MALARIA IN NIGERIA: CONSTRAINED CONTINUOUS-TIME MARKOV MODELS FOR DISCRETE-TIME LONGITUDINAL DATA ON HUMAN MIXED-SPECIES INFECTIONS

Joel E. Cohen[1] and Burton Singer[2]

"A thorough prior knowledge of the endemicity of malaria will be essential in a population which is to receive an acceptable malaria vaccine."

S. Cohen (1979, p. 340)

"In art as in science there is no delight without the detail, and it is on details that I have tried to fix the reader's attention. Let me repeat that unless these are thoroughly understood and remembered, all 'general ideas' (so easily acquired, so profitably resold) must necessarily remain but worn passports allowing their bearers short cuts from one area of ignorance to another."

V. Nabokov (1972, p. 8)

Abstract

A research project on malaria in the Garki district of northern Nigeria included 8 baseline surveys at approximately

---

AMS(MOS) subject classifications (1970). Primary 60J20, 62M05, 92A15; Secondary 60J25, 62P10

[1]Partially supported by National Science Foundation grant DEB74-13276 to The Rockefeller University.

[2]Partially supported by National Science Foundation grant SOC76-17706 to Columbia University.

10 week intervals prior to efforts to control malaria. Using data from these surveys, we define 4 infection statuses for each person: uninfected, singly infected with either Plasmodium malariae (P.m.) or P. falciparum (P.f.), and jointly infected with both species. We study the 4 × 4 estimated transition probability matrices P(s,t) from survey s to survey t during the 8 baseline surveys. We find that the infection histories are representable as a time-series of time-homogeneous continuous-time Markov chains with intensity matrices of a special form, called model Q. This model excludes the possibility that both P.f. and P.m. would either be gained or lost simultaneously. The model makes it possible for the first time to disentangle the transition intensities of one species when a second species is absent from the transition intensities of the first species when the second species is present.

A special form of Q, called model M, which is formally equivalent to Lotka-Volterra competition equations linearized near equilibrium, did not describe the full set of transition tables. We infer that the Lotka-Volterra equations should not be regarded as a general model for the interaction of malarial species in human populations.

The variation of the estimated parameters of model Q, as functions of age and season, is reviewed in detail. A major finding is that adults are generally better than very young children in eliminating infections from peripheral blood. Adults appear to be less susceptible than children only to infection with P.f. when P.m. is absent.

Possible non-Markovian dependence between events separated by at least one survey is not modeled in our time-series representation. We present evidence that initially uninfected individuals remain uninfected, and initially doubly infected individuals remain doubly infected, with higher frequency than would be predicted from the Markovian assumption.

Contents

1. Introduction

Malaria remains one of man's most durable and lethal parasitic diseases. From 1972 to 1976, the number of confirmed autochthonous (i.e., not imported) cases of malaria in the world increased from 3,251,000 to 7,517,000, an increase of 131%. These figures omit China, Kampuchea and Vietnam, for which figures are not available, and all of Africa, for which the figures are unreliable (Director-General of WHO, 1978, p. 226). In Europe, the number of autochthonous cases increased from 21,000 to 39,000 and in The Americas from 284,000 to 379,000. For Africa, the estimated number of annual deaths due to malaria is one million (S. Cohen, 1979, p. 324). By the end of 1976, "the population of originally malarious areas of the world was 2,048 million . . . 352 million (17%) were living in places where no antimalaria measures were undertaken" (Noguer et al., 1978, p. 9).

An improved understanding of the natural dynamics of malaria infections in unprotected human populations may provide a more precise baseline against which the performance of control measures can be assessed and may suggest critical points at which the disease could be attacked.

The research project on the epidemiology and control of malaria conducted in the Garki District, Kano State, jointly by the Government of Nigeria and the World Health Organization, included among its objectives the study of the baseline epidemiology (Molineaux and Gramiccia, 1979). This project collected data uniquely suited to test ecological and evolutionary models of the dynamics of multiple species infections in unprotected human populations (Cohen, 1970). Independently of these empirical and theoretical studies, methods have been developed to analyze longitudinal survey data, such as those collected in Garki, in terms of continuous-time stochastic models like those proposed for malaria (Singer and Spilerman, 1976a, 1976b; Singer and Cohen, in press).

This paper draws together these recent empirical, theoretical, and methodological developments. The new results presented here are both methodological and substantive. These results are summarized in the Discussion, section 10.

2. Materials and Methods

Molineaux and Gramiccia (1979) describe the Garki study comprehensively. The Garki study had three phases: a baseline period, a control period, and a follow-up period. The data discussed in this paper are drawn entirely from the baseline period.

During the 18-month baseline period, from November 1970 to May 1972, a previously unprotected human population was studied without any attempts to interfere with malaria. This

population lived in a rural district (Garki) of northern Nigeria, in the West African Sudan savanna. In this region there is a long dry season and a short wet season. The density of mosquitoes and the transmission of malaria are low in the dry season and high in the wet season. Eight village-clusters (follow-up units, each containing two or more villages or parts of villages) were surveyed every 10 weeks. Eight of these surveys fell within the baseline period.

Sixteen villages were included from survey 1; 6 more villages (or sections of villages) were added at survey 5. At that survey, the 22 villages had 7,423 inhabitants. The surveys aimed at total coverage. The initial survey included a nominal de facto census, updated at each survey.

The dates, number of villages, season, and number of individuals present at each successive pair of baseline surveys may be summarized as follows:

Survey	Approximate date of midpoint*	Number of villages	Season	Population 1† present at both surveys n and n+1
1	11-70	16	wet	1514
2	17-2-71	16	dry	1450
3	23-4-71	16	dry	1784
4	30-6-71	16	dry	2364
5	15-9-71	22	wet	3620
6	6-12-71	22	wet	2720
7	20-2-72	22	dry	2635
8	1-5-72	22	dry	

*Dates of surveys 2-8 from Molineaux, Storey, Cohen and Thomas (in press).

†In addition, there were 2785 individuals from population 2 who were present at all 8 surveys.

At each survey, a thick blood film was collected from each individual. In collecting a thick blood film, a drop of blood is drawn, usually from a finger or ear lobe, and spread in a standardized way on a glass microscope slide. Each film was linked by a code number to the person's identity and examined microscopically by a standardized procedure. Among the characteristics recorded from each blood film examination, the 2 of interest here are the presence or absence of malarial parasites of the species Plasmodium malariae (hereafter abbreviated P.m.) and the presence or absence of a second malarial species Plasmodium falciparum (abbreviated P.f.). We omit discussion of Plasmodium ovale. Each individual in a survey has one of 4 states of infection:

State	Symbol	P.f.	P.m.
0	(-,-)	absent	absent
1	(-,+)	absent	present
2	(+,-)	present	absent
3	(+,+)	present	present

Sometimes we refer to P.m. as species 1 and to P.f. as species 2. With this convention, for i = 1, 2, an individual is in state i when he is infected with species i only.

The number of the state corresponding to an infection status may be computed by replacing - by 0, + by 1 and interpreting the ordered pair of numerals as a binary integer: thus $(+,-) = 10_2 = 2$.

We adopt the convention that an individual with a positive blood smear is infected and that an individual with a negative blood smear is uninfected. Sampling errors and errors in diagnosis are discussed by Molineaux and Gramiccia (1979, Ch. 5). Aside from these sources of error, an individual with a negative blood smear may harbor exoerythrocytic forms of the malaria parasite, e.g., in the liver, without parasites in the peripheral blood. Hence the estimated intensity with which an "uninfected" individual acquires infection may overstate the intensity with which a truly uninfected individual acquires infection, and the estimated intensity with which an infected individual appears to "lose" infection may overstate the true intensity with which an infected individual loses infection.

We refer to the collection of all individuals who were present for at least one of the 8 baseline surveys but who were not present at all of them as population 1. We refer to the 2,785 individuals who were present at all 8 baseline surveys as population 2. We refer to the combination of populations 1 and 2 as population 3.

Each individual falls into one of 7 age groups, according to his age at survey 1. These age groups are:

Age group	Range of ages (in years)
1	infants (less than 1)
2	1-4
3	5-8
4	9-18
5	19-28
6	29-43
7	44 and older

The irregular boundaries of these age groups minimize the effect of age rounding (the tendency of people who are uncertain of their own age to pick round numbers like 5, 10, or 20) and provide greatest detail at the younger ages, where incidence and prevalence are changing most rapidly.

Let u be the number of any one of the first 7 surveys, and v be the number of any one of the baseline surveys later than survey u. There are 28 pairs of surveys (u, v) for which

$u < v$. For each pair (u, v) of baseline surveys, for each of the 3 populations just defined, for each of the 7 age groups, the data of this study are 4×4 tables of transition frequencies. Each entry in such a table gives the number of people who had infection status i at survey u and who subsequently had infection status j at survey v, $i = 0, 1, 2, 3$; $j = 0, 1, 2, 3$. For each of the 3 populations, there are $28 \times 7 = 196$ such 4×4 tables. It is not feasible to present the data here. We shall present illustrative analyses of one 4×4 transition table and summaries of parallel analyses of the remaining tables.

3. Mixed-Species Interactions

We conceptualize the infection histories of persons in each age class as realizations of a 4-state continuous-time stochastic process $\{X(t,w),\ 0 \le t \le \tau\}$, where τ is the duration of the baseline surveys, here approximately 80 weeks, and w is a sample path. We define the transition rates

$$\rho_{ij} = \lim_{t \downarrow s} E(\text{number of } i \to j \text{ transitions in } X(u) \text{ during } [s,t) \mid X(s)=i)/(t-s) \qquad (3.1)$$

for $i \neq j$, $i,j = 0,1,2,3$. By suppression of P.m. by P.f., we mean $\rho_{01} > \rho_{23}$ and $\rho_{32} > \rho_{10}$. On the other hand, we interpret $\rho_{01} < \rho_{23}$ and $\rho_{32} < \rho_{10}$ to mean that P.f. enhances P.m.: the rate of acquisition of P.m. is higher for persons already positive for P.f. than for those who are negative for P.f., and an individual positive for P.m. is more likely to become negative for P.m. in the absence of P.f. than in the presence of P.f. Similar interpretations ensue for comparisons of ρ_{02} with ρ_{13} and of ρ_{20} with ρ_{31} when the roles of P.m. and P.f. are reversed.

Since the data in this study do not allow us to observe $\{X(t,w),\ 0 \le t \le \tau\}$, we cannot directly measure (3.1). Our only recourse is to propose classes of stochastic models to describe the available data

$$t_{ij}(u\Delta, v\Delta) = \text{number of persons who are in state } i \text{ at survey } u+1 \text{ and in state } j \text{ at survey } v+1, \qquad (3.2)$$

where $0 \le u < v \le 7$, and Δ is the sampling interval between successive surveys, here approximately 10 weeks. Next, we ascertain whether any of the proposed models could have generated (3.2). Then we estimate (3.1) and infer competitive or cooperative behavior between malarial species by appropriate comparisons of ρ_{ij} estimated within a model that is not rejected.

The models we shall consider are 4-state homogeneous, continuous-time Markov chains with intensity matrices denoted by

$$\underset{\sim}{R} = \{R : R_{ij} \ge 0,\ i \neq j,\ \sum_{j=0}^{3} R_{ij} = 0\}. \qquad (3.3)$$

We will restrict attention to the sub-set of $\underset{\sim}{R}$ defined by

$$\underset{\sim}{Q} = \underset{\sim}{R} \cap \{Q : Q_{i,3-i} = 0,\ i = 0,1,2,3\}. \qquad (3.4)$$

The zero elements on the minor diagonal exclude the possibility that both P.f. and P.m. would either be gained or be lost simultaneously. Intensity matrices of exactly this form are considered by Coleman (1964, p. 104, his Fig. 4.5). Within this class of models, the transition rates (3.1) are the intensities Q_{ij}, $i \neq j$. We also introduce two alternative and more restrictive specifications of the transition rates (3.4); namely,

$$\underset{\sim}{M} = \underset{\sim}{Q} \cap \{M : M_{01}-M_{23} = M_{32}-M_{10} \text{ and } M_{02}-M_{13} = M_{31}-M_{20}\}$$

and

$$\underset{\sim}{L} = \underset{\sim}{Q} \cap \{L : L_{01}/L_{23} = L_{32}/L_{10} \text{ and } L_{02}/L_{13} = L_{31}/L_{20}\} .$$

$\underset{\sim}{M}$ specifies that the difference in rates of acquisition of a given species of parasite without--in contrast to with--the presence of a second species is the same as the difference in rates of loss of the given species with--in contrast to without—the presence of the second species. $\underset{\sim}{L}$ is a multiplicative specification of the same idea. The notion of a multiplicative formulation here is due to Tukey (see Sørenson, 1975, p. 88) although no prior models coincide with $\underset{\sim}{L}$.

This kind of balance between acquisition rates and loss rates appears in a Markov chain analog of a linearized form of the Lotka-Volterra equations for competition (Cohen, 1970). In particular, for a 4-state continuous-time Markov chain with an intensity matrix $M \in \underset{\sim}{M}$, the differential equations that describe the expected fraction $x_1(t)$ of people infected with P.m. and the expected fraction $x_2(t)$ infected with P.f. are formally identical to the Lotka-Volterra equations

$$\begin{aligned} dx_1/dt &= r_1x_1(1-ax_1-\alpha x_2) \\ dx_2/dt &= r_2x_2(1-\beta x_1-bx_2) \end{aligned} \qquad (3.5)$$

when equations (3.5) are linearized in the neighborhood of the equilibrium where both species are present.

