A model for the sequential dominance of antigenic variants in African trypanosome infections

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Trypanosoma brucei infects various domestic and wild mammals in equatorial Africa. The parasite's genome contains several hundred alternative and highly diverged surface antigens, of which only a single one is expressed in any cell. Individual cells occasionally change expression of their surface antigen, allowing them to escape immune surveillance. These switches appear to occur in a partly random way, creating a diverse set of antigenic variants. In spite of this diversity, the parasitaemia develops as a series of outbreaks, each outbreak dominated by relatively few antigenic types. Host-specific immunity eventually clears the dominant antigenic types and a new outbreak follows from antigenic types that have apparently been present all along at low frequency. This pattern of sequential dominance by different antigenic types remains unexplained. I use a mathematical model of parasitaemia and host immunity to show that small variations in the rate at which each type switches to other types can explain the observations. My model shows that randomly chosen switch rates do not provide sufficiently ordered parasitaemias to match the observations. Instead, minor modifications of switch rates by natural selection are required to develop a sequence of ordered parasitaemias.

Keywords: parasitism; protozoa; *Trypanosoma brucei*; within-host dynamics

1. INTRODUCTION

The infectious protozoan *Trypanosoma brucei* afflicts various domestic and wild mammals in equatorial Africa. The tsetse fly vector also transmits the disease to humans, causing sleeping sickness and eventually death. This parasite has attracted much research because of its ability to change its antigenic surface properties and escape immune surveillance (Vickerman 1989). Each parasite cell is covered with a nearly uniform and strongly antigenic glycoprotein coat. The parasite's genome contains several hundred alternative and highly diverged surface antigens, of which only a single one is expressed in any individual. A parasite switches its antigenic expression and entire coat in each cell generation at a rate of 1/1000 to 1/100 (Barry & Turner 1991; Barry 1997; Turner 1997).

These switches appear to occur in a partly random way, creating a diverse set of antigenic variants (Turner & Barry 1989). In spite of this diversity, the parasitaemia develops as a series of outbreaks, each outbreak dominated by relatively few antigenic types (Barry 1986; Barry & Turner 1991). Host-specific immunity eventually clears the dominant antigenic types and a new outbreak follows from antigenic types that have apparently been present all along at low frequency. The sequence of dominant antigenic variants tends to follow a specific order, although the particular sequence can be influenced by the host immune response and other factors (Gray 1965; Capbern *et al.* 1977; Miller & Turner 1981; Barry 1986; Barry & Turner 1991).

Several hypotheses have been proposed to explain these dynamics within the host (Seed 1978; Kosinski 1980; Agur *et al.* 1989; Antia *et al.* 1996), but none has gained empirical support or widespread acceptance (Vickerman 1989; Barry & Turner 1991). I use a mathematical model of parasitaemia and host immunity to show that small variations in the rate at which each type switches to other types can explain the observations. Variation in switch rates may also play an important role in the wide variety of pathenogenic micro-organisms that undergo programmed antigenic variation, although at present less is known about the dynamics of those other systems (Deitsch *et al.* 1997; Fussenegger 1997; Nash 1997; O'Connor *et al.* 1997; Serkin & Seifert 1998; Zhang *et al.* 1998).

2. MODEL

I simplified the complex interaction between the immune system and an infection to three key components: the number of parasites p_i, of each antigenic type i; the antigen-specific killing capacity of the host's antibodies a_i; and the host's memory of particular antigens m_i.

The dynamics are given by

$$\frac{\mathrm{d}p_i}{\mathrm{d}t} = rp_i(1 - P/c) - kp_ia_i + \sum_j v_{ij}p_j - p_i\sum_j v_{ji}, \qquad (1)$$

$$\frac{\mathrm{d}a_i}{\mathrm{d}t} = \rho a_i m_i\left(\frac{p_i}{p_i + \phi}\right) - \mu a_i + \pi m_i, \qquad (2)$$

$$\frac{\mathrm{d}m_i}{\mathrm{d}t} = \gamma m_i^2(1 - m_i/\delta) + bm_i\left(\frac{p_i}{p_i + \phi}\right) - dm_i + \epsilon, \qquad (3)$$

where there are n possible antigenic types, with all sums from unity to n.

