2.). A time priority campaign aiming at tolerance will present a profile similar to one of cost priority, but with the requirement of intervention at very high infection levels.
(Fig. V.3.), possibly a more acceptable situation but predicated on great cost. Eradication under this priority requires constant intervention (Fig. V.4.) and is implicitly the most expensive. Overall, all control takes place with $h_i_{\text{max}}$ until $x_f, y_f$ is reached; then it takes place with $h_i_{\text{eq}}$. 
Discussion

The choices presented in the analysis are realistic since time and cost priorities are acute issues in field interventions, and eradication is rarely a realistic goal. We hope that our study may provide a basis for the difficult ethical and economic decisions of campaign managers. Epidemiological and ethical considerations of eradication and its alternatives have been considered (Rossignol 1993, Spielman and Rossignol 1984) but not dissected analytically.

In addition to applications in ecology and epidemiology (Wickwire 1977, Mohler 1973), two previous studies of optimal control theory specifically address problems involved in malaria (Dietz 1975, Gonzalez-Guzman 1980). Both studies used Ross' single equation model, which has serious shortcomings when compared with Ross' two equations malaria model (Lotka 1923). Using the single equation model of transmission, Gonzalez-Guzman (1980) concluded that cost optimal control of malaria required constant and intensive intervention, whether the target level was eradication or not. The analysis by Dietz (1975) additionally considers a population of untreatable hosts and considers the permutations of insecticide and drugs treatments. The end-point is again eradication and he concludes that some extensive use of treatments is required at all times. Our results differ markedly in that many conditions exist where intervention is not of value, and in that conditions vary with the objective (eradication or not). We feel that this is a more realistic and useful picture than can be obtained from the single equation model.

Decision making based on optimal control theory relies on knowledge of the present state \((x, y)\) of the system. We realize that this information may be a significant practical limitation in malariology, but modern diagnostic tools are making the attainments of such results both faster and more reliable than before
(Wirth et al. 1986), albeit still neither trivial nor cheap. Observability in optimal control theory is used to assess whether or not the state of a system can be determined by measuring output in a finite time. Entomological parameters have been most often measured in the field to estimate basic reproduction rate. Basic reproduction rate, however, only provides information on the status of the system as a whole, such as its equilibrium, and on the sensitivity of parameters, such as the relative importance of vector longevity and vector density, but yields insufficient information to make decisions on optimal control. Estimates of basic reproduction rate based on sporozoite rate or inoculation rate are similarly limited. Evaluating current prevalences of both host and vector populations, as through sporozoite rate of vectors and gametocyte rate of hosts, are required.

Although the above analysis addresses theoretical considerations based on Ross' two equations model of malaria, it raises practical, and timely, issues surrounding the value of intervention, through insecticides, drugs or vaccines, under realistic conditions. Optimal control theory indicates that the setting of objectives, eradication or tolerance, and of priorities, time or cost, may greatly influence the value of intervention, and raise economic, ethical and epidemiological challenges to campaign agencies and managers. Particularly relevant in an era of limited resources, the conflicts between cost optimization and intervention under certain high levels of human prevalence may pose agonizing problems in justification. Our understanding of malaria parasitism has relied heavily on theory, and it may be rewarding to understand some theoretical aspects of its control as well. A logical basis for analysis can help to make these difficult decisions, although, in the end, human health is the only priority.
Figure V. 1. Graph of cost optimal trajectories (doted), trajectories without control (dashed) and switch lines (solid); a cost optimal strategy aims at the least possible cost and assumes that time considerations are secondary. The objective of this cost optimal control is to drive the system to an acceptable level \((x_f, y_f)\) in area \(0 < x, y < 1\) other than extinction \((x_0, y_0)\); the 'natural' non-zero equilibrium of the system is \((x_e, y_e)\). The trajectories were generated from numerical solutions of equations (1) and (2) with a variant of the Switching Time Variations Method (Mohler 1973), while application at each step of the decision criteria yielded the switch lines. The arrow represents the tolerance threshold of infected hosts; this point is variable and selected in this example for clarity; it could be set elsewhere and not affect the generality of the results. In area A, no intervention is required; in area B, intervention is required immediately even though infections levels are below tolerance; in area C, intervention is not necessary even though infection levels are above tolerance until levels reach area D, where intervention should be undertaken immediately; in area E, no intervention is necessary until the levels return to area D. Strictly speaking, the cost optimal control does not always exist. For a set of initial conditions in area E, keeping control measure at zero results in asymptotically approaching the corresponding equilibrium with additional increment. Therefore it is always possible to get lower cost by waiting for a switch a little longer, even if the cost gain is very small. This of course is a theoretical consideration and in practice, the waiting time will be limited.
Figure V. 2. Graph of cost optimal trajectories (dotted), trajectories without control (dashed) and switch lines (solid); a cost optimal strategy aims at obtain control with the least possible cost and assumes that time considerations are secondary. The objective of this cost optimal control is to drive the system to extinction. In area A, intervention should be instituted, while in area B no intervention is necessary until levels enter area A.
Figure V. 3. Graph of time optimal trajectories (doted), trajectories without control (dashed) and switch lines (solid); a time optimal strategy aims at obtain control as rapidly as possible and assumes that cost considerations are secondary. The objective of this strategy is to drive the system to an acceptable level \((x_f, y_f)\) other than extinction. In area A, no intervention is required; in area B, intervention is required immediately even though infections levels are below tolerance; in area C, intervention is not necessary even though infection levels are above tolerance until levels reach area D, where intervention should be undertaken immediately. This strategy is similar to the cost optimal strategy of Fig. 1.
Figure V. 4. Graph of time optimal trajectories. The objective of this particular strategy is to drive the system to extinction. In this case, immediate intervention is required under all conditions.