The constraints that define $\underset{\sim}{M}$ and $\underset{\sim}{L}$ specify 6-parameter families of intensity matrices, in contrast to the 8-parameter family defined by (3.4). The matrices in $\underset{\sim}{M}$ may be represented as

$$M = \begin{pmatrix} -\lambda_1-\lambda_2 & \lambda_1 & \lambda_2 & 0 \\ \mu_1 & -\mu_1-\lambda_2+\epsilon_2 & 0 & \lambda_2-\epsilon_2 \\ \mu_2 & 0 & -\mu_2-\lambda_1+\epsilon_1 & \lambda_1-\epsilon_1 \\ 0 & \mu_2+\epsilon_2 & \mu_1+\epsilon_1 & -\mu_1-\epsilon_1-\mu_2-\epsilon_2 \end{pmatrix}$$

where $\lambda_i \geq 0$, $\mu_i \geq 0$, and $\lambda_i \geq \epsilon_i \geq -\mu_i$, $i = 1,2$. The matrices in $\underset{\sim}{L}$ may be represented as

$$L = \begin{pmatrix} -b_1d_1-b_2d_2 & b_1d_1 & b_2d_2 & 0 \\ a_1/d_1 & -b_2-a_1/d_1 & 0 & b_2 \\ a_2/d_2 & 0 & -b_1-a_2/d_2 & b_1 \\ 0 & a_2 & a_1 & -a_1-a_2 \end{pmatrix}$$

where $a_i \geq 0$, $b_i \geq 0$, $d_i > 0$, $i = 1,2$.

In these models, suppression of P.m. by P.f. is represented by $\epsilon_1 > 0$ in M and $d_1 > 1$ in L. Suppression of P.f. by P.m. is represented by $\epsilon_2 > 0$ in M and $d_2 > 1$ in L. If the inequalities are reversed, then the two species reinforce or enhance one another.

To test the hypotheses underlying $\underset{\sim}{Q}$, $\underset{\sim}{M}$ and $\underset{\sim}{L}$, we fit each of the 3 model types to the 4×4 tables of counts for each age class

$$T((k-1)\Delta, k\Delta) = (t_{ij}((k-1)\Delta, k\Delta)), \ 1 \leq k \leq 7, \qquad (3.6)$$

and measure the goodness of fit of members of a single model class to this time-series of transition counts.

By modeling each of the 4×4 tables of counts separately, we represent the process $\{X(t,w), 0 \leq t \leq \tau\}$ in the form

$$X(t,w) = Z_k(t,w), \quad (k-1)\Delta \leq t < k\Delta, \quad 1 \leq k \leq 7, \qquad (3.7)$$

where $Z_k(t,w)$ is a homogeneous continuous-time Markov chain that describes transitions only between surveys k and k+1. Possible non-Markovian dependence between events in $X(t,w)$ separated by at least one survey is not modeled in the representation (3.7). The full probability structure of $\{X(t,w),\ 0 \le t \le \tau\}$ is not ascertainable from the time-series of tables of counts (3.6), even when supplemented by the tables

$$T(u\Delta, v\Delta), \quad 0 \le u < v \le 7, \quad \text{with } v - u \ge 2. \tag{3.8}$$

Though our analysis ignores possible dependence among events at points widely spaced in time, it is the first analysis of mixed-species infections in which the rates of acquisition and of loss of one species when a second species is absent can be disentangled from the corresponding rates when a second species is present.

4. Fitting Models to Data

We assume a priori that the infection histories of individuals in each age class are independent realizations of some 4-state continuous-time stochastic process. Thus, conditional on being in state i at time $k\Delta$, an individual has an a priori unknown, multinomial probability $p_{ij}(k\Delta, (k+1)\Delta)$, $j = 0,1,2,3$, $\Sigma_{j=0}^{3} p_{ij}(\cdot) = 1$, of being in state j at the next survey time, $(k+1)\Delta$. We estimate $p_{ij}(\cdot)$ from the transition counts $T(k\Delta, (k+1)\Delta)$ using the maximum likelihood estimator

$$\hat{p}_{ij} = t_{ij}(k\Delta, (k+1)\Delta)/t_{i+}(k\Delta) \tag{4.1}$$

where $t_{i+}(k\Delta) = \Sigma_{j=0}^{3} t_{ij}(k\Delta, (k+1)\Delta)$, $k = 0,1,\ldots,7$.

Since the transition matrices of time-homogeneous Markov chains are of the form

$$(p_{ij}(s,t)) = (\underset{\sim}{P}(X(t)=j \mid X(s)=i)) = e^{(t-s)\underset{\sim}{R}} \tag{4.2}$$

where $0 \le s \le t$ and $R \in \underset{\sim}{R}$ (Doob, 1953), we test our hypotheses about parasite interactions within the class of time-homogeneous Markov chains by seeking to replace R in (4.2) with a matrix of the form Q, M, or L, for $t-s = \Delta$. We measure the goodness of fit of a model R by

$$G_R^2 = -2\ \Sigma\ (\text{observed frequency}) \cdot \log(\text{frequency predicted by model/observed frequency}) \tag{4.3}$$

where the summation is over all cells in the 4 × 4 table $T(k\Delta, (k+1)\Delta)$ with nonzero observed frequencies (Bishop, Fienberg and Holland, 1975). The predicted frequencies under models $\underset{\sim}{Q}$, $\underset{\sim}{M}$ and $\underset{\sim}{L}$ are given by $(T_R)_{ij} = t_{i+}(e^{\Delta R})_{ij}$ for R in $\underset{\sim}{Q}$, $\underset{\sim}{M}$ and $\underset{\sim}{L}$, respectively.

For each table of transition counts in each age class we compute a matrix Q, M, and L that minimizes G_Q^2, G_M^2 and G_L^2, respectively. These estimated minimal values of $G_\cdot^2$ and the corresponding estimated intensity matrices are given in Tables 5, 6 and 7. The numerical methods used to compute $G_\cdot^2$ and the intensity matrices are presented in Appendix 2.

Since $G_\cdot^2$ has asymptotically, for large predicted frequencies, the distribution of a χ^2 variate with degrees of freedom (d.f.) appropriate to the model being fitted, we can find the probability, for each class of models, that a worse agreement between observed and predicted frequencies would have occurred by chance. These probabilities and an appraisal of the performance of the models are given in section 7.

5. A Detailed Example

We now give an example of the analysis of a single 4 × 4 table of data.

Table 1 gives the recorded number of people aged 19 to 28 years old (age group 5) present at both surveys 4 and 5 (population 3) who made each transition from state i = 0, 1, 2, 3 to state j = 0, 1, 2, 3. These data are labelled T (for transitions).

In each cell of Table 1, beneath each datum T, are 5 predicted frequencies for the same transition. The predicted frequencies labelled Q, M, and L are computed, as described previously, from

$$t_{i+}(e^R)_{ij}, \text{ where } R = Q, M, L .$$

The predicted frequencies labelled 1 and 2 are derived from models 1 and 2 of Molineaux et al. (in press). Model 1 assumes complete independence between species in the transitions from one survey to the next; in the 4 × 4 table, the model has 4 fitted parameters and 8 d.f. Model 2 assumes conditional independence: the transitions of each of the 2 species of malaria are independent but occur at rates that depend on the presence or absence of the other species of malaria. In the 4 × 4 table, model 2 has 8 fitted parameters and 4 d.f. For details of parameter estimation, see Molineaux et al. (in press).

Table 1 gives the summary G^2 measure of goodness of fit and d.f. for each model. For these data (though not in general), all 5 models are acceptable. In the absence of other information, one would prefer models with the fewest fitted parameters or greatest d.f. Molineaux et al. (in press) observe that model 1 is not an adequate model in general. In unpublished work, we find that if the entire set of transition

frequencies is viewed as a large multidimensional contingency table, model 2 is also inadequate. We evaluate Q, M and L in general in the next section.

Certain comparisons of the models in Table 1 are possible. Model 2 contains model 1 as a special case. Q contains both M and L as special cases. Because of these "hierarchical" relations, it is valid to subtract values of G^2 and corresponding d.f. to determine whether a model with fewer parameters fits significantly worse. For example, $G^2_M - G^2_Q = 7.483 - 3.764 = 3.719 = G^2_{M-Q}$ with $\text{d.f.}_{M-Q} = \text{d.f.}_M - \text{d.f.}_Q = 6 - 4 = 2$. Thus, in this instance, there is no evidence to favor the 8-parameter Q over the simpler 6-parameter M. However, $G^2_{L-Q} = 10.662 - 3.764 = 6.898$ with $\text{d.f.}_{L-Q} = 2$ is significant at the 5% level, according to a table of the χ^2-distribution. Some people would accept this result as evidence that L might be worse than Q even though it is simpler and even though the fit of L is not significantly bad considered by itself.

Table 2 gives the elements of C = Re(log P), Q, M, and L. The imaginary part of log P in this instance has every element less than 10^{-14}. Thus here no information has been lost by disregarding the imaginary part of log P.

Except in the second row (corresponding to state 1), the elements on the minor diagonal of C are the smallest (in magnitude) elements of the corresponding row. The requirement in Q, M and L that the minor diagonal elements be 0 is thus not Procrustean.

The interval between surveys has been taken as the unit of time. To convert the entries in Table 2 to rates per day, divide by the interval between the midpoints of surveys 4 and 5, approximately 77 days.

By comparing the numerical values in Table 2 with the corresponding matrix elements in the definitions of the models

Table 1. Transitions in infection state between surveys 4 and 5 for all individuals aged 19-28 years present at both surveys (population 3): observed and predicted frequencies

State at survey 4 (30 June 1971)	Entry	State at survey 5 (15 Sept. 1971)			
		0 (P.f.-,P.m.-)	1 (P.f.-,P.m.+)	2 (P.f.+,P.m.-)	3 (P.f.+,P.m.+)
0	T	340	14	171	7
(P.f.-,P.m.-)	Q	340.1	13.6	168.3	10.0
	M	341.7	13.0	164.6	12.7
	L	347.0	12.2	160.4	12.3
	1	337.5	18.1	167.4	9.0
	2	340.0	14.0	171.0	7.0
1	T	21	2	9	0
(P.f.-,P.m.+)	Q	21.6	1.9	8.2	0.4
	M	20.4	1.6	9.1	1.0
	L	21.4	0.8	9.1	0.7
	1	19.1	2.3	9.5	1.1
	2	21.6	1.4	8.4	0.6
2	T	77	3	103	13
(P.f.+,P.m.-)	Q	77.1	3.7	106.1	9.2
	M	76.0	4.3	105.8	9.9
	L	73.0	3.9	109.1	10.0
	1	76.6	4.1	109.4	5.9
	2	73.5	6.5	106.5	9.5
3	T	16	2	20	4
(P.f.+,P.m.+)	Q	15.2	2.0	20.2	4.6
	M	16.4	1.6	21.2	2.7
	L	18.1	1.0	20.8	2.1
	1	15.4	1.9	22.0	2.7
	2	15.4	2.6	20.6	3.4

(Table 1 continued)

Model	Summary measures of fit		d.f.
	G^2	X^2	
Q	3.764	3.419	4
M	7.483	6.170	6
L	10.662	10.835	6
1	11.846	12.876	8
2	5.573	4.658	4

Q, M and L, one can find the estimated parameter values. For example, $\lambda_1 = M_{01} = 0.110$ and $\epsilon_1 = M_{01} - M_{23} = -.218$. The same estimate of ϵ_1 is obtained from $\epsilon_1 = M_{32} - M_{10} = 3.086 - 3.304$. The substantive surprise here is that ϵ_1 is negative. The negative sign of ϵ_1 is the opposite of that expected from the linearized Lotka-Volterra competition equations. However, $\epsilon_2 = 0.179$ is positive as expected.

Q differs from M only in that there is no requirement that $Q_{01} - Q_{23} = Q_{32} - Q_{10}$, and no analogous requirement for ϵ_2. Further insight into ϵ_1 may be gained by comparing the 2 unconstrained estimates of this interaction parameter from Q. Since $Q_{32} - Q_{10} = -1.405$, the rate of loss of P.m. when P.f. is absent at the initial survey exceeds the rate of loss of P.m. when P.f. is present; crudely speaking, the initial presence of P.f. helps P.m. remain present (a cooperative effect). Also, since $Q_{01} - Q_{23} = -0.090$, the rate of acquisition of P.m. when P.f. is present slightly exceeds the rate of gain of P.m. when P.f. is absent (again a cooperative effect). Thus the initial presence of P.f. consistently promotes the acquisition and reduces the loss of P.m. in this example.

On the contrary, the presence of P.m. does not have such a consistent effect on the acquisition and loss of P.f. in this example. Since $Q_{31} - Q_{20} = -0.142$, the rate of loss of P.f. when P.m. is absent at the initial survey exceeds the rate of P.f. loss when P.m. is present (a cooperative effect). But $Q_{02} - Q_{13} = +0.635$, so the rate of P.f. acquisition when P.m. is present initially is less than the rate of P.f. acquisition when P.m. is absent initially (a competitive effect). M has a positive value of ϵ_2 because here the competitive effect dominates the cooperative one.

This discussion illustrates the detailed information available from a comparison of the parameter estimates of the 3

Table 2. Intensity matrices Q, M and L estimated from the transition frequencies in Table 1 for all 19-28 year olds present at surveys 4 and 5; C = real part of log P.

State at survey 4 (30 June 1971)	Entry	State at survey 5 (15 Sept. 1971) 0 (P.f.-,P.m.-)	1 (P.f.-,P.m.+)	2 (P.f.+,P.m.-)	3 (P.f.+,P.m.+)
0	C	-0.782	0.154	0.710	-0.083
(P.f.-,P.m.-)	Q	-0.751	0.116	0.635	0
	M	-0.723	0.110	0.613	0
	L	-0.786	0.208	0.578	0
1	C	3.028	-3.174	0.357	-0.211
(P.f.-,P.m.+)	Q	3.351	-3.351	0	0
	M	3.304	-3.738	0	0.434
	L	7.205	-7.451	0	0.246
2	C	0.840	-0.095	-1.141	0.395
(P.f.+,P.m.-)	Q	0.764	0	-0.970	0.206
	M	0.734	0	-1.063	0.328
	L	0.626	0	-1.046	0.420
3	C	-0.049	0.828	2.216	-2.995
(P.f.+,P.m.+)	Q	0	0.621	1.946	-2.567
	M	0	0.914	3.086	-4.000
	L	0	1.469	3.561	-5.029

models.

