THE ROYAL SOCIETY

Virulence in malaria: an evolutionary viewpoint

Margaret J. Mackinnon[*] and Andrew F. Read

School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT, UK

Malaria parasites cause much morbidity and mortality to their human hosts. From our evolutionary perspective, this is because virulence is positively associated with parasite transmission rate. Natural selection therefore drives virulence upwards, but only to the point where the cost to transmission caused by host death begins to outweigh the transmission benefits. In this review, we summarize data from the laboratory rodent malaria model, *Plasmodium chabaudi*, and field data on the human malaria parasite, *P. falciparum*, in relation to this virulence trade-off hypothesis. The data from both species show strong positive correlations between asexual multiplication, transmission rate, infection length, morbidity and mortality, and therefore support the underlying assumptions of the hypothesis. Moreover, the *P. falciparum* data show that expected total lifetime transmission of the parasite is maximized in young children in whom the fitness cost of host mortality balances the fitness benefits of higher transmission rates and slower clearance rates, thus exhibiting the hypothesized virulence trade-off. This evolutionary explanation of virulence appears to accord well with the clinical and molecular explanations of pathogenesis that involve cytoadherence, red cell invasion and immune evasion, although direct evidence of the fitness advantages of these mechanisms is scarce. One implication of this evolutionary view of virulence is that parasite populations are expected to evolve new levels of virulence in response to medical interventions such as vaccines and drugs.

Keywords: malaria; virulence; evolution; parasite fitness; transmission; immunity

1. INTRODUCTION

Why do parasites kill their hosts? Malaria, one such parasite that does, ranks high on the list of world health problems by causing massive human and economic loss (Sachs & Malaney 2002). Just why malaria parasites cause so much mortality and morbidity is a question that can be addressed from different viewpoints. One view is that of the clinician, who wants to understand the pathogenesis and pathology of the disease, and thus takes a proximate view of the causes and mechanisms of virulence. Another is that of the evolutionary biologist, who wants to know what selective forces shape the parasite's virulence evolution. Recent advances in both fields are leading us towards a deeper understanding of what makes this parasite so harmful to its host. In this review, we focus on the evolutionary view now emerging from experimental studies, and link these findings to current knowledge on the molecular mechanisms of virulence.

Several features of human malaria are relevant to this discussion. First, the amount of variation in disease severity observed in the field is remarkably high. Infections range from being asymptomatic to life-threatening with case fatality rates in untreated non-immune individuals being *ca.* 1% and sometimes exceeding 20% (Alles *et al.* 1998). Much of this variation can be attributed to prior exposure and acquired immunity, but clearly other factors operate too. These include parasite species (most mortality is due to infection with *Plasmodium falciparum*), age, nutrition, inoculum size, socioeconomic factors, host

genetics and parasite genetics (reviewed in Greenwood *et al.* 1991; Marsh 1992; Baird 1998; Phillips 2001; Miller *et al.* 2002). The relative importance of these different factors has not been well quantified. In one study in an area of low endemicity in Sri Lanka where *P. vivax*—a less virulent human malaria species than *P. falciparum*—predominates, of the 30% of variation in disease severity explained by known factors, approximately one-third was explained by prior exposure, one-third by host genetics and another third by other non-genetic factors pertaining to the host (Mackinnon *et al.* 2000). The fact that 70% remained unexplained highlights the complex nature of the epidemiology and physiology of the disease. It also leaves room for a strong contribution from parasite genetics. Similar studies in areas where the more virulent *P. falciparum* predominates have not been performed.

A second feature of human malaria is that immunity is virtually never sufficient to prevent infection. In endemic areas, hosts are typically reinfected throughout their lives, although rarely become ill after experiencing 5–10 symptomatic infections (reviewed in Baird 1998). This ineffective protection against reinfection appears to be partly attributable to parasite variation in immunogenic antigens, the strain-specific nature of the immune response to the parasite, and the relatively short-lived immune memory (reviewed in Day & Marsh 1991; Marsh 1992; Hviid 1998; Richie & Saul 2002).