The parasite dynamics in equation (1) have four components. The first term describes logistic growth, with intrinsic rate of increase r, carrying capacity c, and total parasite abundance over all types P. The second term is the killing effect of host immunity, determined by the kill rate per contact k. The third term is the switch from other antigenic types to type i, where v_{ij} is the switch rate from type j to type i. The final term is the switch rate of type i to other types.

Three components determine the antibody dynamics in equation (2). The first is the growth of antibody production, which increases according to an intrinsic rate ρ, the stimulation from memory-related signals m_i, and the saturation kinetics of the antigens such that ϕ is the antigenic level that provides one-half maximum stimulation. The second term describes the rate of antibody loss, μ. The final term is the low rate at which antibody-producing capacity is made from the memory component.

Memory dynamics in equation (3) maintain low numbers of specific memory cells when not stimulated and, when stimulated above a threshold, settle to a steady-state with a high abundance of memory. The first term controls the rise to the upper steady-state near abundance δ when stimulated above a threshold. The second term gives the saturating response to antigen. The third term is the decay rate of memory. The final term is the intrinsic rate at which specific memory is produced in absence of antigenic stimulation.

I first calibrate the immune response to follow the two key features of specific immunity—slow initial response to challenge by a novel antigen and fast response to repeat challenge by the same antigen. Figure 1a shows response to initial challenge; figure 1b shows response to repeat challenge. I use the parameters in figure 1 for the remainder of the paper.

Given this immune response by the host, I studied how natural selection influences the switching rates between antigenic types. For n antigenic types, I analysed the $n \times n$ matrix with entries v_{ij} for the rate at which type j switches to type i. I express the switch entries non-dimensionally as v_{ij}/r, the switch rate per unit time divided by the parasite's intrinsic rate of increase r. Thus $v_{ij}/r \ln 2$ is the switch rate of type j to type i per doubling time of the parasite population during exponential growth.

I initialized the switch matrix to values of 1×10^{-3} for all entries. I then calculated fitness as follows. First, I numerically integrated equations (1)–(3) over T time units. Second, I took the average parasitaemia level as parasite fitness under the assumption that fitness (transmission) increases proportionally with parasite load in the blood (Vickerman 1989). I then iterated through the following cycle for $\mathcal{N}$ evolutionary steps. (i) Mutate the switch matrix: randomly choose an entry from the matrix, each of which has the form $M \times 10^{-E}$; randomly choose an integer from the range [1, 9] for the mantissa M, and randomly choose an integer from the range [3, S] for the exponent E. The parameter $S = 3, \ldots, 6$ sets the limit on the orders of magnitude over which switch rates vary. (ii) Calculate fitness for the mutated switch matrix. (iii) If the mutant fitness is higher than the original, use the mutant matrix for the next iteration, otherwise use the previous matrix.
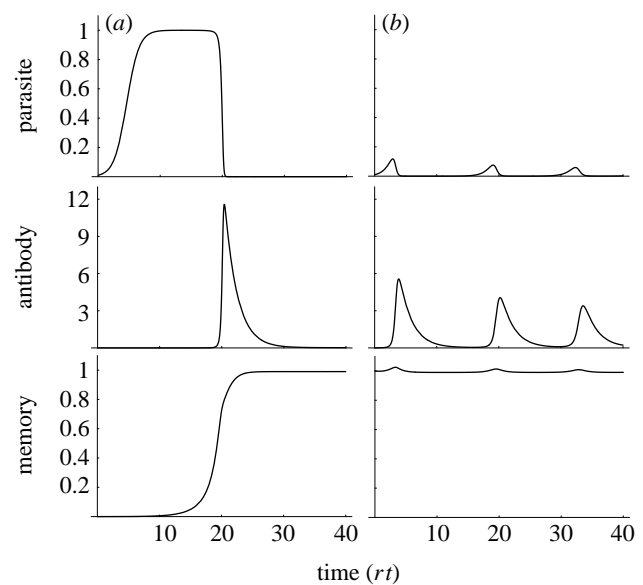
Figure 1. Immune response to initial challenge (a) and repeat challenge (b). Curves were obtained by numerical integration of equations (1)–(3) for a single parasite antigen ($i=1$) and no switching ($v=0$). The long-term dynamics follow damped oscillations to a stable equilibrium. Time is measured in non-dimensional units $\tau = rt$, where r is the intrinsic rate of increase for the parasite. On the non-dimensional scale, the doubling time of the parasite population in the absence of competition is $\ln 2 \approx 0.7$. The heights of curves are abundances, expressed non-dimensionally as: fraction of carrying capacity, p/c, for the parasites; normalized killing ak/r, for antibodies; and fraction of carrying capacity m/δ, for memory. For the other parameters I also used non-dimensional normalizations to reduce the number of parameters and emphasize the scaling relationships (Segel 1972; Murray 1989): $\rho\delta/r = 10$, $\phi/c = 0.2$, $\mu/r = 0.3$, $\pi\delta k/r^2 = 0.01$, $\gamma\delta/r = 1$, $b/r = 0.5$, $d/r = 0.01$ and $\epsilon/r\delta = 10^{-4}$. The initial conditions are (a) $p/c = 0.01$, $a = m = 0$ and (b) $p/c = 0.01$, $a = 0$, $m/\delta = 1$.