6. Is Pooling Legitimate?

Many of the 4 × 4 transition tables for individuals who are present at all 8 baseline surveys (population 2) have some cells with small counts. It would be desirable, when testing models, to pool these tables with the corresponding tables from the population of persons who missed at least one survey (population 1). In this section we investigate whether there are systematic differences in transition proportions between these 2 populations.

To this end, consider the numbers in the first line of Table 3. In columns 1 and 2, the numbers "1" and "2" mean that we are examining the transitions in infection status from an initial survey, number 1, to a final survey, number 2. Columns 3, 4, and 5 pertain to all individuals who were initially (that is, at survey 1 in line 1) infected with both species (in state 3, (+,+)). These individuals were in one of 4 states at the final survey (survey 2 in line 1). We wished to test whether the proportions in each of the 4 states in population 1 differed significantly from the corresponding proportions in population 2. The numbers in columns 3, 4, and 5 describe the results of a homogeneity test on a 2 × 4 contingency table that was constructed as follows.

Starting with the youngest age group (infants, group 1), we entered the numbers of individuals from population 1 in each state at the final survey in row 1 of the 2 × 4 table, and the corresponding numbers from population 2 in row 2 of the 2 × 4 table. We then observed that at least one cell in the 2 × 4 table had fewer than 5 individuals. We then added the numbers of individuals from the next age group; individuals from population 1 were added to row 1 and from population 2 were added to row 2. We again observed that at least one cell had

Table 3. Homogeneity of transition frequencies between individuals absent from at least one of the 8 baseline surveys (population 1) and individuals present at all 8 of the baseline surveys (population 2). High values of G^2 or low values of P indicate lack of homogeneity. Text describes each column in greater detail.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
		Initial state (+,+)			Initial state (+,-)			Initial state (-,+)			Initial state (-,-)			Row sum		
Surveys		G^2	d.f.	P	G^2	d.f.	P	G^2	d.f.	P	G^2	d.f.	P	G^2	d.f.	P
1	2	0.883	3	0.830	10.632	6	0.100	0.121	3	0.989	0.130	3	0.988	11.766	15	0.697
1	3	1.119	3	0.772	12.939	9	0.165	0.315	3	0.960	1.328	3	0.676	23.900	18	0.156
1	4	7.048	3	0.070	4.518	12	0.972	2.714	3	0.438	10.933	9	0.280	25.213	27	0.563
1	5	3.464	3	0.325	14.859	9	0.095	1.910	3	0.591	12.242	9	0.006	32.525	18	0.019
1	6	0.169	3	0.982	6.621	3	0.085	2.313	3	0.510	15.725	3	0.001	24.831	12	0.016
1	7	1.906	3	0.592	4.910	6	0.621	3.223	3	0.358	7.247	3	0.064	16.786	15	0.332
1	8	2.657	3	0.448	7.907	3	0.048	2.398	3	0.494	0.247	3	0.970	13.210	12	0.354
2	3	4.345	3	0.227	10.791	6	0.095	1.159	3	0.763	5.241	3	0.513	21.536	18	0.253
2	4	0.236	3	0.972	4.560	6	0.601	3.088	3	0.378	1.959	9	0.992	9.843	21	0.981
2	5	4.524	3	0.210	8.616	6	0.196	0.470	3	0.925	5.754	3	0.124	19.364	15	0.198
2	6	3.717	3	0.294	5.332	3	0.149	2.364	3	0.500	10.283	3	0.016	21.695	12	0.041
2	7	1.912	3	0.591	1.643	3	0.650	3.468	3	0.325	14.244	3	0.003	21.268	12	0.047
2	8	3.966	3	0.265	10.055	3	0.018	10.670	3	0.014	1.099	3	0.777	25.789	12	0.011
3	4	5.437	3	0.142	4.553	6	0.602	3.575	3	0.311	15.253	12	0.228	28.818	24	0.227
3	5	6.686	3	0.083	6.391	3	0.094	5.943	3	0.114	13.090	6	0.042	32.110	15	0.006
3	6	9.932	3	0.019	1.878	3	0.598	4.859	3	0.182	20.856	3	0.000	37.524	12	0.000
3	7	6.382	3	0.094	7.473	6	0.279	10.613	3	0.014	17.547	6	0.007	42.015	18	0.001
3	8	0.298	3	0.960	2.598	3	0.458	3.462	3	0.326	4.334	6	0.632	10.692	15	0.774
4	5	5.803	3	0.185	7.708	6	0.260	0.459	3	0.932	6.635	6	0.356	20.184	18	0.323
4	6	3.926	3	0.270	4.149	3	0.246	6.076	3	0.108	8.166	6	0.226	22.317	15	0.100
4	7	0.298	3	0.960	9.607	3	0.022	4.748	3	0.191	27.642	3	0.000	42.295	12	0.000
4	8	2.324	3	0.508	9.927	3	0.128	1.780	3	0.619	4.902	6	0.556	18.933	18	0.396
5	6	4.077	3	0.253	7.773	9	0.557	0.296	3	0.961	4.655	6	0.588	16.801	21	0.723
5	7	1.712	3	0.634	13.593	6	0.035	6.393	3	0.094	13.375	6	0.037	35.073	18	0.009
5	8	3.940	3	0.268	13.384	9	0.146	5.538	3	0.136	6.554	6	0.364	29.375	21	0.105
6	7	2.030	3	0.917	4.813	6	0.568	0.677	3	0.878	8.360	6	0.213	15.881	21	0.776
6	8	1.536	3	0.675	1.746	6	0.942	0.361	3	0.948	9.359	6	0.154	13.002	18	0.791
7	8	1.905	3	0.706	1.424	6	0.965	2.404	3	0.493	10.889	6	0.092	16.112	18	0.585
Total		91.330	87	0.334	199.850	156	0.010	99.374	84	0.121	258.304	144	0.000	648.858	471	0.000

fewer than 5 individuals. So we continued adding age groups, stopping only when there were no more age groups to add. In this case, we added all 7 age groups together to obtain a single 2×4 table for individuals who were initially (+,+) .

To assess homogeneity between populations 1 and 2, we computed the G^2 statistic (4.3). In this instance, the predicted frequencies are those that would be expected supposing the transition probabilities to be the same in population 1 as in population 2. The numerical value of G^2 in this case was 0.883, as shown in column 3. This statistic has in theory the distribution of a χ^2 variate with (in this instance) 3 d.f. as shown in column 4. The probability of a greater departure from homogeneity due to sampling variation alone is 0.830, as shown in column 5. We find no evidence of a statistically significant difference between populations 1 and 2 in the distribution of the final state at survey 2 of the individuals who were (+,+) at survey 1.

Columns 6, 7, and 8 show the results of the same kind of homogeneity test for individuals who were initially in state 2, (+,-), that is, infected with P.f. but not infected with P.m. In this case, there are 6 d.f., as shown in column 7. This means that it was possible to partition the 7 age groups into 2 classes, young and old, and to construct 2×4 tables for each class, such that no frequency in either table was less than 5. There were 3 d.f. for each table separately, and the sum of G^2 calculated separately for each 2×4 table was 10.632, as shown in column 6. From the distribution of a χ^2 variate with 6 d.f., we infer that the probability of a greater departure from homogeneity due to sampling fluctuation alone is 0.100, as shown in column 8.

Columns 9, 10, 11 present the corresponding results for individuals initially in state 1, (-,+), that is, infected with P.m. but not P.f. Columns 12, 13, and 14 do the same for individuals initially in state 0, uninfected with either species.

Columns 15, 16, and 17 summarize the homogeneity tests for all 4 initial states. The entry $G^2 = 11.766$ in column 15 is the sum of the entries in columns 3, 6, 9 and 12. The 15 d.f. shown in column 16 is the sum of the d.f. in columns 4, 7, 10, and 13 and is calculated on the assumption that the transitions made by individuals with different initial starting states are independent. The probability that a χ^2 variate with 15 d.f. would exceed 11.766 is 0.697, given in column 17.

From line 1, we infer that there is no strong evidence that the transitions observed in population 1 from survey 1 to survey 2 were different from the transitions observed in population 2 in the same interval.

The remaining lines of Table 3, except the last line, report parallel computations for all other possible pairs of surveys in the baseline period.

Just as the last 3 columns of Table 3 are a summary of the homogeneity tests according to initial and final survey, the last line of Table 3 is a summary according to initial infection state. In column 3, $G^2 = 91.330$ is the sum of the entries above it; similarly for the values of G^2 in columns 6, 9, 12, and 15 and for the d.f. in columns 4, 7, 13, and 16. The probability that a χ^2 variate with 87 d.f. would exceed 91.330 is 0.354, shown in column 5. The probability values in columns 8, 11, 14, and 17 are calculated in the same way.

We now summarize the conclusions drawn from Table 3. The entry in the lower right corner of Table 3, P = 0.000, indicates that somewhere there are significant differences between population 1 and population 2 in the transition proportions from an initial to a final state. A scan of the columns 5, 8, 11, and 14, which contain P values for specific initial states and specific pairs of surveys, shows that all P

values smaller than 0.010 appear in column 14. Moreover, in column 14, only 6 of the 28 P values for specific pairs of surveys are less than 0.010. In 5 of these 6 pairs of surveys, the final survey is either survey 6 or survey 7. In the remaining pair of surveys, the final survey is number 5. Thus populations 1 and 2 differ in the transition proportions of individuals who are initially uninfected and who are subsequently observed during or near the end of the wet season (1971) or the beginning of the dry season (1972).

To show that the transition proportions of initially uninfected individuals in populations 1 and 2 differ only in the 6 instances with $P < 0.01$, we find the sum of G^2 and of d.f. for the 28 - 6 = 22 remaining comparisons in columns 12 and 13 of Table 3. With $G^2 = 149.99$ and d.f. = 123, we find $P = 0.049$, which is not significant evidence of heterogeneity.

In the 6 sets of transition frequencies where populations 1 and 2 differ, there is a consistent pattern to the difference (Table 4). In every case, at the final survey, the initially uninfected members of population 1 have a higher probability of being infected with P.f. only (+,-), a higher probability of double infection (+,+), and a lower probability of remaining uninfected (-,-), than do the initially uninfected members of population 2. Compared to the individuals present at all 8 surveys, there is an association between being absent from at least one survey and acquiring infection with P.f. (entering states 2 or 3). Put differently, initially uninfected individuals who appear in all 8 surveys have a reduced probability of becoming infected with P.f. and an increased chance of remaining uninfected, compared to individuals who miss at least one survey. For those 6 pairs of surveys where this difference is statistically significant, its magnitude is on the order of 0.06 to 0.10. The differences between the 2

Table 4. Distribution of final infection status in individuals initially uninfected, for 6 pairs of surveys in which population 1 (individuals absent from at least one baseline survey) differs from population 2 (individuals present at all 8 baseline surveys)

Survey		Population	Proportions with each final infection state				Total
Initial	Final		(+,+)	(+,-)	(-,+)	(-,-)	individuals
1	5	1	0.021	0.366	0.027	0.587	632
		2	0.016	0.293	0.020	0.671	1115
1	6	1	0.024	0.336	0.014	0.627	581
		2	0.022	0.259	0.033	0.685	1115
2	7	1	0.031	0.228	0.025	0.716	447
		2	0.020	0.157	0.033	0.790	1427
3	6	1	0.039	0.358	0.016	0.588	825
		2	0.019	0.301	0.031	0.649	1494
3	7	1*	0.077	0.365	0.021	0.536	233
		2*	0.044	0.247	0.021	0.688	430
		1+	0.014	0.158	0.026	0.802	349
		2+	0.012	0.134	0.036	0.818	1064
4	7	1	0.034	0.258	0.024	0.684	737
		2	0.018	0.176	0.035	0.771	1451

*Ages 0-28 (age groups 1-5)

+Ages 29+ (age groups 6-7)

populations in the proportions who change from uninfected to doubly infected or to infected with P.m. only are relatively minor.

This analysis provides some assurance that, except for the 6 transitions that appear in Table 4, we may accept the transition proportions of population 2 as representative of the transition proportions of all individuals who were observed at any time during the baseline. With the same exceptions, we may also combine the transition frequencies of populations 1 and 2 to obtain overall transition proportions for population 3 without overlooking significant heterogeneity.

7. General Performance of Models Q, M, and L

To assess the overall usefulness of the models, we now examine 3 sets of 4 × 4 tables of transition frequencies.

Set A is the transitions from survey 4 to survey 5 of population 3 in all 7 age groups separately (Table 5). Set B is the transitions of individuals aged 0 to 4 (pooled age groups 1 and 2) in population 2 at all 7 pairs of successive surveys (survey 1 to survey 2, survey 2 to survey 3, etc.) plus the transition from survey 1 to survey 8 (Table 6). Set C is the transitions among individuals aged 44 and older (age group 7) in population 2 at all 7 pairs of successive surveys plus the transition from survey 1 to survey 8 (Table 7).

These 3 sets of tables were selected before any models were fitted to the data. The selection was not biased by the success or failure of any of the models.

Set A is cross-sectional: it includes all age groups at a given pair of surveys. The transition from survey 4 to survey 5 was chosen because the wet season began in the interval and changes in infection status seemed very likely. Sets B and C are longitudinal: exactly the same set of individuals (population 2) were followed throughout the baseline period.