Third, transmission intensity in the field is highly variable both temporally and geographically (Molineaux & Gramiccia 1980; Hay *et al.* 2000). These three features of malaria, in addition to the necessary limitations on performing experimental studies of disease in humans or primate models, have obscured our understanding of why

Phil. Trans. R. Soc. Lond. B (2004) **359**, 965–986
DOI 10.1098/rstb.2003.1414

965

© 2004 The Royal Society

malaria parasites cause disease, and why the outcome is so variable. Nevertheless, it is clear from several lines of evidence that there is considerable parasite genetic variation in virulence and transmissibility in the field, and that this can help explain some of the patterns of disease epidemiology (Gupta *et al.* 1994; Gupta & Day 1996). In this review, we pursue the notion that parasite-based virulence–transmissibility relationships drive epidemiological and evolutionary patterns. We show how knowledge of these relationships can help predict how the parasite is likely to evolve when subjected to selection pressure by control programmes.

2. THE VIRULENCE EVOLUTION HYPOTHESIS

In the past two decades, there has been a realization that evolutionary biology should be able to help explain patterns of human disease (Ewald 1994; Nesse & Williams 1994; Stearns 1999). Virulence—defined as the damage done to the host—has received most attention in this regard. Although virulence is an outcome of parasite, host and environmental factors, most evolutionary theory centres on the parasite, and we follow that tradition. The idea is that parasites do not evolve to zero virulence (i.e. to cause no reduction in host fitness) because virulence incurs benefits as well as costs to the lifetime transmission success of the parasite, i.e. its Darwinian fitness. The benefit of virulence is higher transmission rate: by extracting more resources from the host, the parasite is able to make more transmissible forms per unit time. Another benefit is slower parasite clearance rate and hence a longer infection from which to transmit. However, the cost of virulence is premature host death which shortens the lifetime of the infection. Thus there is a trade-off between 'how fast' and 'how long' the parasite transmits. This leads to maximum lifetime transmission occurring in parasites that have intermediate virulence, and this is therefore the outcome of natural selection (see Frank (1996) for a review of the theory, and figure 1 for a diagrammatic explanation).

This trade-off view of virulence evolution has generated a large body of theory, and elaborations of it continue to multiply. These theoretical studies are often pinned on claims that 'virulence management' will be helpful in combating diseases of medical and veterinary importance (Dieckmann *et al.* 2001). Unfortunately, the pace of theory is not matched by data to support it. Even the most basic assumption of a positive virulence–transmissibility relationship implicit in the trade-off argument (figure 1) remains largely untested, and is currently justified by only a few observations. Direct evidence that there is a positive relationship between transmission rate and virulence among natural pathogen isolates has been found for myxomatosis virus in rabbits (Fenner *et al.* 1956), typhoid bacteria in mice (Greenwood *et al.* 1936), trypanosomes in mice (Turner *et al.* 1995), microsporidia in *Daphnia* (Ebert 1994) and malaria in mice (Mackinnon & Read 1999*a*; Ferguson *et al.* 2003*b*). Indirect evidence has also been found from experimental evolution studies (Bull *et al.* 1991; Ebert & Mangin 1997; Turner *et al.* 1998; Mackinnon & Read 1999*b*; Messenger *et al.* 1999; Elena 2001) or cross-species comparisons (Herre 1993; Clayton & Tompkins 1994; Ewald 1994). Evidence that host

death reduces lifetime transmission is rare (Fenner *et al.* 1956) and there are pathogens that are claimed to not fit the trade-off model (Lipsitch & Moxon 1997; Weiss 2002; Ebert & Bull 2003), although direct data are lacking in these cases. Further, there are alternative hypotheses that challenge the adaptive hypothesis. For example, virulence may be an accidental and rare by-product of normally asymptomatic infection (Levin & Svanborg-Eden 1990), or the outcome of short-term within-host evolution for rapid proliferation, but with detrimental effects on between-host transmission and hence evolutionary stability (Levin & Bull 1994).

The lack of data on genetic variation and covariation among the key life-history traits of parasites—virulence, transmission rate and clearance rate—is surprising given that these traits determine the population-level burden of disease. In fact, the aim of control programmes (e.g. drugs and vaccines) is to reduce the burden by acting directly on these traits. The success of such programmes will depend on what parasite and host factors influence these traits and how they are biologically related to each other. Importantly, if these traits really are the prime determinants of parasite fitness, then control programmes will do more than just reduce disease: they will bring about evolutionary change in the parasite population with long-term consequences. With these considerations in mind, and given our firm belief that evolutionary models need to be grounded and tested in specific disease contexts, we set out to examine the virulence adaptive trade-off hypothesis using the *P. chabaudi* rodent malaria as a model. Our immediate aims were twofold: first, to test the assumptions underlying the evolutionary theory in a microparasite of medical importance, and second, to help us understand the evolutionary forces driving malaria parasite virulence. Our ultimate objective is to determine the key parasite properties that cause virulence in human malaria.