Figure 2 shows the dynamics of total parasitaemia after evolution of the switch matrix. The rows are, from (a)–(d), for $S = 3$, 4, 5, 6. For example, in the middle column of (a) there are $n = 10$ antigenic types and the switch rates vary from 1×10^{-3} to 9×10^{-3}. In the middle column of (b) the rates vary from 1×10^{-4} to 9×10^{-3}. With a wider range of switch rates, the parasite is able to spread its peaks of parasitaemia over longer time periods and obtain higher fitness. These patterns match in a qualitative way the observations that many antigenic types are present at any time and the parasitaemia follows waves in which successive types dominate. In the model here, each peak corresponds to the increase of a particular type followed by immune control and reduction of that type's abundance. At any time, most types are present in low abundance and a few types are rapidly increasing or decreasing.

The evolved switch rates separate the transitions into an ordered sequence of parasitaemias. Figure 3 shows the switch matrices for four of the corresponding time-series in figure 2 ((a,b) middle and right columns). The diameter of each circle in figure 3 is proportional to the switch rate, with a minimum rate of 10^{-3} in figure 3a(i,ii) and 10^{-4} in figure 3b(i,ii). A quick glance shows that the transitions

$n = 5$ $n = 10$ $n = 20$

(a)

(b)

(c)

(d)

P/c

0   100 0   100 0   200
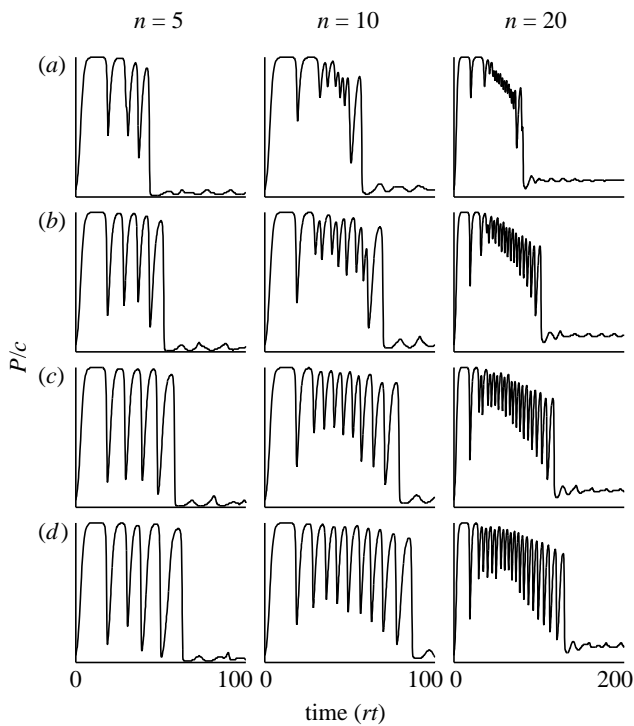
time (rt)