Table 5. Estimated parameters and goodness of fit of models Q, M and L for transition frequencies from survey 4 (end of dry season 1971) to survey 5 (start of wet season 1971) for all individuals present at both surveys, in 7 age groups. The unit of time is the interval (77 days) between survey 4 and survey 5.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Age group	Model	R_{01}	R_{02}	R_{10}	R_{20}	$R_{32}-R_{10}$	$R_{01}-R_{23}$	$R_{31}-R_{20}$	$R_{02}-R_{13}$	G^2	X^2	FT^2
1: <1 yr	Q	.000	1.719	.000	.351	.258	-.112	-.053	-.594	5.249	4.247	3.793
	M*	.000	1.755	.293	.359	-.084		-.081		6.547	6.261	4.905
	L	.041	1.842	.457	.409	-.245	-.047	-.119	-.753	8.528	7.421	6.552
2: 1-4 yr	Q	.000	1.920	1.919	.174	-1.352	-.262	.029	.360	7.306	8.061	5.960
	M*	.000	2.004	.902	.202	-.274		.003		8.894	8.734	7.539
	L	.009	2.583	8.350	.222	-8.018	-.215	.124	.924	19.902	23.596	18.639
3: 5-8 yr	Q	.072	1.672	1.439	.271	-.266	-.363	-.165	-.642	5.799	5.262	5.509
	M	.080	1.705	1.487	.278	-.335		-.186		5.959	5.342	5.608
	L	.140	1.660	3.352	.257	-2.239	-.282	-.111	-1.271	7.258	6.797	7.251
4: 9-18 yr	Q	.228	.840	2.681	.327	-1.363	-.102	-.098	-.489	8.498	8.671	7.957
	M	.147	.871	1.836	.336	-.272		-.132		10.906	10.728	10.154
	L	.201	.841	2.306	.331	-.939	-.138	-.117	-.458	8.641	8.793	8.014
5: 19-28 yr	Q	.116	.635	3.351	.764	-1.405	-.090	-.142	.635	3.764	3.419	3.241
	M	.110	.613	3.304	.734	-.218		.179		7.483	6.170	7.131
	L	.208	.578	7.205	.626	-3.644	-.212	.843	.332	10.662	10.835	9.967
6:29-43 yr	Q	.112	.685	2.508	1.276	-.231	.010	-.581	-.976	18.288	17.251	18.611
	M*	.104	.677	2.508	1.236	-.047		-.629		19.627	17.743	20.316
	L	.094	.703	2.413	1.297	-.410	-.019	-.602	-.608	17.468	15.593	18.027
7: 44+ yr	Q	.044	.846	.521	1.606	4.405	-.214	7.920	-6.009	10.504	11.134	9.758
	M*	.215	.880	2.963	1.808	.111		-1.144		28.663	32.063	27.732
	L	.100	2.081	1.914	4.011	.269	.012	-.725	-.459	22.993	26.527	21.378

Q has 4 d.f., M has 6 d.f., L has 6 d.f. *See Appendix 2.

Table 6. Estimated parameters and goodness of fit of models Q, M and L for transition frequencies from survey 1 to survey 8 and for successive pairs of surveys, for all individuals 0-4 yr old present at all 8 baseline surveys. The unit of time is the interval between the initial and final survey of each pair.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Surveys	Model	R_{01}	R_{02}	R_{10}	R_{20}	$R_{32}-R_{10}$	$R_{01}-R_{23}$	$R_{31}-R_{20}$	$R_{02}-R_{13}$	G^2	X^2	FT^2
1 to 8	Q	.000	1.274	1.180	.235	-.063	-.908	.145	-4.538	3.392	2.914	2.644
	M*	1.243	7.633	.603	1.722	.560		-1.521		14.652	16.448	12.933
	L	.040	4.425	17.101	.531	-16.362	-.877	.075	.548	19.631	23.506	17.485
1 to 2	Q	.000	.720	.000	.180	.377	-.257	-.142	-.074	4.402	2.825	3.402
	M*	.000	.722	.586	.176	-.243		-.129		12.499	8.670	11.757
	L	.188	.563	.422	.161	-.076	-.041	-.095	-.809	17.453	11.837	16.487
2 to 3	Q	.037	.534	.000	.117	.237	-.172	-.059	-.282	3.390	3.393	2.760
	M	.049	.565	.368	.111	-.153		-.046		5.777	4.031	4.879
	L	.274	.521	.169	.118	.063	.074	-.054	-.443	13.114	9.836	11.650
3 to 4	Q	.100	.202	.805	.234	-.427	-.173	-.130	-6.389	2.889	2.683	2.083
	M	.196	8.926	.432	3.696	-.050		-3.566		65.261	75.073	62.584
	L	.111	.437	.908	.279	-.533	-.158	-.245	-3.129	7.355	6.437	6.412
4 to 5	Q	.000	1.643	2.423	.112	-1.836	-.206	.067	1.643	4.172	4.700	3.116
	M	.000	1.626	.882	.134	-.206		.100		7.540	6.628	6.362
	L	.013	1.624	7.629	.006	-7.211	-.221	.373	1.600	19.592	22.811	18.004
5 to 6	Q	.343	1.502	.320	.267	.477	-.141	-.184	-.197	5.770	5.119	5.197
	M*	.417	1.472	.813	.255	-.056		-.162		5.938	4.723	5.555
	L	.516	1.346	.661	.241	.099	.067	-.099	-.940	7.136	5.952	6.642
6 to 7	Q	.000	1.301	.644	.134	-.375	-.284	.000	.259	7.454	6.402	6.965
	M	.000	1.262	.557	.132	-.284		.018		7.633	6.571	7.217
	L	.031	1.492	1.510	.136	-1.324	-.223	.037	.320	11.315	10.395	10.277
7 to 8	Q	.192	.383	.680	.093	-.431	.019	.015	-3.274	11.790	10.290	9.848
	M	.403	6.492	.000	1.224	.268		-1.077		50.549	70.849	43.487
	L	.137	.456	.333	.116	-.069	-.036	-.092	-1.758	13.184	13.286	10.958

Q has 4 d.f., M has 6 d.f., L has 6 d.f. *See Appendix 2.

Table 7. Estimated parameters and goodness of fit of models Q, M and L for transition frequencies from survey 1 to survey 8 and for successive pairs of surveys, for all individuals 44+ yr old present at all 8 baseline surveys. The unit of time is the interval between the initial and final survey of each pair.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Surveys	Model	R_{01}	R_{02}	R_{10}	R_{20}	$R_{32}-R_{10}$	$R_{01}-R_{23}$	$R_{31}-R_{20}$	$R_{02}-R_{13}$	G^2	X^2	FT^2
1 to 8	Q	.102	.339	2.139	1.773	1.131	-.378	1.424	-.830	5.039	3.832	4.604
	M*	.176	.337	2.882	1.964	-.124		-.726		6.853	6.395	5.959
	L	.169	.360	3.070	1.887	-1.207	-.109	-.368	-.087	6.376	5.714	5.691
1 to 2	Q	.136	.382	3.111	1.471	-2.183	-.063	-.049	.347	2.294	2.366	2.103
	M	.104	.371	2.486	1.457	-.245		.110		4.099	4.435	3.328
	L	.116	.367	2.857	1.398	-1.580	-.143	.622	.113	3.300	3.483	2.765
2 to 3	Q	.053	.343	1.531	1.249	5.692	-.976	.863	.240	4.040	3.958	3.644
	M*	.349	.328	5.564	1.385	-.370		-.149		16.370	21.502	14.149
	L	.117	.319	2.036	1.274	-.942	-.101	.332	.066	7.567	7.319	6.886
3 to 4	Q	.117	.442	1.251	1.820	2.196	-.861	-1.439	.442	9.362	9.343	8.143
	M	.534	.374	5.662	1.439	-.767		-.078		27.729	38.742	22.899
	L	.230	.375	3.594	1.031	-2.591	-.595	1.652	.231	25.777	34.244	21.586
4 to 5	Q	.032	.807	.874	1.363	2.917	-.198	4.343	-3.526	7.346	7.405	6.999
	M*	.088	.874	1.824	1.549	.039		-.548		13.160	17.245	11.458
	L	.080	1.294	2.026	2.320	-.241	-.011	-.357	-.235	13.855	15.974	12.806
5 to 6	Q	.080	.661	.641	1.683	4.082	-.090	.505	-.976	3.071	2.709	2.624
	M*	.120	.701	1.688	1.746	.081		-.307		6.220	5.662	5.751
	L	.108	.780	1.338	1.889	.979	.045	-.483	-.268	5.136	4.617	4.620
6 to 7	Q	.291	.302	7.314	1.638	-3.056	-.248	6.730	-2.768	4.158	3.366	2.959
	M*	.354	.343	8.715	1.911	-.630		-.112		5.808	4.769	4.450
	L	.313	.674	7.971	3.537	-3.648	-.264	-.536	-.120	13.726	13.746	12.241
7 to 8	Q	.202	.326	3.688	1.488	-3.389	.137	.455	-.639	5.643	5.019	5.069
	M*	.255	.350	4.705	1.635	-.396		-.368		12.988	19.662	9.763
	L	.141	.350	2.335	1.659	-.535	-.042	-.823	-.344	8.707	7.686	8.000

Q has 4 d.f., M has 6 d.f., L has 6 d.f. *See Appendix 2.

The youngest and oldest age groups were selected to make possible a comparison of estimated rates for children, before they could acquire immunity, with estimated rates for adults, who had survived long exposure to infection and who had had a maximum opportunity to develop immunity. According to S. Cohen (1979, p. 333), "Infants born to immune mothers are relatively resistant during the first three months of life and thereafter all children suffer severe and recurrent attacks of the disease. Clinical malaria becomes infrequent in later childhood and among adults is rarely seen in acute form."

None of the transitions in sets A, B, or C displayed any difference in proportions between populations 1 and 2 (Table 3). The rates estimated for population 2 in sets B and C therefore pertain to the entire population. However, in each of the 23 sets of transition frequencies analyzed in Tables 5, 6 and 7, there were several non-zero transition frequencies (absolute numbers of people making a specified change of infection status) less than 5. The transition proportions, or estimated probabilities, and the transition intensities or rates given in Tables 5, 6 and 7 are therefore not to be regarded as highly stable.

We now explain the columns of Tables 5, 6, and 7. Column 1 specifies from which age group or from which pair of surveys the transition frequencies are taken. Column 2 specifies the model fitted, Q, M or L. In the rows marked M, the starting guesses for the parameters are obtained from the final values of the Q parameters. In the rows marked M*, the starting guesses are least-squares approximations to C = Re(log P), as described in Appendix 2. Let R stand for any of the final matrices Q, M, or L. Column 3 is R_{01}, the inferred rate of acquisition of P.m. in the absence of P.f. Column 4 is R_{02}, the inferred rate of acquisition of P.f. in the absence of P.m. Column 5 is R_{10}, the inferred rate of loss of infection with P.m. in the absence of P.f. Column 6 is R_{20}, the inferred rate of loss of infection with P.f. in the absence of P.m. Column 7 is $R_{32} - R_{10}$, the inferred rate of loss of P.m. when P.f. is present minus the rate of loss of P.m. when P.f. is absent. In model M, column 7 gives ϵ_1. Column 8 is $R_{01} - R_{23}$, the inferred rate of acquisition of P.m. when P.f. is absent minus the rate of acquisition of P.m. when P.f. is present. In model M, the entry in column 8 would by definition be equal to that in column 7 and is therefore omitted here. Column 9 is $R_{31} - R_{20}$, the inferred rate of loss of P.f. infection when P.m. is present minus the rate of loss of P.f. infection when P.m. is absent. In model M, column 9 gives ϵ_2. Column 10 gives $R_{02} - R_{13}$, the inferred rate of acquisition of P.f. when P.m. is absent minus the rate of acquisition of P.f. when P.m. is present. For model M, the entry in column 10 would be identical to that in column 9 and is therefore omitted.

All of these rates assume that the interval between surveys is one unit of time. Even the interval from survey 1 to survey 8 is treated as one unit of time. The time scale has no effect on the goodness of fit of the models. But the numerical values in Tables 5, 6 and 7 must be divided by the appropriate number of days between surveys before they can be interpreted as transition intensities per day.

Columns 11, 12 and 13 give 3 measures of goodness of fit of the predicted transition frequencies to the observed transition frequencies. These are not measures of the goodness of fit of the constrained parameters of the models to Re(log P). G^2 for each model has been defined earlier.

$$X^2 = \Sigma(\text{observed} - \text{expected})^2/\text{expected},$$

where the sum is over all cells of the 4 × 4 table, and terms in

which the expected frequency is 0 are taken as 0. FT refers to Freeman-Tukey deviates and is calculated as

$$FT^2 = \Sigma(\text{observed}^{1/2} + (\text{observed} + 1)^{1/2} - (1 + 4 \times \text{expected})^{1/2})^2.$$

Bishop, Fienberg and Holland (1975) show that when the underlying model is correct and the expected frequencies are estimated in any "reasonable" way (including, as in this instance, by maximum likelihood via minimum G^2), the 3 goodness of fit statistics all have the same distribution in the limit as the number of observations in each cell becomes large. A cursory comparison of columns 11, 12, and 13 confirms the general numerical similarity of the 3 measures. Our remaining discussion of goodness of fit will be based entirely on G^2.

To summarize the performance of the 3 models Q, M, L, we add the values of G^2 corresponding to each model for the 7 age groups in Table 5 and for the 7 pairs of successive surveys n to n+1 in Tables 6 and 7. (In Tables 6 and 7, we omit from this sum the transitions from survey 1 to survey 8.) The corresponding d.f. for Q is 7 × 4 = 28 and for M and L is 7 × 6 = 42. The results are:

Data set	G^2 for model		
	Q	M	L
A. All age groups, survey 4 to survey 5 (Table 5)	59.408**	88.079***	95.452***
B. Ages 0-4, all pairs of surveys n to n+1 (Table 6)	39.867	155.197***	89.149***
C. Ages 44+, all pairs of surveys n to n+1 (Table 7)	35.934	86.374***	78.068**
Degrees of freedom	28	42	42

** means $10^{-3} > P > 10^{-4}$; *** means $10^{-4} > P$

Model Q performs admirably with the longitudinal histories of sets B and C (Tables 6 and 7). Table 5 shows that G_Q^2 is large enough to give $0.001 < P < 0.01$ for only one of the 7 age groups, age group 6, 29-43 years old. A comparison of observed frequencies with predicted frequencies for each individual cell in this 4 × 4 table shows that only one of the Freeman-Tukey deviates

$$\text{observed}^{1/2} + (\text{observed} + 1)^{1/2} - (1 + 4 \times \text{expected})^{1/2}$$

is greater in magnitude than 2.0. The number, 8, of individuals observed making the transition from state 0 (-,-) to state 3 (+,+), was considerably less than the number, 18.6, predicted by Q. Otherwise, predicted and observed frequencies were close, cell by cell.