Time optimal trajectories for $r_{1\text{max}}=0.375$; target - circle of radius 0.001
LITERATURE CITED


CHAPTER VI

POPULATION DYNAMICS OF MALARIA AS MACROPARASITE OF THE VECTOR

Introduction

Population dynamics models of parasites have been divided into two broad categories, namely, those of 'microparasites' and of 'macroparasites' (Anderson and May 1979; May and Anderson 1979). An important aspect of this division is the absence or presence of density-dependent effects on morbidity and mortality. If little exists, then transmission is best approximated with a prevalence model; organisms of this category are called microparasites. If intensity of infection of host and/or vector is an overriding consideration, then modelling will incorporate parasite distribution and density in the host population; these are the so-called macroparasites.

Many mathematical models have been developed, or at least elaborated, to describe the dynamics of malaria ever since the first model was developed by Ronald Ross and, later, by others (Ross 1911; Macdonald 1957; Aron and May 1982; Bailey 1982; Dietz 1988; Dye 1992). Although these theoretical models have been of importance and usefulness in practical malariology, as with the concept of vectorial capacity (Garrett-Jones 1964), a common assumption has been that both infected mosquitoes and infected hosts suffer no density-dependent effect.

Ever since Buxton's (1935) early work suggesting parasite-induced mortality in mosquitoes, recent laboratory studies have investigated the problem of morbidity and mortality to mosquito vectors caused by malaria parasites (Klein et al. 1982 gives a brief review of pre-1972 literature). Results have been contradictory. In particular, a correlation between mortality and oocyst
number may exist with *Plasmodium cynomolgi* in *Anopheles dirus A* (Klein et al. 1982, 1986). This mortality was most evident at high loads, vectors with less than 10 oocysts not being different than non-infected vectors. Laboratory investigations of *P. berghei*-infected *An. stephensi* point to a mortality effect (Gad et al. 1979a); this observation was supported by a mechanistic explanation, namely, amino acid loss (Gad et al. 1979b). The pathological effects of malaria on vectors has been reviewed (Maier et al. 1987) and it was concluded that vector morbidity and mortality are significant; the authors did not discuss epidemiologic consequences in detail. Specific pathology, as of salivary glands, is well documented (James and Rossignol 1991), but associated mortality has not been so. These studies, however, appear contradicted by two others that reported no difference in survival of *Aedes aegypti* infected with *P. gallinaceum* (Freier and Friedman 1987), a situation also anecdotally reported elsewhere (Rossignol et al. 1986), or anophelines infected with *P. falciparum* (Chege and Beier 1990). Despite the different parasite and vector species, these studies would be flawed, however, if parasite aggregation occurred because the standard statistical comparisons of means of non-normal distributions would then be incorrect. Analysis of mortality in different load classes (as in the Klein et al. 1986), is a more valid approach under such conditions; none of the other studies take parasite load into account.

Laboratory studies often deal with heavy infections that may not be representative of natural conditions. One field study in Tanzania of *An. gambiae* indicates no evidence of parasite-induced mortality in the vector (Lines et al. 1991), while another field study on the same species of vector and parasite in the same country (and one shared author) reported a lowered and density-dependent survival rate in heavily oocyst-infected mosquitoes (Lyimo and Koella 1992). These studies demonstrated the difficulties in documenting
mortality of such parasites; the effects can be subtle and require careful mathematical analysis and interpretation of parasite distribution.

In the light of the hypothesis that malaria may display macroparasite-like characteristics, and keeping in mind that the evidence is still highly arguable, we constructed a set of differential equations that incorporates parasite density, density-dependent mortality on vector and host populations, and an aggregated distribution. We were unable to solve these non-linear equations analytically and therefore used numerical methods to reach solutions within specific limits. We demonstrate that equilibrium and stability can occur under reasonable circumstances and that there exist optima in aggregation and parasite fecundity, leading to potentially paradoxical consequences in intervention.
Model Equations

Description of system dynamics

The basic approach of our model is based on Anderson and May (1978) and Dobson (1988). We added and incorporated parasite populations and their density-dependent mortality and transmission effects to malaria. The schematic of the model is represented in Fig. VI.1.

We assume the following scenario. A proportion of a non-infected mosquito population ingests malaria gametocytes randomly from an infected host population. The number of gametocytes ingested is directly proportional to the gametocyte load in the host. In turn, the number of sporozoites will be correlated to that number of gametocytes; similarly the production of gametocytes is determined by the number of the sporozoites that a host received. Mosquitoes randomly locate a host and no attraction bias exists towards infected or non-infected hosts. The absolute number of parasites in the population of hosts and vectors will obviously be extremely large, and we therefore graded loads as units, as is common in field studies. Our parasite populations are sized in such units.

Model components and justifications

Model parameters are listed and defined in Table VI.1. Parasites in the mosquito population is recruited at a rate,

\[ \lambda \left( \sum_{i}^{\infty} \frac{H_1}{H_2 + H_2} P_2(i) S_2 i^2 \right) \]

which is the product of the rate and efficiency of contact with the infected host population, incorporating the number of gametocytes and parasite fecundity
(number of sporozoites that a pair of gametes produces). Gametocyte sex ratio may not be equal (see Read et al. 1992); we assume equality but this problem could be addressed by modifying Eq. 1.