Figure 2. Time-series of parasitaemia dynamics. The rows
vary in minimum switch rate, determined by the parameter S,
which varies over 3, 4, 5, 6 from (a)–(d). The number of
evolutionary iterations for $n = 5,10$ is $\mathcal{N} = 20\,000$, and for
$n = 20$ is $\mathcal{N} = 40\,000$. For each case, the antigenic type 1 has
initial non-dimensional abundance $p_i/c = 0.1$, all other initial
abundances are zero for parasites, antibodies and memory.
The non-dimensional time units can be translated into days as
follows. The number of population doublings of the parasite in
the absence of competition is $\tau/0.7$, as explained in the legend
of figure 1. If we use an approximate doubling time of 5 h
(Turner & Barry 1989), then the number of hours on the
time-scale is $5\tau/0.7 \approx 7\tau$. Thus 100 non-dimensional time
units is approximately 700 h, or about 29 days. This matches
the order of magnitude for the time-course of observed
parasitaemias (Barry 1986).

are not precisely defined by the switch matrices. Instead,
there is sufficient separation that each type gets, in turn,
a head start of approximately an order of magnitude over
competitors, which provides a sufficient lead for that type
to dominate until controlled by specific immunity. For
example, if one follows types by the column of the switch
matrix, the sequence of dominance for figure 3a(i) is 1, 9,
7, 4, 3, 8, 6, 2, 5, 10 (see figure legend).

3. MECHANISM OF VARIABLE SWITCH RATES

The model suggests that natural selection may favour
some switch matrices over others. This raises two
questions: What is the mechanism by which switch
probabilities are encoded in the genome? Is it plausible to
assume that mutations can modify particular switch prob-
abilities for changing from one antigenic type to another?

Some of the molecular details of antigenic switching
are known (reviewed by Borst *et al.* 1997; Pays & Nolan
1998). The expressed surface antigen is encoded as part of
a large telomeric transcription unit. There are approxi-
mately 20 such transcription units in the genome, but

usually only one site is active at any particular time. Borst
et al. (1997) list five mechanisms that cause change of the
expressed antigenic variant, of which I discuss three.

(a) *Gene conversion of the expressed site by silent telomeric genes*

The genome is estimated to have 100–200 loci
encoding alternative antigenic variants located in
telomeric regions. Most of these telomeric loci have
70 bp repeats upstream that provide homology with the
long 70 bp repeat segment in front of the antigenic
variant in the telomeric expression site. It seems
plausible that the probability of conversion by one of
these sites depends in part on the homology of the loci
and the flanking regions.

(b) *Gene conversion of the expressed site by internal genes*

The trypanosome genome contains up to 1000 silent
loci that encode alternative surface variants. These loci
are called 'internal' because they are not located in
telomeric regions. When an internal locus converts the
currently expressed site in a telomeric expression unit,
the surface antigen changes. Again, it seems plausible that
the probability of conversion by a particular internal site
depends in part on the homology of the loci and the
flanking regions.

(c) *Switch among the 20 transcription units*

This requires activation of a new unit and inactivation
of the current unit. Borst (1991) summarized indirect
evidence and suggested that activation and inactivation of
expression sites occur by stochastic processes. Stochastic
switching may be subject to modification such that the
probability of activation of particular sites depends on base
sequence properties of the site or its flanking regions.

4. EVOLUTIONARY DYNAMICS

My analysis demonstrates that some switch matrices
cause greater total parasitaemias than other matrices. I
searched for a locally optimal matrix by sequentially
comparing a given matrix and a randomly perturbed
(mutated) matrix. When the perturbed matrix produced
a higher total parasitaemia it was used in the next round
of comparison. The final result of many such pairwise
comparisons is a matrix that approaches a locally optimal
parasitaemia when compared with matrices perturbed at
a single entry. Clearly this search method has no relation
to realistic evolutionary dynamics. Thus one must
consider what sort of solution has been obtained.