Subtracting the poor fit of Q in this age group yields $G_Q^2 = 41.120$ with 24 d.f. for the remaining 6 age groups and $P > 0.01$. Therefore, Q provides an acceptable description of the transition data for 22 of the 23 sets of data in Tables 5, 6, and 7. It is not surprising to find one of 23 significance tests significant at the 0.01 level. We conclude that there is little evidence against Q. For more formal ways of carrying out such multiple comparisons, see Miller (1966). If the transitions in infection status result from the operation of a time-homogeneous Markov chain, one may assume that the 2 potentially present species of malaria do not change status simultaneously.

The value of G_Q^2 obtained for age group 6, 29-43 years, in Table 5 is not the global minimum, because G_L^2 is lower for these transition data. Since L is a special case of Q, this could not happen if G_Q^2 were a global minimum. Comparison of G_Q^2 with G_M^2 and G_L^2 shows that this anomaly, which results from limitations of the numerical minimization procedure, arises

nowhere else in the data analyzed.

Model M describes acceptably 18 of the 23 sets of data in Tables 5, 6 and 7, if one abandons the constraints on the signs of ϵ_1 and ϵ_2 suggested by the competitive Lotka-Volterra interpretation of M.

In Table 5, if the values of G_M^2 associated with the 2 significantly bad fits (in age groups 6 and 7) are subtracted from the total G_M^2 for Table 5, the remaining $G_M^2 = 39.789$ with 30 d.f. is acceptable. In Table 6, if the values of G_M^2 associated with the 2 significantly bad fits (in the transitions from survey 3 to survey 4 and from survey 7 to survey 8) are subtracted from the total G_M^2 for Table 6 (which omits the transition from survey 1 to survey 8), the remaining $G_M^2 = 39.387$ with 30 d.f. is also acceptable. However, in Table 7, if the value of G_M^2 associated with the only significantly bad fit (in the transition from survey 3 to survey 4) is subtracted from the total G_M^2 for Table 7, the remaining $G_M^2 = 58.645$ with 36 d.f. has $0.001 < P < 0.01$. This indicates a generally poor fit among the remaining sets of data in Table 7, even though they are not significantly bad individually. Since age group 7 (44+ years old) is poorly described by M in Table 5, which describes the transitions from survey 4 to survey 5, it is indeed not surprising that the transition from survey 4 to survey 5 in Table 7, which describes the transitions of individuals 44+ years old, is not very well described by M, even though G_M^2 does not quite attain the $P = 0.01$ critical value. Because of these failures of M, we must finally admit that this model is not in general an adequate description of the interactions in continuous time between P.f. and P.m. in this human population in Nigeria, though it may describe many individual transition tables economically.

To determine whether there was a consistent pattern in the discrepancies between the observed frequencies and those predicted by M, we examined the Freeman-Tukey residuals cell by cell for each of the five 4×4 transition tables with a significantly large value of G_M^2. The Freeman-Tukey deviates exceeded 2.0 in the following cases. In Table 5, age group 6, fewer transitions from state 0 to state 3 were observed than predicted (the same discrepancy was observed previously with Q). In Table 5, age group 7, the same discrepancy was observed; in addition more transitions from state 3 to state 1 were observed than predicted, but here both the observed and predicted frequencies were small (5 vs. 1.2). In Table 6, the transitions from survey 3 to survey 4 and from survey 7 to survey 8 had an identical pattern of discrepancies, different from the above: there were more transitions from state 0 to state 0 observed than predicted, and fewer transitions from state 0 to state 3 and from state 2 to state 0 observed than predicted. Finally, in Table 7, the transitions from survey 3 to survey 4 had yet another pattern of discrepancies: there were more transitions from state 1 to state 1 observed than predicted.

Because the large residuals between observed and predicted frequencies did not conform to a consistent pattern, we are unable to identify a specific point at which the assumptions of M are inadequate.

Though the details are slightly different, L suffers the same fate as M. L describes acceptably 16 of the 23 sets of data in Tables 5, 6, and 7. If the significantly bad data sets in Table 5 (age groups 2, 6 and 7) and Table 7 (the transition from survey 3 to survey 4) are removed, the remaining data sets do not reject model L. In Table 6, the transitions from survey 1 to 8, 1 to 2, and 4 to 5 are not described acceptably ($0.001 < P < 0.01$ in each case) by L. If the values of G_L^2 associated with the latter 2

transitions are removed from the total G_L^2 for Table 6, the remaining $G_L^2 = 52.104$ with 30 d.f. still has $0.001 < P < 0.01$. In a sense, L here suffers from double jeopardy, since the rejection of L by age group 2 in the transition from survey 4 to survey 5 appears once in Table 5 and once in Table 6. Nonetheless, the data force us to admit that this model, too, is not in general an adequate description of the interactions in continuous time between P.f. and P.m. in this study.

Given that only Q survives, we now examine in detail the estimated values of the parameters of Q in each of the 3 sets of data in Tables 5, 6 and 7.

8. Effects of Age and Season on Intensities of Acquiring and Losing Infections

The estimated Q parameters vary as a function of age group at the transition from survey 4 to survey 5 (Fig. 1, based on Table 5) and as a function of season within the youngest age groups (Fig. 2, based on Table 6) and within the oldest age group (Fig. 3, based on Table 7).

Throughout these figures, o denotes the intensity of acquisition, and * the intensity of loss, of infection with a particular species.

In Figs. 1, 2 and 3, panels (a), (b), (c) and (d) are organized in the same way:

panel	elements of Q	table columns	description
(a)	Q_{01}, Q_{10}	col. 3, col. 5	Rates for P.m. when P.f. is absent at first survey of pair
(b)	Q_{23}, Q_{32}	col. 4, col. 6	Rates for P.m. when P.f. is present at first survey of pair
(c)	Q_{02}, Q_{20}	col. 3 - col. 8 col. 5 + col. 7	Rates for P.f. when P.m. is absent at first survey of pair
(d)	Q_{13}, Q_{31}	col. 4 - col. 10 col. 6 + col. 9	Rates for P.f. when P.m. is present at first survey of pair

When P.f. is not present at the beginning of the transition from the dry season to the wet season, the rates of loss of P.m. are far more variable as a function of age than are the rates of acquisition of P.m. (Fig 1(a)). Intensities of both acquisition and loss appear to peak in the middle age groups and to decline in the youngest and oldest age groups. The presence of P.f. at the beginning of the transition (Fig. 1(b)) markedly alters this pattern: the rates of loss of P.m. increase monotonically with age to a high level in the oldest groups. The concomitant presence of P.f. is associated in some important way with the increasing ability of older individuals to lose P.m. infections. As before, the variability by age in rates of loss far exceeds the variability in rates of acquisition.

When P.m. is not present at the beginning of the transition from the dry season to the wet season, there is substantial variation by age in the rates of both acquisition and loss of P.f. (Fig. 1(c)). Rates of acquisition drop from high levels among individuals up to 8 years old (age groups 1,

2, 3) to levels half as large in the older population. Rates of loss of infection are low among individuals up to 18 years old (age groups 1-4) and increase monotonically thereafter. The presence of P.m. at the beginning of the transition (Fig. 1(d)) leaves the increase in rates of loss of P.f. with age qualitatively unchanged, but dilutes or reverses the decline in rates of acquisition of P.f. with increasing age.

In Figs. 2 and 3, an annual seasonal cycle corresponds to abscissae labelled 4 to 8, that is, from the transition from survey 3 to 4 up to the transition from survey 7 to 8. Surveys 3 to 8 include substantially more people than the first 2 surveys, which we do not discuss here.

In individuals up to 4 years old at survey 1, rates of loss of P.m. are generally far more variable during an annual cycle (Figs. 2(a,b)) than rates of acquisition, while for P.f., rates of acquisition are far larger and more variable than rates of loss (Figs. 2(c,d)). The complementarity between P.f. and P.m. that has often been noted clinically acquires quantitative detail from a comparison of Fig. 2(a) with 2(c) and of Fig. 2(b) with 2(d). In both cases, the transition with the greatest rate of P.f. acquisition corresponds to the transition with the least or close to least rate of P.m. acquisition. What is surprising is that the peak rate of acquisition of either species in all 4 panels of Fig. 2 occurs during the dry season transition from survey 3 to survey 4 in Fig. 2(d), one step ahead of the peak rate of acquisition in Fig. 2(c). The transition from survey 3 to survey 4 is one of the transitions where model M fails most egregiously.

Comparison of each panel of Fig. 3 with the corresponding panel of Fig. 2 reveals details of how adults (44 years and older) resist malaria infections better than very young children. In most cases, rates of loss are larger in the older age group. The increase in loss rates

CAPTIONS FOR FIGURES 1, 2 AND 3

Fig. 1. Estimated transition intensities for model Q by age groups, based on all individuals present at survey 4 (end of dry season 1971) and survey 5 (beginning of wet season 1971). The abscissa is the number of the age group: 1 = infant, 2 = 1-4 yr, 3 = 5-8 yr, 4 = 9-18 yr, 5 = 19-28 yr, 6 = 29-43 yr, 7 = 44+ yr.

Fig. 2. Estimated transition intensities for model Q by season, based on all individuals not more than 4 yr old at survey 1 who were present at all 8 surveys. Abscissa 1 = transition from survey 1 to survey 8; for n = 2, 3, . . . , 8, abscissa n = transition from survey n-1 to survey n.

Fig. 3. Estimated transition intensities for model Q by season, based on all individuals at least 44 yr old at survey 1 who were present at all 8 surveys. Abscissae are the same as in Fig. 2.

o = Intensity of acquisition of infection.

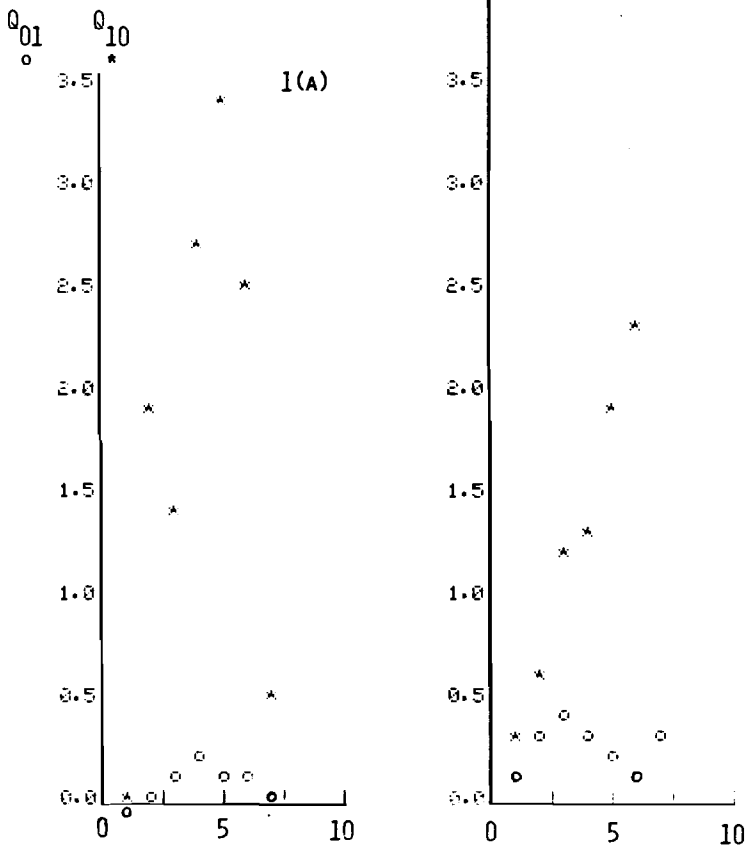
* = Intensity of loss of infection.

(a) Rates for P. malariae in individuals not infected with P. falciparum at the first survey of the pair.

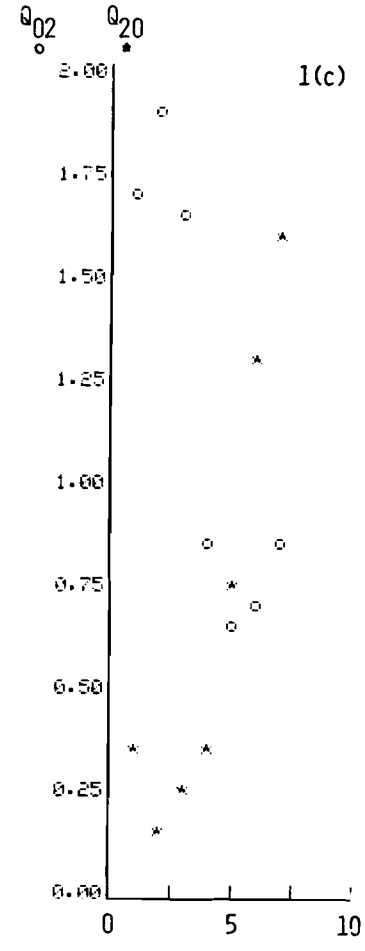
(b) Rates for P. malariae in individuals infected with P. falciparum at the first survey of the pair.

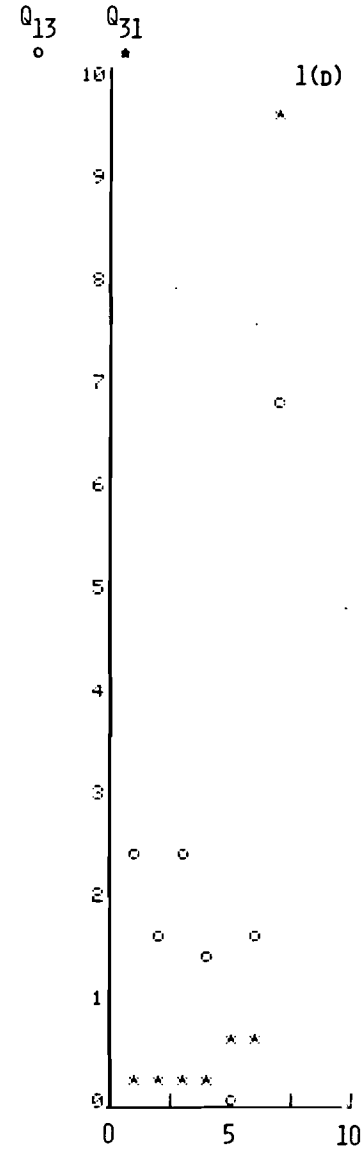
(c) Rates for P. falciparum in individuals not infected with P. malariae at the first survey of the pair.