Numerous studies suggest that oocyst numbers, and ultimately sporozoite numbers, are positively correlated to gametocyte density in the vertebrate host (Boyd 1949; Eyles 1952; Carter and Graves 1988; Gamage-Mendis et al. 1993), albeit not clearly in a linear fashion (Rosenberg and Koontz 1984). A positive relationship has also been demonstrated to exist between gametocytes and ookinetes (Janse et al. 1985). In *P. falciparum*, a significant difference exists in the infectiousness of different classes of gametocyte density (Graves et al. 1988). A recent review suggests that, early in host infection, oocyst density reflects gametocyte density, but that, over the whole period of infection, this correlation does not hold well (Sinden 1991). Indeed, recent studies suggest that oocyst load may vary nonlinearly with gametocyte load (Fig 7.18 in Carter and Graves 1988) (hence our approximation with $i^2$), and that sporozoite number has a positive relationship to oocyst number (Sattabongkot et al. 1991).

Losses to this population of parasites occur due to natural mortality of the vector,

$$\sum b_2 P_i (i) H^i$$

and to parasite-induced vector mortality,

$$\sum H^i P_i (i) \alpha ^i$$

The evidence for this loss (Klein et al. 1986, Maier et al. 1987) is discussed in detail in the introduction above.
Next, we shall deal with vector populations, both noninfected and infected. We thus assume that the noninfected mosquito population satisfies the logistic equation, namely,

\[
r_1 H_1 \left( \frac{H_1}{1 - \frac{H_1}{\mu}} \right)
\]

(4)

with a natural mortality rate of,

\[
b_2 H_1
\]

(5)

and that acquires parasites, that is, becomes infected and incurs losses to the noninfected population, at a rate,

\[
f_1 \sum H_2 \frac{H_2}{H_2 + H_2} P_2(i) \beta_1 i^2
\]

(6)

In turn, losses to the infected mosquito population are imputed to two causes. First, natural mortality,

\[
b_2 H_1'
\]

(7)

and second, parasite-induced mortality,

\[
\sum P_1(i)\frac{H_1'}{\alpha_1} i^2
\]

(8)

After sporozoites enter the host, gametocytes are eventually produced. We assume that the density of first-generation asexual parasites in the blood probably reflects the dose of sporozoites received by the host (McGregor 1965; Vanderberg 1977). Studies on experimentally induced infections of P. falciparum (Kitchen and Putnam 1942) and on natural infections of this species (Thomson 1914) suggest that gametocytemias in such infections depend closely upon the intensity of asexual parasitaemia. Christophers (Christophers 1924) and Sinton and co-workers (Sinton et al. 1926) found that the number of
gametocytes produced was proportional to the overall parasitaemia. Although previous workers (Cantrell and Jordan 1946) reported much fluctuations in the ratio of gametocytes to asexual stages, the fluctuations only occurred in extreme conditions. We therefore assume that the infection of a host depends upon the total number of sporozoites received. Gametocytes are produced at the rate,

\[ \lambda_2 f_2 \sum \frac{H_2}{H_2+H_2} P_1(i) S_1 i^2 \]  \hspace{1cm} (9) 

Two sources of parasite loss exist. First, there is loss due to parasite-induced host mortality. Instantaneous death rate of laboratory mice infected by *P. vinckei* is nonlinear and positively correlated to parasite load (Cox 1966). We therefore assume parsimoniously that host death rate varies with the square of the number of parasites in a host, that is, \( \alpha_2 i^2 \). Thus, the number of parasite lost is,

\[ \sum H_2 P_2(i) \alpha_2 i^2 \]  \hspace{1cm} (10) 

Second, losses to the parasite population occur due to host recovery. We assumed that the recovery rate of a host is inversely related with the square of the number of parasites in the host. The loss of parasites is,

\[ \sum H_2 P_2(i) \left( 1 - \frac{i^2}{R_c} \right) \]  \hspace{1cm} (11) 

Gametocytes may also lose their infectivity over time (Dearsly et al. 1990) and this factor could also be introduced by simply adding another term to loss of parasites, if desired.

The noninfected host population increases at a net rate \( r_2 \) (birth rate minus natural death rate). The 'recovered' hosts are assumed to be added to the
noninfected population. The non-infected population of hosts increases as the sum of recovery (eq. 14) and birth, this last being,

\[ r_2 H_2 \quad (12) \]

Boyd (1940) and Vanderberg and Nawrot (1968) demonstrated that, in general, the higher the sporozoite inoculum the greater the percentage of hosts infected. We therefore assume for simplicity that the probability of a host infection is dependent on the square of the number of sporozoites that the host received, that is \( \beta_2 i^2 \). Then noninfected hosts are infected at a rate,

\[ f_2 \sum \frac{H_2}{H_2 + H} P(i) \beta_2 i^2 \quad (13) \]

Similar as in equation 11 above, the recovery rate increasing levels in the noninfected host population is,

\[ \sum H_2 P_2(i) \left(1 - \frac{i^2}{R_c}\right) \quad (14) \]