The switch matrix represents the probabilities of anti-
genic transition per cell division for a particular genotype. I
have assumed that a genotype (matrix) that confers high
total parasitaemia has a higher fitness than a genotype that
confers a relatively low total parasitaemia. Thus a locally
optimal matrix represents a genotype that, once established
in a population, cannot be displaced by a rare mutant geno-
type with slightly differing switch properties. The locally
optimal genotype, once established, is evolutionarily stable.
This method of finding an evolutionarily stable genotype
has proved to be a very powerful method in the study of
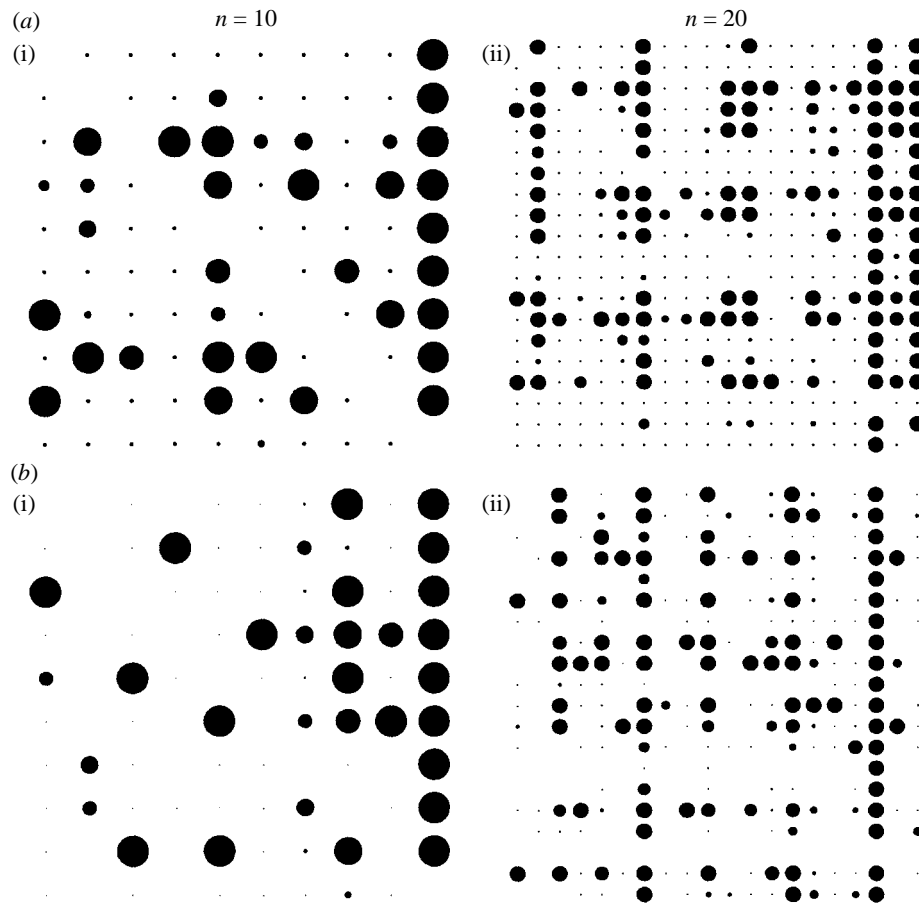complex adaptations (Maynard Smith 1982).

Figure 3. Matrices of switching for change from type j in columns to type i in rows. The sequence given in the text for the dominant parasitaemias of $(a(\mathrm{i}))$ can be explained as follows. The initial parasitaemia grows from type 1 because of the initial conditions. This type switches most frequently to types 7 and 9 (first column). Type 7 has a slightly higher switch rate than type 9 (sum of all switches in columns 7 and 9), thus 9 peaks slightly sooner than 7. These two types both switch most frequently to type 4, which in turn favours a transition to type 3, which changes most often to 8. Type 8 switches to 6, and now it is difficult to follow the last changes. Note that the final types (2, 5, and 10) switch frequently to other types. In this model, high antigenic diversity is favoured at the end of the peak parasitaemias in order to minimize immune attack. Note particularly in the right matrices $(a(\mathrm{ii}), b(\mathrm{ii}))$ with $n = 20$, that each type switches at a high rate to several other types. This fact and the minimum switch rate of 10^{-3} or 10^{-4} shows that there is no rigidly defined switch hierarchy but rather a sequential pattern of small variations in initial frequency.

Evolutionary stability leaves open the question of how a population evolves to a particular stable state—the problem of evolutionary dynamics. This is a particularly complex problem for switch matrices of antigenic variation. If a mutant genotype were to arise during the course of parasitaemia within a host, the success of that genotype would depend on competition with the resident genotype and on the history of immune stimulation in that host. A mutant that would, by itself, produce a higher total parasitaemia, may have lower fitness in the environment of its parental competitor and the host immune response.