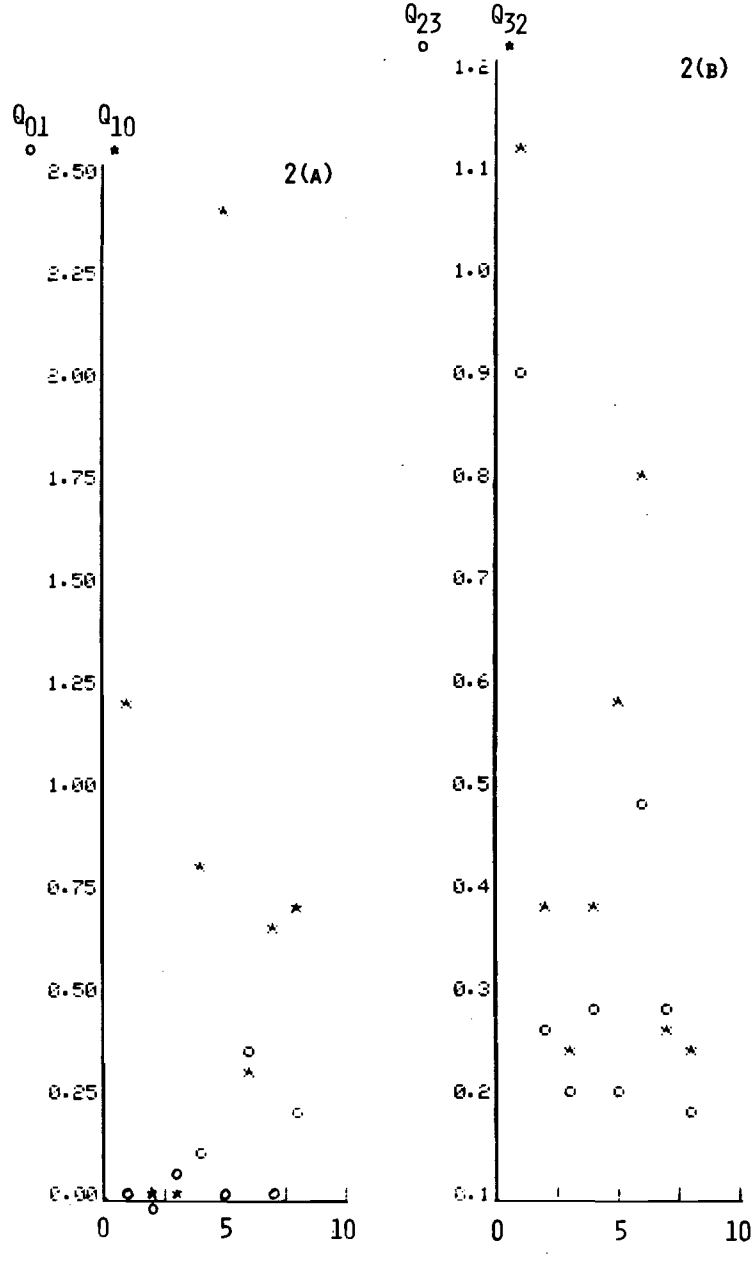
(d) Rates for P. falciparum in individuals infected with P. malariae at the first survey of the pair.


Q_{01}
Q_{10}
1(A)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10

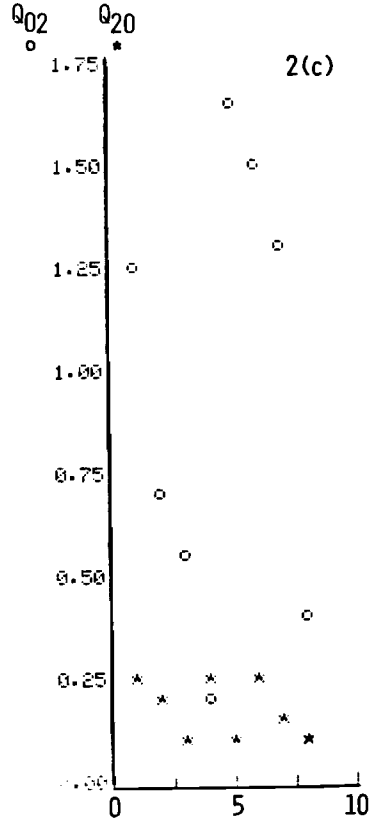
Q_{23}
Q_{32}
1(B)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10

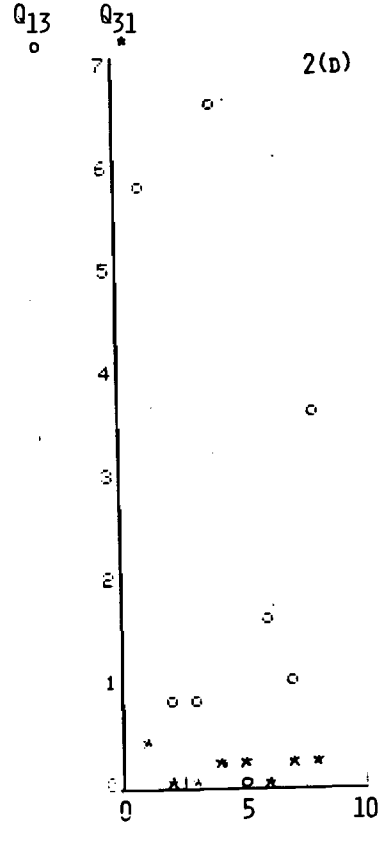

Q_{02}
Q_{20}
1(c)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
5
10

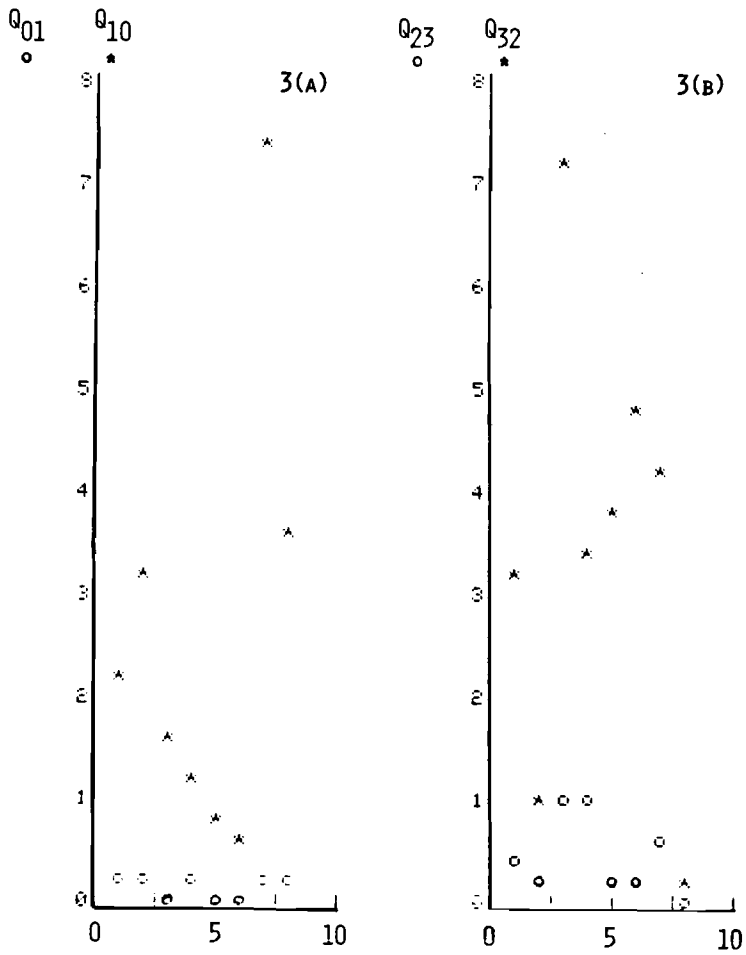

Q_{13}
Q_{31}
1(D)
10
9
8
7
6
5
4
3
2
1
0
0
5
10


Q_{01}
Q_{10}
2(A)
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
5
10

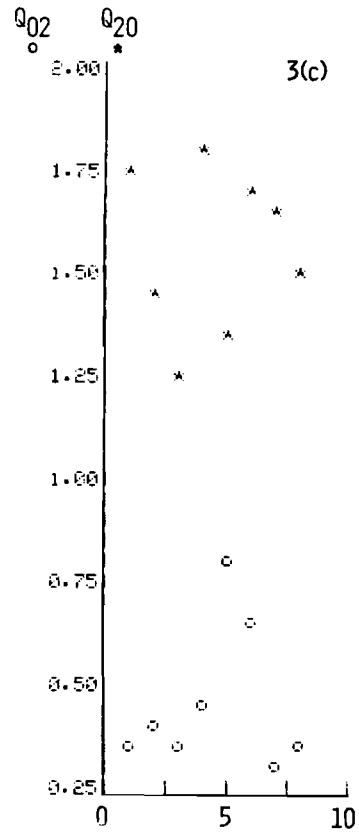
Q_{23}
Q_{32}
2(B)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5
10

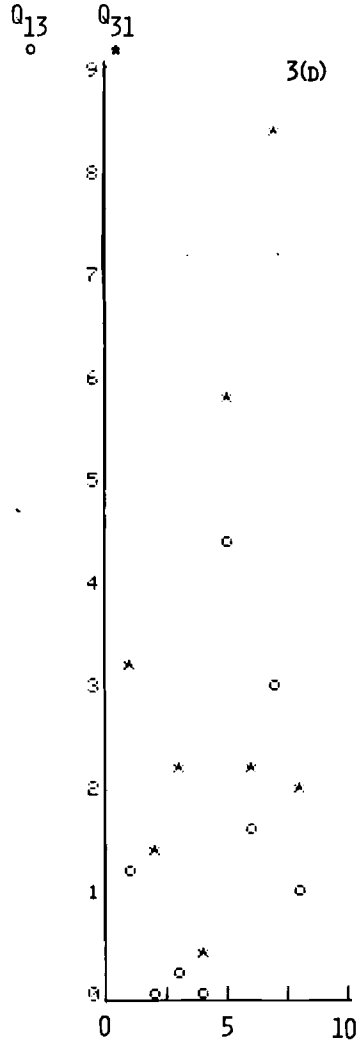

Q_{02}
Q_{20}
2(C)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
5
10


Q_{13}
Q_{31}
2(D)
7
6
5
4
3
2
1
0
0
5
10


Q_{01}
Q_{10}
3(A)
0
1
2
3
4
5
6
7
8
0
5
10

Q_{23}
Q_{32}
3(B)
0
1
2
3
4
5
6
7
8
0
5
10


Q_{02}
Q_{20}
3(C)
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
0
5
10


Q_{13}
Q_{31}
3(D)
0
1
2
3
4
5
6
7
8
9
0
5
10

with age is accompanied by a pronounced decrease in rates of acquisition only for P.f. when P.m. is absent (compare Fig. 3(c) with Fig. 2(c)). Thus adults are generally better than very young children in eliminating established infections from peripheral blood, but appear to be better than children at avoiding infection only with P.f. when P.m. is absent.

9. Are Transitions of Infection Status Markovian?

We have assumed so far that the probabilities of future changes in infection status depend on present infection status, but are independent of past infection status. In assuming that transitions of infection status are Markovian, we joined Molineaux and Gramiccia (1979). We now wish to test that assumption.

We mention at the outset that precise statistical methods are lacking to evaluate the hypothesis that transitions are Markovian, using the data available for this study. But there is evidence that transitions are not Markovian.

What does the Markovian assumption imply? Let s, t, u be the numbers of any 3 of the first 8 surveys ordered so that $s < t < u$. Let P(s,t) be the estimated transition probability matrix from survey s to survey t. If the transitions were Markovian and each P(s,t) were precisely equal to the underlying transition probabilities, i.e., if there were no sampling variability, the Chapman-Kolmogorov equations would imply

$$P(s,t)P(t,u) = P(s,u), \text{ for all } 1 \le s < t < u \le 8.$$

For all 57 increasing triples $s < t < u$ we computed a matrix of residuals

$$D(s,t,u) = P(s,t)P(t,u) - P(s,u).$$

We used all individuals present at both surveys s and t to estimate P(s,t), regardless of whether those individuals were present at survey u, and similarly for P(t,u) and P(s,u). We added the raw transition frequencies for all 7 age groups together to get a single P(s,t) for each pair s,t.

In every one of the 57 cases, $D_{00}(s,t,u)$, $D_{22}(s,t,u)$, and $D_{33}(s,t,u)$ were negative and $D_{03}(s,t,u)$ and $D_{30}(s,t,u)$ were positive. $D_{11}(s,t,u)$ were negative in 47 of 57 cases. Individuals who were in any of states 0 (uninfected), 2 (infected with P.f. only), or 3 (infected with P.f. and P.m.) at any survey s always appeared in that same state at any later survey u more often than predicted by the Markovian assumption. Fewer individuals actually changed from doubly infected to uninfected or vice versa between any surveys s and u than were predicted from the Markovian assumption.

This striking pattern might result from adding together the transition frequencies of age groups, which have different transition probabilities.

To remove this possible artifact, D(4,5,6) was computed for each age group separately. However, the transition frequencies of age groups 1 and 2 (individuals up to 4 yr old) were combined. In all 6 cases, $D_{00}(4,5,6)$ and $D_{33}(4,5,6)$ were negative and $D_{03}(4,5,6)$ were positive, as before. $D_{30}(4,5,6)$ were positive except in the youngest (0-4 yr) age group. If one were to suppose naively that, under the Markovian assumption, the sign of an element of the residual matrix D(4,5,6) would be positive or negative with equal probability (we do not affirm that this supposition is correct), then the probability that the residual would have the same sign in 6 of 6 cases is fairly low: 2^{-6}. We lack a statistical test of the hypothesis that D(s,t,u) = 0, which can allow for the dependence among the observations used to construct P(s,t), P(t,u) and P(s,u).