Finally, we describe the dynamics of infected host population. three components contribute to the death of infected hosts. First is natural death, which we ignored due to its very low rate in relation to vector and parasite populations. Second, pathogen-induced mortality, the estimation of which is similar to the second part in equation 4 above,

\[ \sum H_2 P_2(i) \alpha_2 i^2 \quad (15) \]

and third, loss due to recovery, identical to equation 14 above,
Model equations

We took the negative binomial distribution as the appropriate one for a parasite such as malaria which has a very low prevalence in the vector (see our analysis of field data by Collins et al. 1984 and Rosenberg et al. 1990 in Table 2); it uses the parameter $k$ as a measure of aggregation. Were it more abundant, the variance to mean ratio might be more appropriate (Scott 1987). We therefore propose 6 equations to describe the population dynamics of vectors, hosts and parasites, namely,

\[
\frac{dP_1}{dt} = \lambda P_1 S \frac{P_2^2 k' + P_2}{H_2 + H_2'} - \alpha \left( \frac{P_1^3 J^2}{H_1^2} + \frac{P_1^3 J'}{H_1 J'} + \frac{P_1^2 J'}{H_1'} + P_1 \right) - b_2 P_1 \quad (17)
\]

\[
\frac{dH_1}{dt} = r H_1 \left( 1 - \frac{H_1}{\mu} \right) - f \beta H_1 \frac{P_2^2 k' + P_2}{H_2 + H_2'} - b_2 H_1 \quad (18)
\]
\[
\begin{align*}
\frac{dH'_1}{dt} &= f_1 \beta_1 \frac{y k'}{H'_2} + P_2 - b_2 H'_1 - \alpha_1 \frac{P_1}{H'_1} + P_1 \\
\frac{dP_2}{dt} &= \lambda_2 f_2 \frac{S H_2}{H_2 + H'_2} - \left( \frac{1}{R_c} \right) \left( \frac{P_1}{H'_1} + P_1 \right) - \left( \frac{P_2}{H'_2} + \frac{P_2}{H'_2} \right) - P_2 \\
\frac{dH_2}{dt} &= r_2 H_2 - f_2 H_2 \beta_2 \frac{1}{H_2 + H'_2} - \frac{1}{R_c} \left( \frac{P_2}{H'_2} + P_2 \right) + H'_2 \\
\frac{dH'_2}{dt} &= f_2 H_2 \beta_2 \frac{1}{H_2 + H'_2} - \left( \alpha_2 - \frac{1}{R_c} \right) \left( \frac{P_2}{H'_2} + P_2 \right) - H'_2
\end{align*}
\]
System Dynamic Behaviors

Dynamics of malaria transmission

The complexity of the above system has prohibited us from exploring the dynamic characters analytically. We therefore used somewhat less elegant numerical methods to achieve this purpose, concentrating on three potentially novel parameters in malariology, namely, parasite distribution, parasite fecundity and parasite-induced mortality. Some parameter values of the simulation analyses were taken from the literature.

First, we suggest that there is an optimal distribution pattern for parasites (Fig. VI. 2.). Intuitively, this situation may be interpreted as a trade-off between number of hosts or vectors infected and parasite load. A high aggregation means very few infected vectors while a low aggregation results in an extremely low parasitaemia or parasite inoculum.

Our results differ slightly from other analyses in that our simulations suggest an optimal degree of aggregation, rather than concluding that aggregation in general enhances parasitaemia. Crofton (1971) concluded that the influence of aggregation on the host population level is greatest when the coefficient of the negative binomial was smaller than 2. May and Anderson (1978), based on their 'model E', suggested that when parasite fecundity is high, the extinction of both host and parasite population may be avoided if aggregation is high as well. At least one field-based study in parasitology, dealing with honey bee-mite relationships, has demonstrated an optimal degree of parasite aggregation that has functional significance (Burgett et al. 1990). In addition, our preliminary analyses (Rossignol, Saengtharatip and Li, unpublished) of recent field studies of human malaria oocyst distribution in
vectors suggest a negative binomial distribution, as would be expected for a macroparasite (Table VI. 2).

Second, we propose that parasite fecundity may be a valuable determinant of malaria dynamics. Crofton (1971) first quantified the effects of parasite reproduction under an 'achievement factor' ($A_f$), a composite parameter incorporating both the reproductive capacity of the parasite and the probability of individuals establishing themselves in a host; he stated that when $A_f$ is low the production of both parasites and hosts are maintained at a high level and that, with an increase in the value of $A_f$, the levels of these populations fall. May and Anderson (1978) studied macroparasites that reproduce within their intermediate invertebrate host and reported that too large a value of parasite birth rate produces extinction of both host and parasite populations, while too small a value leads to unregulated host population growth. Our simulations (Fig. VI.3.) yield a optimal parasite fecundity, leading us to a similar conclusion as May and Anderson's.

Third, we found that intermediate pathology of malaria parasite could lead to peaks in infected cases, albeit not an optimum equilibrium level (Fig. VI.4.). Based on simulations and assuming a negative binomial distribution, Crofton (1971) reported that reduction in parasite pathology might increase the tendency for oscillation in population levels of parasite and host. Similarly in our simulations, when parasite-induced mortality of the vector was increased ten-fold, an oscillation in parasite level occurred, but further increases tended to dampen it and bring a dramatic decrease in the number of cases, although not extinction (Fig. VI.4.).

Finally, we present the dynamic relationship between populations of infected vectors, non-infected vectors and infected hosts (Fig. VI.5.). An
asymptotic stable oscillation could be observed. The system reaches and maintains a stable state at the center of the spiral.