How can an improved mutant become established? There are several stages in the life cycle during which the population of parasites within a host may come to be dominated by a new mutant. During uptake of parasites by the vector, relatively few parasites from a single location in the host's body are transmitted. Within the vector, sampling and stochastically influenced differential success may occur. There is again a sampling process of parasites upon injection into a new host. Finally, among

the parasites transmitted, only a subset will be the progenitors of the early parasitaemias that form the ancestral population within that host.

During any of these sampling phases, a new mutant can become established as the dominant genotype within a host. That mutant will then compete with other genotypes according to its success in transmission to new hosts, which I have assumed to be influenced by the total parasitaemia. By such a process, those mutants that produce higher parasitaemias will eventually dominate the population until replaced by a superior mutant.

5. DISCUSSION

Many authors have noted that multiple antigenic types must be used sequentially to provide full advantage to the parasite (Vickerman 1989; Barry & Turner 1991). If all types become abundant early, then specific immunity will develop against all types and the infection will quickly be controlled by the host. By contrast, sequential waves of parasitaemia stretch the time period over which the

parasite can evade host immunity. The puzzle is how a large population of parasites can control expression sufficiently to present a temporally ordered set of antigens.

One possibility is a strongly regulated sequence of expression, each new type being expressed only after a prior type has succeeded through its rise and subsequent control by immunity. However, switching appears to be partly random, with a large number of antigenic types expressed throughout infection (Barry & Turner 1991; Barry 1997). Another possibility is that antigenic types vary in their intrinsic rate of increase (Seed 1978). The types would then switch in dominance, with the faster growing ones earlier in the sequence of parasitaemias. This idea has failed to gain theoretical support (Kosinski 1980; Agur *et al*. 1989), and one experiment designed to test this idea failed to find variation in growth rates among types (Aslam & Turner 1992).

The final, clearly stated theory concerns the mechanism by which surface antigens are replaced (Agur *et al*. 1989). If the process occurs sufficiently slowly, then many cells will express two antigens while in transition. If the immune response against these double expressors varies, then transitions from one dominant antigenic type to the next will be biased by variable immune suppression of double expressors. This process may lead to sequential dominance of types. Although the model of Agur *et al*. has failed to gain empirical or popular support, it has not been properly tested and remains an alternative to my own hypothesis. (For further discussion of this model, see Barry & Turner 1991, 1992; Agur 1992; Muñoz-Jordán *et al*. 1996; Borst *et al*. 1997.)

The idea that small variations in transition rate can explain loosely ordered parasitaemias has been overlooked for two reasons. First, a randomly constructed switch matrix does not provide sufficiently ordered parasitaemias to match the observations. My results depend on minor modifications of switch rates by natural selection. Second, small variations in switch rate, for example, between 10^{-3} and 10^{-4}, do not seem sufficient at first glance to explain antigenic dominance. But an order of magnitude difference in initial frequency allows an antigenic type to gain temporary dominance over competing variants.

REFERENCES

Agur, Z. 1992 Mathematical models for African trypanosomiasis. *Parasitol. Today* **8**, 128–129.

Agur, Z., Abiri, D. & Van der Ploeg, L. H. T. 1989 Ordered appearance of antigenic variants of African trypanosomes explained in a mathematical model based on a stochastic switch process and immune-selection against putative switch intermediates. *Proc. Natl Acad. Sci. USA* **86**, 9626–9630.

Antia, R., Nowak, M. A. & Anderson, R. M. 1996 Antigenic variation and the within-host dynamics of parasites. *Proc. Natl Acad. Sci USA* **93**, 985–989.

Aslam, N. & Turner, C. M. R. 1992 The relationship of variable antigen expression and population growth rates in *Trypanosoma brucei*. *Parasitol. Res*. **78**, 661–664.

Barry, J. D. 1986 Antigenic variation during *Trypanosoma vivax* infections in different host species. *Parasitology* **92**, 51–65.

Barry, J. D. 1997 The relative significance of mechanisms of antigenic variation in African trypanosomes. *Parasitol. Today* **13**, 212–218.