The predominantly negative diagonals in D(s,t,u) may be summarized by saying that trace D(s,t,u) < 0. A wide variety of panel surveys in sociology and economics (Singer and Spilerman, 1977) have a similar pattern of residuals,

$$\text{trace } P^k(0,\Delta) - \text{trace } P(0,k\Delta) < 0.$$

This kind of deviation from time-homogeneous Markov chains has often been accounted for by modeling the panel data with mixtures of Markov chains. It remains for a future investigation to determine whether infection histories in the Garki baseline surveys can be represented by a simple, interpretable mixture of inhomogeneous Markov chains.

The evidence we have presented against the Markovian hypothesis is not a definitive disproof. Molineaux and Gramiccia (1979, §5.2.1.3, §4.6) observe that different villages differ in parasitology and in entomology. The non-Markovian behavior might disappear with finer disaggregation of the data, for example, by age and village or by the presence or absence of P. ovale.

The non-Markovian behavior might also disappear if a finer state space were used. The state "infected" includes densities of infection ranging from low to high. An individual with a high density of infection might have a lower chance of becoming uninfected than an individual with a low density of infection. The dichotomous state space we have used treats all "infected" individuals as homogeneous.

These possibilities cannot be resolved by 4 × 4 tables of transition frequencies defined by age and survey only. Progress awaits examination of more detailed underlying data.

10. Discussion--What Has Been Accomplished?

We have defined 4 infection statuses: uninfected, singly infected with either P. malariae or P. falciparum, and jointly infected with both. We have studied the 4 × 4 estimated transition probability matrices P(s,t) from survey s to survey t during the 8 surveys of the baseline period of the Garki study. Our principal results partly concern malaria and partly concern methods for studying longitudinal panel data. We summarize these results here.

The transition proportions from one survey to the next of individuals who were present at all 8 surveys were the same as the transition proportions of the individuals who were absent from at least one survey. The exception to this general pattern is that initially uninfected individuals who appear in all 8 surveys have a reduced probability of becoming infected with P.f. and an increased chance of remaining uninfected, compared to individuals who miss at least one survey, when the second survey at which the individuals are observed is near the end of the wet season or the beginning of the dry season.

New computer algorithms were developed for obtaining from two-wave panel data the maximum likelihood estimates of the intensity parameters in 3 models, named Q, M and L, of a time-homogeneous continuous-time Markov chain. Previously, the intensities of acquisition and loss of each species of malaria were estimated from a time-series of two-wave panel surveys modeled by 2-state continuous-time Markov chains without regard to the possible effect of the simultaneous presence or absence of another species of malaria (Bekessy et al., 1976; Molineaux and Gramiccia, 1979). The methods developed here make it possible for the first time to disentangle the transition intensities of one species when a second species is absent from the transition intensities of the first species when the second species is present.

The signs of interaction parameters in model M were frequently contrary to the sign predicted by an interpretation of the model in terms of Lotka-Volterra competition equations. The observed signs of the interaction parameters suggested cooperative effects between malarial species rather than competitive ones.

When the 3 models were fitted to 23 sets of transition frequencies, Q performed admirably. Q excludes the possibility that both P.f. and P.m. would either be gained or be lost exactly simultaneously: the elements on the minor diagonal of Q are 0. Though both M and L, which are special cases of Q, described the observed transition frequencies in a majority of the 23 sets well, they did not describe a considerable number of cases acceptably and are therefore considered inferior to Q here.

The estimated parameters of Q vary as a function of age at the transition from the end of the dry season to the beginning of the wet season 1971 and, within the youngest and oldest age groups, as a function of season from the end of April 1971 (dry season) to the beginning of May 1972 (dry season).

As a function of age, whether P.f. is present or absent, the variability of rates of loss of P.m. far exceeds the variability of rates of gain of P.m. The presence of P.f. appears to be associated with a monotonic increase in the rate of loss of P.m. with increasing age of individuals.

Except possibly for infants, rates of loss of P.f. also appear to increase monotonically with age. Rates of gain of P.f. are higher for individuals up to 18 yr old than for older individuals when P.m. is absent, but no such clearcut pattern is evident when P.m. is present.

Among very young children (up to 4 yr old), seasonal variation in P.m. is associated primarily with variation in rates of loss. Seasonal variation in P.f. is associated primarily with variation in rates of acquisition.

Among adults (44+ yr old), seasonal variation in P.m. is, as with young children, associated primarily with variation in rates of loss. Unlike young children, seasonal variation in P.f., in the absence of P.m., is also associated primarily with variation in rates of loss. When P.m. is present, rates of both acquisition and loss of infection with P.f. vary widely and over comparable ranges.

Adults are generally better than very young children in eliminating infections from peripheral blood, but appear to be better than children at avoiding infection only with P.f. when P.m. is absent.

Model Q, like many malaria models before it, assumes that the process of change in infection status is Markovian. Strictly speaking, Q also assumes that the Markovian process is time-homogeneous. Since the parameters of Q change with season, one can piece together a time-series of homogeneous Markov models by assuming, as a first approximation, that the parameters are constant during the interval between surveys.

The question of whether the full baseline process is Markovian seems rarely, if ever, to have been confronted with data. Precise statistical methods are lacking to evaluate the hypothesis that transitions are Markovian, using the data available for this study. But some evidence suggests that transitions are not Markovian. In general, initially uninfected individuals tend to remain uninfected with higher frequency, and initially doubly infected individuals tend to remain doubly infected with higher frequency, than would be predicted from the Markovian assumption.

In conclusion, we remark briefly on the relation of this work to ecological models of the entire transmission cycle of malaria, to previous studies of mixed-species infections of

malaria in human populations, and to previous analyses of these same data.

The present analysis concentrates on one detail of malarial transmission: the intensities of acquisition and loss of malaria species when other species of malaria may or may not be present in the same people. The methods presented make it possible to separate overall intensities of transition of P.f., for example, into intensities of transition for P.f. in the presence of P.m. and intensities of transition for P.f. in the absence of P.m. Such details could be valuable for ecological models of the entire malaria transmission cycle that sought to apply the transition intensities observed for P.f. in the Garki project to locales or times when the prevalance of P.m. might be substantially different. (See Dietz et al., 1974 and Bailey, 1975 for examples of ecological models of malaria.) These details about intensities of acquisition and loss may lead to more nearly correct interpretations of age-prevalence curves, which are often of interest to ecological modelers (see Fig. 1 and accompanying text). The present study is thus complementary to efforts to model overall transmission dynamics.

Model M was originally proposed as a means of testing the Lotka-Volterra competition equations empirically (Cohen, 1970). At that time, no longitudinal data comparable to those of the Garki project existed for malaria. Analysis of the prevalence of single-species and mixed-species malarial infections in human populations suggested that phenomenologically something like competition between malarial species might occur. There were fewer than random mixed-species infections in humans who had enlarged spleens, a clinical sign of immunological arousal (Cohen, 1973).

The prevalences of single-species and mixed-species infections in the Garki project have yet to be analyzed by

level of immune response (e.g., titer of immunoglobulin). But, contrary to the surveys analyzed by Cohen (1973), there are overall more mixed-species infections than expected at random from the prevalences of the species separately. Without doubt, the microscopic technique of the Garki project was more tightly controlled and of higher quality than that of any malaria survey before it. Nevertheless, when the frequency distribution of the positive (infected) blood films was analyzed by the number of positive microscope fields, there were more films with 2 fields positive than with one field positive. "A plausible explanation is that, once a positive field is found, the remaining fields are examined more carefully" (Molineaux and Gramiccia, 1979, §5.1.4.3). If this explanation were correct, it might also explain a more than random prevalence of mixed-species infections.

If one takes at face value the estimates of the interaction parameters ϵ_1, ϵ_2 for M where that model succeeds in predicting the observed transition frequencies (Tables 5, 6, 7), the high frequency of negative signs also argues against competition.

Since M does not succeed very well overall, the linearized Lotka-Volterra equations, with or without the competitive interpretation, should not be regarded as a general model for the interaction of malarial species in human populations.

Incidentally, there is a remark, which has been attributed to Joseph Bertrand, to the effect that "if you give me n parameters, I will fit an elephant; and if you give me n+1 parameters, I will make him wave his trunk," where n is usually 4 or 5. This remark, frequently and often justly aimed at statisticians, is true only if one looks at few data--a small elephant. Here we have rejected the 6 parameter models M and L by evaluating their performance against a large number of sets

of data.

Finally, we relate our results to some findings of Molineaux and Gramiccia (1979), which are based on independent analyses of the same data. We confirm in general their inference (§5.2.1.1) "that the main effect of immunity is to increase recovery and/or to decrease detectability, rather than to decrease susceptibility." We interpret "recovery" as intensity of loss of infection and "susceptibility" as intensity of gain of infection. Molineaux and Gramiccia (1979, Figs. 5.11 and 5.12) graph the intensities per day of acquiring and of losing infection with P.f., P.m. and P. ovale as a function of age. Our results in Fig. 1 suggest that these patterns by age may depend significantly on the simultaneous presence or absence of malarial species other than the one of interest. The same point arises from comparing our Figs. 2 and 3 with their Fig. 5.13, a plot for each age group of the estimated daily intensity of acquiring infection with P.f. by season. Our panels (c), showing the intensity of P.f. acquisition in the absence of P.m., agree very well qualitatively with the shape of the seasonal distributions from survey 3 to survey 8 shown in their Fig. 5.13. The very different pattern in our panels (d) has no counterpart in Fig. 5.13. If a substantial fraction of a population were jointly infected with P.m. and P.f., the marginal intensity of acquisition of P.f. might have a distribution by season quite different from that shown in Fig. 5.13.

In offering our own analysis of the results of the Garki project, we aspire only to take one more step down the road Molineaux and Gramiccia (1979) pioneered.

Appendix 1. Row Sums of log P

The following theorem is basic to the computational algorithms in Appendix 2.

Theorem: Over the field of complex numbers, let M be an $n \times n$ matrix whose minimal polynomial (Gantmacher, 1960, I:89) has only simple roots $\lambda_1, \lambda_2, \ldots, \lambda_m$, $m \le n$. Let λ be one of these roots with corresponding right eigenvector x (so that $Mx = \lambda x$), and let f be a complex-valued function of a complex number such that $f(\lambda) = 0$. If f(M) is defined by the Lagrange-Sylvester interpolation formula (Gantmacher, 1960, p. 101), then $f(M)x = 0$.

Proof. By definition, if I is the $n \times n$ identity matrix

$$f(M) = \sum_{k=1}^{m} f(\lambda_k) \prod_{j \neq k} (M-\lambda_j I)(\lambda_k-\lambda_j)^{-1} .$$

The justification for using $\prod$ without indicating the sequence of factors is that for any complex z and w, $(M-zI)(M-wI) = (M-wI)(M-zI)$. Labelling the roots so that $\lambda_1 = \lambda$, we have $f(\lambda_1) = 0$, so

$$f(M) = \sum_{k=2}^{m} f(\lambda_k)\Big[\prod_{\substack{j \neq k \\ j \neq 1}} (M-\lambda_j I)(\lambda_k-\lambda_j)^{-1}\Big](\lambda_k-\lambda_1)^{-1}(M-\lambda_1 I)$$

$$= A(M-\lambda_1 I)$$

where A is defined by the last equality. Then $f(M)x = A(Mx-\lambda_1 x) = 0$.

Corollary. If P is a non-negative stochastic matrix whose minimal polynomial has only simple roots, then both Re(log P) and Im(log P) have 0 row sums.

Proof. Such a P has eigenvalue 1 with corresponding right eigenvector $\underset{\sim}{1}$ in which each element is 1. Now $\log 1 = 0$. By the theorem, $(\log P)\underset{\sim}{1} = \underset{\sim}{0}$, and each element of the vector

(log P)$\underset{\sim}{1}$ is just the sum of the corresponding row of log P. The corollary is proved.

Empirically, the eigenvalues of the transition matrices estimated from observed transition frequencies were distinct in every case, so the corollary is relevant to the data.

Appendix 2. Computational Algorithms

We describe procedures for finding the matrices in $\underset{\sim}{Q}$, $\underset{\sim}{M}$, and $\underset{\sim}{L}$ that give the best fit of predicted frequencies to observed frequencies. By minimizing G^2, we obtain the maximum likelihood estimates of the parameters of each model (Bishop, Fienberg, and Holland, 1975).

The fitting procedure consists of 2 parts: first, obtain initial parameter estimates; second, perturb these estimates, subject to the constraints of the particular model, to minimize G^2.

The second part of the fitting procedure is the same for all 3 models and will be explained first. The initial value of G^2 is minimized using the method of successive adjustment of variables (Lance, 1960, p. 130). Our algorithm for minimizing a function

AXMIN(FUNCT, X, N, DELTA, EPS, MAXFN, IER, CTFN, CTITR, F)

is a double-precision PL/1 subroutine.

FUNCT is the name of the function to be minimized; it must have 2 arguments, (X,F), where X, a vector of length N, is the input to FUNCT, and F is the scalar result of evaluating FUNCT at X.

X, as input, carries the initial estimate of the argument of FUNCT that minimizes F; as output, carries the final estimate of the argument of FUNCT that minimizes F.

N is the length of the vector X. For model Q, N = 8;

for M and L, N = 6.

DELTA is a vector of length N. On input, DELTA informs AXMIN of the initial estimate of the perturbation to be used for each element of X; here we set DELTA(I) = max(0.1, 0.1 × absolute value of X(I)), for I = 1,...,N.

EPS, a scalar input to AXMIN, is the convergence criterion. Each final X(I) should be within EPS of the actual minimizing argument of FUNCT when AXMIN stops (unless IER = 1 or the minimum obtained is not global); we set EPS = 0.0005.

MAXFN, a scalar integer input, is the maximum number of times AXMIN is permitted to evaluate FUNCT; we set MAXFN = 600.

IER, an output error parameter, is 0 if convergence occurs with not more than MAXFN function evaluations, and is 1 otherwise.

CTFN, on output, counts the actual number of evaluations of FUNCT.