System sensitivity and equilibrium perturbation analysis

Because of the large dimension of the model and its nonlinearity, analytical methods for studying sensitivity and stability are not practical and we therefore used simulations to explore it. First, we varied 3 parameters at equilibrium to observe the effects of change to the behavior of the system (Fig. VI.6). We found that even when the magnitude of parameters was increased by 20%, the system still could regain its initial state or at least maintain a new stable state around the equilibrium; similar results were also observed when the magnitude of the three parameters were decreased by 20%. Secondly, we observed that perturbations in initial conditions also stabilized around the initial state (not shown). Based on the results, it appears that the model is stable for a considerable range of perturbations under the conditions that the simulations were made. We must caution however that some, if not all of the parameters are of theoretical value and their measurements will be required for a realistic assessment.
Discussion

Our purpose was to investigate the potential impact of parasite aggregation on the population dynamics of malaria. We chose to do so by considering density-dependent effects that intuitively and experimentally appear to occur but have not been considered in classical models of malaria. Based on simulations, we found first, that introducing density-dependent vector mortality, parasite aggregation and parasite fecundity into a model of malaria, transmission will still achieve equilibrium as well as a high degree of stability under reasonable conditions and values. Second, not only may the classical considerations of proportions of infected hosts and vectors have a great effect on transmission, but so do parasite distribution patterns, either in host or vector populations. For equal numbers of parasites, different distribution patterns could result in quite different epidemiological profiles. Finally, vector-induced mortality and parasite proliferation in host or vector play an important role although not as counterintuitive as other factors.

We must stress that this study is strictly theoretical and does not imply that density-dependent effects occur or, if they do, that they are epidemiologically significant. Many of the empirical observations that we marshall in support of our analyses are contradicted by others in the vast literature of malaria. The important point to remember, however, is that any supporting or contradicting data is suspect to some degree. We suggest that density-dependent effects in malaria can lead to stable transmission under theoretical simulations and that it may therefore be worth investigation; it should not be discarded off-hand. Unfortunately, the equations appear unsolvable analytically and we can only provide a numerical, inherently partial, and less elegant solution.
Dobson (1988) analysed the impact of parasite-induced vector mortality in the Ross-Macdonald model and concluded that equilibrium level was lowered and stability heightened. That study however did not address the impact of density-dependent mortality and aggregation effects in the malaria vector. Such effects are addressed in his models of intermediate host transmission. In this last case he concluded that two non-zero equilibria arise and that the high one introduces oscillation into the system due to its unstable nature. We cannot achieve the generality of his models since our equations are not solvable analytically, but our model nevertheless demonstrates that malaria could possess macroparasite tendencies, still achieve stability, and display unexpected behavior.

In an important study, Dietz (1988) specifically addressed the problem of 'density-dependence' in malaria. In his insightful analysis, he assumed that density-dependence is a consequence of malaria parasites self-regulating either through input or output from host or vector. He defined density-dependence as an immune or physiological phenomenon wherein a host or vector is unable to acquire concurrent infections or so-called superinfections. No other density-dependent effect of the parasites was assumed on the vector or host and his models are still therefore of prevalence. He demonstrated that one of Macdonald's conclusion, namely, that entomological control is more effective than measures directed at the host, does not always hold if prevalence bottlenecks occur. Our assumptions are quite different in that we consider the effects of density-dependence on transmission and our model is one of distribution and not prevalence. Our model differs most however in that we postulate that a crucial determinant of transmission may be parasite distribution and intensity and not only prevalence of infected hosts and vectors. Ultimately,
both aspects will probably be of importance, but we emphasize that parasite distribution has not been considered so far but should be assessed.

In addition, we observed that optimal values of transmission occurred for some parameters. This last conclusion is of particular interest since counterintuitive results may be observable when the parameters are manipulated. Thus, if one were to intervene and purposefully or incidentally reduced parasite fertility, one could possibly increase transmission in the long run. Similarly, if genetic modification of vectors led to a change in parasite aggregation, transmission might again increase. On the positive side, our model indicates that lower transmission can occur on both sides of these optima, rather than only one. Our model also suggests parameters that could be monitored with the diagnostic tools being developed by modern biotechnology.

As powerful residual insecticides, such as DDT, were becoming readily available during and after the Second World War, Macdonald was elaborating Ross's model and pointing out that the hierarchy in entomological parameters could be used with some effectiveness, and this theoretical discovery was to be a cornerstone of control, at least until eradication programs were abandoned. Of the six parameters in Macdonald's version of the model, five are entomological (mortality, host preference, density, efficiency, extrinsic incubation period); only one is related to the host (recovery rate); all parasitological considerations are a function of one of the above. The Ross-Macdonald model was thus useful in the worldwide eradication campaign initiated in the 1950's because its theoretical predictions converged with two recent practical discoveries, namely, residual indoor spraying of chlorinated hydrocarbons and age grading of mosquitoes; the first allowing control programs to reduce adult mosquito life expectancy, and the last to evaluate results.
Recent discoveries and endeavours appear to be adding a parasitological approach to control in addition to an entomological one. Vaccines and new drugs are being developed and tested, and modern monitoring techniques allowing us to quantify parasite loads are emerging (Wirth et al. 1986). This is all well, except that theoretical considerations may have lagged behind and that clear targets of control and evaluation may be absent, admittedly a point of contention. As an initial step, our model introduces the concept that malaria parasites may be a major factor in determining the profile of transmission, and offers three parameters relating to the parasite which are measurable with modern biotechniques. Without supplanting previous models, it may offer targets better suited for the provider of vaccines or drugs; one might thus use these tools in a fashion that differs from insecticide. For example, it may allows us to identify the populations of infected hosts or vectors that are most responsible for maintenance, to monitor effects of parasite-directed interventions, and hopefully to devise new and sound strategies.