Barry, J. D. & Turner, C. M. R. 1991 The dynamics of antigenic variation and growth of African trypanosomes. *Parasitol. Today* **7**, 207–211.

Barry, D. & Turner, M. 1992 Mathematical models for African trypanosomiasis—reply. *Parasitol. Today* **8**, 129.

Borst, P. 1991 Molecular genetics of antigenic variation. *Immunol. Today* **12**, A29–A33.

Borst, P., Rudenko, G., Blundell, P. A., Van Leeuwen, F., Cross, M. A., McCulloch, R., Gerrits, H. & Chaves, I. M. F. 1997 Mechanisms of antigenic variation in African trypanosomes. *Behring Inst. Mitt*. **99**, 1–15.

Capbern, A., Giroud, C., Blatz, T. & Mattern, P. 1977 *Trypanosoma equiperdum:* étude des variations antigéniques au cours de la trypanosomose expérimentale du lapin. *Exp. Parasitol*. **42**, 6–13.

Deitsch, K. W., Moxon, E. R. & Wellems, T. E. 1997 Shared themes of antigenic variation and virulence in bacterial, protozoal, and fungal infections. *Microbiol. Mol. Biol. Rev.* **61**, 281–293.

Fussenegger, M. 1997 Different lifestyles of human pathogenic procaryotes and their strategies for phase and antigenic variation. *Symbiosis* **22**, 85–153.

Gray, A. R. 1965 Antigenic variation in a strain of *Trypanosoma brucei* transmitted by *Glossina morsitans* and *Glossina palpalis*. *J. Gen. Microbiol*. **41**, 195–214.

Kosinski, R. J. 1980 Antigenic variation in trypanosomes: a computer analysis of variant order. *Parasitology* **80**, 343–357.

Maynard Smith, J. 1982 *Evolution and the theory of games.* Cambridge University Press.

Miller, E. N. & Turner, M. J. 1981 Analysis of antigenic types appearing in first relapse populations of *Trypanosoma brucei*. *Parasitology* **82**, 63–80.

Muñoz-Jordán, J. L., Davies, K. P. & Cross, G. A. M. 1996 Stable expression of mosaic coats of variant surface glycoproteins in *Trypanosoma brucei*. *Science* **272**, 1795–1797.

Murray, J. D. 1989 *Mathematical biology*. New York: Springer.

Nash, T. E. 1997 Antigenic variation in *Giardia lamblia* and the host's immune response. *Phil. Trans. R. Soc. Lond*. B **352**, 1369–1375.

O'Connor, R. M., Lane, T. J., Stroup, S. E. & Allred, D. R. 1997 Characterization of a variant erythrocyte surface antigen (VESA1) expressed by *Babesia bovis* during antigenic variation. *Mol. Biochem. Parasitol*. **89**, 259–270.

Pays, E. & Nolan, D. 1998 Expression and function of surface proteins in *Trypanosoma brucei*. *Mol. Biochem. Parasitol*. **91**, 3–36.

Seed, J. R. 1978 Competition among serologically different clones of *Trypanosoma brucei gambiense in vivo*. *J. Protozool*. **25**, 526–529.

Segel, L. A. 1972 Simplification and scaling. *SIAM Rev.* **14**, 547–571.

Serkin, C. D. & Seifert, H. S. 1998 Frequency of pilin antigenic variation in *Neisseria gonorrhoeae*. *J. Bacteriol*. **180**, 1955–1958.

Turner, C. M. R. 1997 The rate of antigenic variation in fly-transmitted and syringe-passaged infections of *Trypanosoma brucei*. *FEMS Microbiol. Lett*. **153**, 227–231.

Turner, C. M. R. & Barry, J. D. 1989 High frequency of antigenic variation in *Trypanosoma brucei rhodesiense* infections. *Parasitology* **99**, 67–75.

Vickerman, K. 1989 Trypanosome sociology and antigenic variation. *Parasitology* **99**, S37–S47.

Zhang, J. R., Hardham, J. M., Barbour, A. G. & Norris, S. J. 1998 Antigenic variation in Lyme disease borreliae by promiscuous recombination of VMP-like sequence cassettes. *Cell* **89**, 275–285.