CTITR, on output, counts the number of iterations of the main loop in AXMIN.

F, on output, gives the value of FUNCT at the final value of X.

In operation, AXMIN increments the index I cyclically through 1, 2, ..., N. Each complete cycle counts as one iteration of CTITR. For each I, X(I) is increased by DELTA(I). If this increment increases F, X(I) is decreased by DELTA(I). If this decrement also increases F, then DELTA(I) may be too large to detect the minimum. So DELTA(I) is replaced by 1/2 × DELTA(I); the increment and decrement tests are then repeated, continuing if necessary until DELTA(I) becomes less than or equal to 0.6 × EPS.

If the initial change of X(I) by DELTA(I) decreases F, DELTA(I) may be too small to locate the minimum rapidly. So AXMIN multiplies DELTA(I) by 1, 2, 4, 8, and 16 successively,

increases the previous DELTA(I) by this amount, and evaluates F with X(I) replaced by X(I) + DELTA(I) until the adjustment in DELTA(I) no longer decreases F. Using the latest DELTA(I), the interval between X(I) and X(I) + DELTA(I) is then repeatedly bisected to see if the previous lowest value of F can be lowered further; the old value of X(I) is then replaced by the new value that gives the lowest F.

In this application, FUNCT takes the 6 or 8 parameters in X, constructs an intensity matrix of appropriate form (Q, M, or L) satisfying the corresponding constraints, exponentiates that intensity matrix, multiplies each row of the result by the corresponding row sum of T in order to obtain expected transition frequencies, and then computes G^2.

We now describe procedures for obtaining initial parameter estimates for Q, M, and L. We take P as the transition probability matrix estimated by (4.1). Because all row sums of P are 1, P has one eigenvalue equal to 1. P has 3 other real or complex eigenvalues of smaller modulus.

We assume henceforth that these 3 other eigenvalues are distinct. In the computations to be described, we always computed the eigenvalues and confirmed that they were distinct. In the context of panel surveys, whenever P is estimated from frequencies large enough to be of scientific interest, repeated eigenvalues are extremely unlikely.

A 4 × 4 matrix log P is any matrix that satisfies $\exp(\log P) = P$.

Following Singer and Spilerman (1976a), let $z = a + bi$ be any non-zero complex number with modulus $|z| = (a^2 + b^2)^{1/2}$ and argument $\theta = \tan^{-1} b/a$. For each value of $k = 0, \pm 1, \pm 2, \ldots$, $\log z = \log |z| + i(\theta + 2\pi k)$ is a branch of the logarithm of z, and the branch with $k = 0$ is the principal branch. Since $\log |z|$ is the logarithm of a real number, it is unique. Clearly, $\arg(\log z) = \tan^{-1} [(\theta + 2\pi k)/\log |z|]$.

We say that a branch of log z is admissible if it satisfies

$$(3\pi)/4 \leq \arg(\log z) \leq (5\pi)/4 .$$

(For 4 × 4 matrices, this is a necessary condition for an eigenvalue of a stochastic matrix P to be generated by a continuous-time Markov process; see Singer and Spilerman, 1976a, p. 12, eq. 3.3.)

Numbering the eigenvalues so that $1 = \lambda_0 > |\lambda_1| \geq |\lambda_2| \geq |\lambda_3|$, we define log P by the Lagrange-Sylvester interpolation formula (Gantmacher, 1960, I:101):

$$\log P = \Sigma_{k=0}^{3} \log(\lambda_k)[\Pi_{j \neq k}(P-\lambda_j I)]/[\Pi_{i \neq k}(\lambda_k-\lambda_i)],$$

where I is the 4 × 4 identity matrix, and log λ is an admissible branch of the logarithm.

If any eigenvalue of P is such that its logarithm has more than one admissible branch, then each possible combination of the admissible branches, one for the logarithm of each eigenvalue, yields, by definition, an admissible branch of log P. At most a finite number of branches of log z can satisfy the inequalities above, so log P has at most a finite number of admissible branches. In the sets of data analyzed here, log P never had more than one admissible branch. We henceforth assume log P is unique. For a detailed discussion of the problem of choosing among the admissible branches of log P when more than one branch is admissible, see Singer and Spilerman (1976a,b).

An admissible branch is not necessarily an intensity matrix. An intensity matrix is a square real matrix with zero row rums and nonnegative elements off the main diagonal.

The matrix Re(log P), which has ijth element equal to the real part of $(\log P)_{ij}$, has row sums equal to 0 (for

proof, see Appendix 1) but may have some negative elements that are not on the main diagonal. Let $C = \mathrm{Re}(\log P)$ be a 4×4 matrix with elements c_{ij}.

For Q, we seek the minimum of the sum of squared deviations

$$\min \sum_{i=0}^{3} \sum_{j=0}^{3} (c_{ij}-Q_{ij})^2 = \sum_{i=0}^{3} \min \sum_{j=0}^{3} (c_{ij}-Q_{ij})^2,$$

subject to the constraints that Q be an intensity matrix with 0 minor diagonal. The above equality holds because the elements of different rows of Q are independent. Thus it suffices to minimize the sum of squared deviations for each i independently. We illustrate with i = 0. Since

$$Q_{00} \le 0, \quad Q_{01} \ge 0, \quad Q_{02} \ge 0, \quad Q_{00} = -(Q_{01}+Q_{02}), \quad Q_{03} = 0,$$

we have

$$\min \sum_{j=0}^{3} (c_{0j}-Q_{0j})^2 = c_{03}^2 + \min f(Q_{01},Q_{02})$$

where

$$f(Q_{01},Q_{02}) = (-(c_{01}+c_{02}+c_{03}) + (Q_{01}+Q_{02}))^2 + (c_{01}-Q_{01})^2 + (c_{02}-Q_{02})^2.$$

Then setting $\partial f/\partial Q_{01} = \partial f/\partial Q_{02} = 0$ yields

$$Q_{01} = c_{01} + c_{03}/3; \quad Q_{02} = c_{02} + c_{03}/3 .$$

To ensure that $Q_{01} \ge 0$, $Q_{02} \ge 0$, we take

$$Q_{01} = \max(0, c_{01} + c_{03}/3),$$
$$Q_{02} = \max(0, c_{02} + c_{03}/3),$$
$$Q_{00} = -Q_{01} - Q_{02},$$
$$Q_{04} = 0.$$

The other rows of Q are obtained by an appropriate rearrangement of indices. Q obtained this way provides a reasonable starting value for numerical minimization.

For M, we again seek a least-squares approximation to C. We no longer have independence among rows because the same parameters occur in different rows. Let

$$\begin{aligned} f = {} & (c_{00}+\lambda_1+\lambda_2)^2 + (c_{01}-\lambda_1)^2 + (c_{02}-\lambda_2)^2 \\ & + (c_{10}-\mu_1)^2 + (c_{11}+\mu_1+\lambda_2-\epsilon_2)^2 + (c_{13}-\lambda_2+\epsilon_2)^2 \\ & + (c_{20}-\mu_2)^2 + (c_{22}+\mu_2+\lambda_1-\epsilon_1)^2 + (c_{23}-\lambda_1+\epsilon_1)^2 \\ & + (c_{31}-\mu_2-\epsilon_2)^2 + (c_{32}-\mu_1-\epsilon_1)^2 + (c_{33}+\mu_1+\epsilon_1+\mu_2+\epsilon_2)^2 . \end{aligned}$$

Then we obtain 6 linear equations

$$0 = \partial f/\partial\lambda_1 = \partial f/\partial\lambda_2 = \partial f/\partial\mu_1 = \partial f/\partial\mu_2 = \partial f/\partial\epsilon_1 = \partial f/\partial\epsilon_2 .$$

Explicitly, these equations may be written

$$AX + K = 0$$

where

$$A = \begin{pmatrix} 4 & 1 & 0 & 1 & -2 & 0 \\ 1 & 4 & 1 & 0 & 0 & -2 \\ 0 & 1 & 4 & 1 & 2 & 0 \\ 1 & 0 & 1 & 4 & 0 & 2 \\ -2 & 0 & 2 & 0 & 4 & 1 \\ 0 & -2 & 0 & 2 & 1 & 4 \end{pmatrix},$$

$$X = \begin{pmatrix} \lambda_1 \\ \lambda_2 \\ \mu_1 \\ \mu_2 \\ \epsilon_1 \\ \epsilon_2 \end{pmatrix}, \qquad K = \begin{pmatrix} C_{00} - C_{01} + C_{22} - C_{23} \\ C_{00} - C_{02} + C_{11} - C_{13} \\ -C_{10} + C_{11} - C_{32} + C_{33} \\ -C_{20} + C_{22} - C_{31} + C_{33} \\ -C_{22} + C_{23} - C_{32} + C_{33} \\ -C_{11} + C_{13} - C_{31} + C_{33} \end{pmatrix}.$$

Thus

$$X = -A^{-1}K ,$$

and then we set to 0 any λ_i or μ_i for which a negative estimate is obtained. ϵ_i may be either positive or negative, and we impose $\lambda_i \geq \epsilon_i \geq -\mu_i$. A virtue of this method is that A^{-1} needs to be computed only once. The final value of the intensity matrix obtained using these least-squares initial values is called M*.

Another obvious way of obtaining initial guesses for M is to ride piggyback on the final values of Q as follows:

$$\lambda_i = Q_{0i},\ \mu_i = Q_{i0},\quad i,j = 1, 2;$$
$$\epsilon_i = (1/2)(Q_{0i} - Q_{j3} + Q_{3j} - Q_{i0}),\quad i \neq j.$$

We shall refer to the final numerical values obtained by

minimizing G^2 starting from these piggybacked initial estimates as M. We computed both M and M* and chose whichever result gave the lower G^2.

For L, we obtain initial estimates by again riding piggyback on Q. From the definition of L, we have

$$d_1 = [(L_{01}/L_{23})(L_{32}/L_{10})]^{1/2},$$
$$d_2 = [(L_{02}/L_{13})(L_{31}/L_{20})]^{1/2},$$
$$a_1 = [d_1 L_{10} L_{32}]^{1/2},$$
$$a_2 = [d_2 L_{20} L_{31}]^{1/2},$$
$$b_1 = [L_{01} L_{23}/d_1]^{1/2},$$
$$b_2 = [L_{02} L_{13}/d_2]^{1/2}.$$

These identities become initial estimates of the parameters a_i, b_i, d_i if each L_{ij} on the right is replaced by Q_{ij}.

Acknowledgments

The parasitology on which this study is based was done by or under the supervision of J. Storey. The raw data were tabulated into 4 × 4 tables of transition frequencies by A. Thomas. The World Health Organization Division of Malaria and Other Parasitic Diseases (L. Molineaux) made available many documents of the Garki project and portions of the monograph by Molineaux and Gramiccia (1979) in advance of publication. Our initial contact with the World Health Organization, which supported all the work up to and including the preparation of the transition frequencies, was mediated by K. Dietz. Without these collaborators from 4 different countries, this study could not have started.

We thank T. Mueller for computer programming and A. Whittaker for assistance in preparing the manuscript.

REFERENCES

N. T. J. Bailey, The mathematical theory of infectious diseases, 2nd ed., Macmillan, New York, 1975.

A. Bekessy, L. Molineaux and J. Storey, The estimation of incidence and recovery rates of Plasmodium falciparum parasitemia, from longitudinal data, Bull. World Health Org. 54 (1976), 685-693.

Y. M. M. Bishop, S. E. Fienberg and P. W. Holland, Discrete multivariate analysis: theory and practice, M.I.T. Press, Cambridge, Mass., 1975.

J. E. Cohen, A Markov contingency-table model for replicated Lotka-Volterra systems near equilibrium, Amer. Naturalist 104 (1970), 547-560.

_____, Heterologous immunity in human malaria, Quart. Rev. Biol. 48 (1973), 467-489.

S. Cohen, Immunity to malaria, Proc. Royal Soc. Lond. B 203 (1979), 323-334.

J. S. Coleman, Introduction to mathematical sociology, Free Press of Glencoe, New York, 1964.

K. Dietz, L. Molineaux and A. Thomas, A malaria model tested in the African savannah, Bull. World Health Org. 50 (1974), 347-357.

Director-General of the World Health Organization, Malaria control--a reoriented strategy, WHO Chronicle 32 (1978), 226-230.

J. L. Doob, Stochastic processes, Wiley, New York, 1953.

F. R. Gantmacher, The theory of matrices, Chelsea, New York, 1960.

G. N. Lance, Numerical methods for high speed computers, Iliffe and Sons, London, 1960.

R. G. Miller, Simultaneous statistical inference, McGraw-Hill, New York, 1966.

L. Molineaux and G. Gramiccia, The Garki project: research on the epidemiology and control of malaria in the Sudan savannah of West Africa, World Health Organization, Geneva, 1979.

L. Molineaux, J. Storey, J. E. Cohen and A. Thomas, A longitudinal study of human malaria in the West African savanna in the absence of control measures: relationships between P. falciparum and P. malariae, submitted.

V. Nabokov, Translator's introduction to Pushkin's Eugene Onegin, Bollingen Ser., Pantheon, New York, 1972.

A. Noguer, W. Wernsdorfer, R. Kouznetsov and J. Hempel, The malaria situation in 1976, WHO Chronicle 32 (1978), 9-17.

B. Singer and J. E. Cohen, Estimating malaria incidence and recovery rates from panel surveys, J. Amer. Statist. Assoc., in press.

B. Singer and S. Spilerman, The representation of social processes by Markov models, Amer. J. Sociol. 82 (1976a), 1-54.

_____, Some methodological issues in the analysis of longitudinal surveys, Ann. Econ. Social Measurement 5 (1976b), 447-474.

_____, Trace inequalities for mixtures of Markov chains. Adv. Appl. Prob. 9 (1977), 747-764.

A. B. Sørensen, Models of social mobility, Social Sci. Res. 4 (1975), 65-92.

THE ROCKEFELLER UNIVERSITY, 1230 YORK AVENUE, NEW YORK, NY 10021 (J.E.C., B.S.)

COLUMBIA UNIVERSITY, NEW YORK, NY 10027 (B.S.)