The model that we present above is based on theoretical considerations, although the initial instigation was based on laboratory and field studies by various workers that strongly suggest, although do not prove, density-dependent effects as well as an aggregated distribution in vector, at least under certain circumstances. In addition, the simulations used to evaluate our model rely on parameter values that are derived in part from the literature. We caution nevertheless that the model is still untested and requires intensive experimental and field validation, but that nevertheless it appears refutable.
Table VI. 1. Definitions of parameters used.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_1$, $f_2$</td>
<td>Biting rate of non-infected and infected mosquitoes, respectively</td>
</tr>
<tr>
<td>$H_1$, $H'_1$</td>
<td>Populations of non-infected and infected vectors, respectively</td>
</tr>
<tr>
<td>$H_2$, $H'_2$</td>
<td>Populations of non-infected and infected hosts, respectively</td>
</tr>
<tr>
<td>$i$</td>
<td>Number of parasite units in an individual host or vector</td>
</tr>
<tr>
<td>$J$</td>
<td>Aggregation coefficient of negative binomial distribution of parasite in vector population</td>
</tr>
<tr>
<td>$J'$</td>
<td>Abbreviation for $1/(1+J)$</td>
</tr>
<tr>
<td>$k$</td>
<td>Aggregation coefficient of negative binomial distribution of parasite in host population</td>
</tr>
<tr>
<td>$k'$</td>
<td>Abbreviation for $1/(k+1)$</td>
</tr>
<tr>
<td>$P_1$, $P_2$</td>
<td>Populations of sporozoites and gametocytes, respectively</td>
</tr>
<tr>
<td>$P_1(i)$, $P_2(i)$</td>
<td>Populations of infected vectors and hosts with $i$ parasite units, respectively</td>
</tr>
<tr>
<td>$r_1$, $r_2$</td>
<td>Instantaneous birth rate of mosquito and host, respectively</td>
</tr>
<tr>
<td>$R_e$</td>
<td>Square of parasite loading unit per most heavily infected host class</td>
</tr>
<tr>
<td>$S_1$, $S_2$</td>
<td>Density-dependent coefficient of parasite transmission from mosquito to host and host to mosquito, respectively</td>
</tr>
<tr>
<td>$\alpha_1$, $\alpha_2$</td>
<td>Instantaneous vector and host death rate which caused by the parasite(vector or host/unit time), respectively</td>
</tr>
<tr>
<td>$\beta_1$, $\beta_2$</td>
<td>Instantaneous vector or host infection rate as function of parasite load, respectively</td>
</tr>
<tr>
<td>$\lambda_1$, $\lambda_2$</td>
<td>Mean number of sporozoite units produced by one oocyst or of gametocytes resulting from one sporozoite per unit time, respectively</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Carrying capacity of mosquito population</td>
</tr>
</tbody>
</table>
Table VI. 2. Comparison of observed and expected (Rossignol, Saengtharatip and Li, unpub.) numbers of *An. gambiae* (Collins et al. 1984) and *An. dirus* (Rosenberg et al., 1990) with *P. falciparum* oocysts; expected numbers were calculated as in Royce and Rossignol (1990), in turn based on Crofton (1971). Seven additional *An. dirus* had over 20 oocysts each, and of these, three with over 100; these outliers were not used in the calculations. Another field study suggests a similar distribution although we have not analysed it (Burkot et al. 1988). The parameter k is the aggregation coefficient of the negative binomial distribution.

<table>
<thead>
<tr>
<th>Number of oocysts per vector</th>
<th>Number of vectors</th>
<th>An. gambiae</th>
<th>An. dirus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected</td>
<td>Observed</td>
</tr>
<tr>
<td>0</td>
<td>1186</td>
<td>1186.0</td>
<td>1744</td>
</tr>
<tr>
<td>1</td>
<td>41</td>
<td>37.7</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>17.6</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>10.8</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.4</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>5.4</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>4.1</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>3.2</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2.6</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>2.1</td>
<td>1</td>
</tr>
<tr>
<td>&gt;10</td>
<td>&gt;3</td>
<td>&gt;3</td>
<td>&gt;4 (below)</td>
</tr>
<tr>
<td>k</td>
<td>.035</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure VI.1. Schematic representation of model equations. Each number corresponds to an equation in the text.
Figure VI. 2. Simulations of number of cases (vertebrate hosts) over time under three different degrees of aggregation. Values of $k$ are .0351 (highly aggregated and as calculated in Table 2), .351 and 3.51 (essentially not aggregated), from front to back. Approximate initial conditions for Fig. 2-6 are 1,000 infected host, $10^8$ non-infected hosts, 20,000 infected vectors and 44,000 non-infected vectors, 600 unit numbers of sporozoites (as discussed in text), $5 \times 10^4$ unit numbers of gametocytes; these points are close to equilibrium according to numerical solutions and the programme was run until equilibrium was reached. Time units are arbitrary, but approximately weeks. Other parameters were unchanging.
Figure VI. 3. Simulations of number of cases over time under three different fecundity rates for parasites with initial condition of 60,000 unit numbers of sporozoites (as discussed in text), $5 \times 10^6$ unit numbers of gametocytes. Values of $\lambda_1$ are 10, 100, 150 and $\lambda_2$ are 500, 5,000, 7,500 from front to back. The values are approximations derived from published studies (Rosenberg et al. 1990; Dearsly et al. 1990).
Figure VI. 4. Simulations of number of cases over time under three different rates of parasite-induced mortality with initial conditions as in Fig. 3. Values for $\alpha_1$ (vector mortality) range from .025, .005 and .0005 from front to back (note reversed order). The values are derived from the literature (Klein et al. 1986).
Figure VI. 5. Simulated dynamic behaviour of the system starting from an arbitrary point and displaying dampened oscillatory movement toward an equilibrium point. Values of $\alpha_1 = .0005$, $\lambda_1 = 100$, $\lambda_2 = 5,000$ and $k = .055$. Both vector populations are $\times 10^5$ and the number of cases is $\times 10^3$; these data are speculative.
Figure VI. 6. Simulation of number of cases over time after increasing vector mortality (dashed line), fecundity (solid line) and aggregation (dotted line) by 20%.
LITERATURE CITED


Thomson, D. 1914. The origin and development of gametes (crescents) in malignant tertian malaria: Some observations on flagellation etc. Annals of Tropical Medicine & Parasitology 8: 85-104.


BIBLIOGRAPHY


APPENDICES
APPENDIX A

List of important parameters

<table>
<thead>
<tr>
<th>a</th>
<th>human biting habit</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>proportion of infected hosts</td>
</tr>
<tr>
<td>F</td>
<td>proportion of noninfected hosts</td>
</tr>
<tr>
<td>h</td>
<td>number of blood-feeding attempts</td>
</tr>
<tr>
<td></td>
<td>1: infected</td>
</tr>
<tr>
<td></td>
<td>2: noninfected</td>
</tr>
<tr>
<td>H</td>
<td>probability of host acquiring infection</td>
</tr>
<tr>
<td>J</td>
<td>number of attempts at blood location</td>
</tr>
<tr>
<td>k</td>
<td>number of bites received</td>
</tr>
<tr>
<td>m</td>
<td>relative number of vectors</td>
</tr>
<tr>
<td></td>
<td>1: infected</td>
</tr>
<tr>
<td></td>
<td>2: noninfected</td>
</tr>
<tr>
<td>N</td>
<td>number of hosts</td>
</tr>
<tr>
<td></td>
<td>1: infected</td>
</tr>
<tr>
<td></td>
<td>2: noninfected</td>
</tr>
<tr>
<td>P</td>
<td>probability of mosquito locating blood</td>
</tr>
<tr>
<td>S'</td>
<td>number of successful blood-feeding</td>
</tr>
<tr>
<td></td>
<td>attempts (engorgements)</td>
</tr>
<tr>
<td></td>
<td>1: from infected and noninfected</td>
</tr>
<tr>
<td></td>
<td>mosquitoes on infected host</td>
</tr>
<tr>
<td></td>
<td>2: ...on noninfected host</td>
</tr>
<tr>
<td></td>
<td>11: from infected mosquitoes on infected</td>
</tr>
<tr>
<td></td>
<td>host</td>
</tr>
<tr>
<td></td>
<td>12: from noninfected mosquitoes ...</td>
</tr>
<tr>
<td></td>
<td>21: from infected mosquitoes on noninfected host</td>
</tr>
<tr>
<td></td>
<td>22: from noninfected mosquitoes...</td>
</tr>
<tr>
<td>S&quot;</td>
<td>number of bites</td>
</tr>
<tr>
<td></td>
<td>1: parasite transfer to vector</td>
</tr>
<tr>
<td></td>
<td>2: ...to host</td>
</tr>
<tr>
<td>T</td>
<td>time</td>
</tr>
<tr>
<td>W</td>
<td>probability of host acquiring successive</td>
</tr>
<tr>
<td></td>
<td>broods</td>
</tr>
<tr>
<td>X</td>
<td>mosquito probing parameters</td>
</tr>
<tr>
<td>Y</td>
<td>number of hosts contacted</td>
</tr>
<tr>
<td>δk</td>
<td>probability of acquiring infection</td>
</tr>
</tbody>
</table>
APPENDIX B

The equations are:

\[ Y_1 = 298.054 + 581.816X_3 + 5566.28X_2X_3 - 8625.905X_1X_2X_3 - 
5665.563X_2^2X_3 + 3663.577X_1^3X_3 \]  

\( (r^2 = 0.78) \)  

\[ Y_2 = 282.977 + 6506.152X_3 + 323.3031X_1X_3 + 2283.961X_2X_3 - 386.365X_3^2 - 
7051.384X_1X_2X_3 - 8730.401X_2^2X_3 + 785.2355X_1^3X_3 \]  

\( 451.788X_1^2X_3^2 + 5556.385X_1^2X_3 \)  

\( (r^2 = 0.997) \)  

\[ Y_3 = 303.576 + 6652.24X_3 + 68.00002X_3^2 \]  

\( (r^2 = 0.998) \)  

where \( Y \) is the number of hosts contacted (\( Y_1 \) when probability of hematoma location, \( Ph \), has a linear relationship with vector gland function or apyrase level \( C \), \( Y_2 \) when \( Ph \) has exponential relationship with \( C \), and \( Y_3 \) when \( Ph \) has a natural base logarithmic relationship with \( C \), and \( X_1, X_2 \) and \( X_3 \) are vector gland function or apyrase level, \( C \), geometric factor, \( G \), and host switch factor, \( SWT \), respectively). (See derivations of these parameters in Ribeiro et al., 1985a; Rossignol and Rossignol, 1988).
APPENDIX C

Given that $P_i$ is the probability of a mosquito locating blood during an attempt on a particular host, where $i=1$ for infected and $i=2$ for noninfected mosquitoes, then the probability of locating blood on the $J^{th}$ attempt following $J-1$ unsuccessful attempts is,

$$P_i, (1-P_i)P_i, \ldots, (1-P_i)^{(J-1)}P_i, \quad J=1,2,\ldots \quad (4)$$

Then, the $J^{th}$ attempt on which a mosquito successfully locates blood with probability $P_v$ can be calculated as follows,

$$P_i+(1-P_i)P_i+(1-P_i)^2P_i+\ldots+(1-P_i)^{(J-1)}P_i \geq P_v \quad (5)$$

that is,

$$\frac{P_i[(1-P_i)^J -1]}{(1-P_i)-1} \geq P_v \quad (6)$$

which yields,

$$J_i \geq \frac{\log(1-P_v)}{\log(1-P_i)} \quad (7)$$
APPENDIX D

Assume that, if $\alpha$ and $\beta$ are the probabilities of locating blood by any vector on infected and noninfected hosts, respectively, then $E$ and $F$ are the proportions of infected and noninfected hosts ($E+F=1$). The parameters $\alpha$ and $\beta$ will vary according to the state of infectivity of host and vector (see Table III.3.). Assume that a vector population, infected and not, lands on a host population also mixed, and that no attraction difference exists. Distribution between hosts will be identical. Mosquitoes will have differing success depending on their infection status or their host's. After a certain period, mosquitoes will have either engorged or desisted (Ribeiro et al., 1985a). If the unsuccessful mosquitoes are then grouped together again and split between the various hosts, we see that a bias can occur towards certain groups.

We have then a series for the number of infected mosquitoes successfully engorging on infected hosts, for every step,

$$
E_1 \alpha_1, E_1 \alpha_1 [E(1-\alpha_1)+F(1-\beta_1)], E_1 \alpha_1 [E(1-\alpha_1)+F(1-\beta_1)]^2, E_1 \alpha_1 [E(1-\alpha_1)+F(1-\beta_1)]^3, \ldots \ E_1 \alpha_1 [E(1-\alpha_1)+F(1-\beta_1)]^{h_1}
$$

the sum of which yields $S'_{11}$. The number of infected mosquitoes remaining after each step is,

$$
m_1, m_1 [E(1-\alpha_1)+F(1-\beta_1)], m_1 [E(1-\alpha_1)+F(1-\beta_1)]^2, m_1 [E(1-\alpha_1)+F(1-\beta_1)]^3, \ldots m_1 [E(1-\alpha_1)+F(1-\beta_1)]^{h_1}
$$

Assume that after $h_1$ steps all infected mosquitoes engorge except for one (for logarithmic calculations). We then have that,

$$
m_1 [E(1-\alpha_1)+F(1-\beta_1)]^{h_1} = 1
$$

from which the number of steps $h_1$ needed for infected mosquito population to obtain blood is,

$$
h_1 = \frac{-\log m_1}{\log [E(1-\alpha_1)+F(1-\beta_1)]}
$$
For infected mosquitoes on noninfected hosts, the series is,

\[ F_{m} \beta_{1}, \quad F_{m} \beta_{1}[E(1-\alpha_{1})+F(1-\beta_{1})], \quad F_{m} \beta_{1}[E(1-\alpha_{1})+F(1-\beta_{1})]^{2}, \quad F_{m} \beta_{1}[E(1-\alpha_{1})+F(1-\beta_{1})]^{3}, \ldots, \quad F_{m} \beta_{1}[E(1-\alpha_{1})+F(1-\beta_{1})]^{h_{1}} \]

the sum of which yields \( S'_{21} \).

Similarly, we can obtain series for noninfected mosquitoes on infected hosts and noninfected hosts which yield \( S'_{12} \) and \( S'_{22} \).

Similarly for noninfected mosquitoes,

\[ h_{2} = \frac{-\log m_{2}}{\log[E(1-\alpha_{2})+F(1-\beta_{2})]} \]
APPENDIX E

For a noninfected mosquito however blood must be successfully obtained for transmission to occur. The number of bites resulting in parasite transfer to the vector is,

\[ \text{Em}_2\alpha_2, \text{Em}_2\alpha_2\langle E(1-\alpha_2)+F(1-\beta_2)\rangle, \text{Em}_2\alpha_2\langle E(1-\alpha_2)+F(1-\beta_2)\rangle^2, \text{Em}_2\alpha_2\langle E(1-\alpha_2)+F(1-\beta_2)\rangle^3, \ldots, \text{Em}_2\alpha_2\langle E(1-\alpha_2)+F(1-\beta_2)\rangle^{h_2} \]

the sum of which is \( S''_1 \).

We assume that sporozoites can be delivered when a mosquito probes and salivates and that engorgement is not necessary for infection. The number of infections will be,

\[ \text{Fm}_1, \text{Fm}_1\langle E(1-\alpha_1)+F(1-\beta_1)\rangle, \text{Fm}_1\langle E(1-\alpha_1)+F(1-\beta_1)\rangle^2, \text{Fm}_1\langle E(1-\alpha_1)+F(1-\beta_1)\rangle^3, \ldots, \text{Fm}_1\langle E(1-\alpha_1)+F(1-\beta_1)\rangle^{h_2} \]

the sum of which is \( S''_2 \).