3. *PLASMODIUM CHABAUDI* AS A MODEL FOR VIRULENCE EVOLUTION

All *Plasmodium* species have an asexual haploid life stage that replicates rapidly in the red blood cells of their vertebrate host, thereby causing anaemia and other general pathology. These asexual stages occasionally produce sexual stages (gametocytes) which themselves do not replicate, but when ingested by *Anopheles* mosquitoes, fuse to from a diploid zygote, undergo meiosis, then proliferate to yield forms that are transmitted back to vertebrate hosts. Gametocytes are produced throughout the infection, and are generally rare, typically constituting *ca.* 1% of the total parasite population. Asexual parasite densities reach a maximum of 10^8 to 10^9 ml^{-1} after about one week of becoming detectable in the blood, and then decline in a log-linear fashion over the next two to six months. The similarity in infection pattern across *Plasmodium* species is illustrated in figure 2.

The rodent malaria species, *P. chabaudi*, is the laboratory model that has been most used for addressing evolutionary questions. This is because it shares many infection characteristics with *P. falciparum*, the most virulent of the human-infecting species. Both species have a relative lack of preference for young red blood cells (reticulocytes) over older ones (normocytes) (Pasvol *et al.*
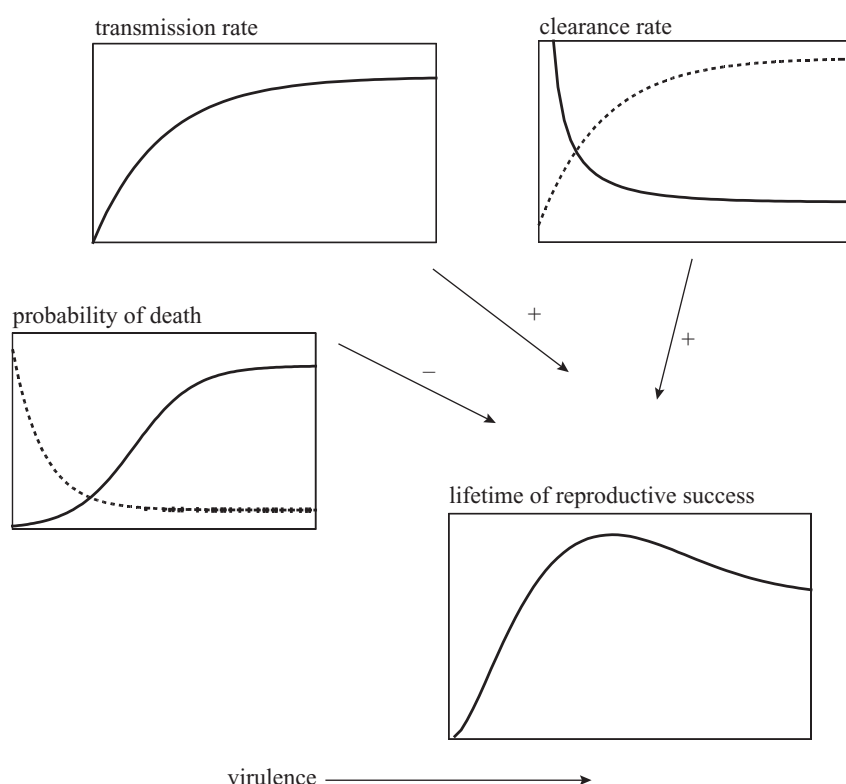
Figure 1. Relationships between virulence (*x*-axis), transmission rate, clearance rate and host mortality (*y*-axes, solid lines) as assumed in the trade-off model for the evolution of parasite virulence. The parasite's lifetime reproductive success (parasite fitness) increases with virulence at the lower end of the spectrum because of its positive relationship with transmission rate and negative relationship with clearance rate. However, at the upper end of the spectrum, the negative effect of host mortality, and hence infection length, outweighs these benefits. Thus maximum parasite fitness occurs at an intermediate level of virulence. The dashed lines show the relationships between virulence and infection length.

1980; Jarra & Brown 1989; Clough *et al.* 1998*a*) compared with many other species (e.g. *P. vivax*, *P. berghei* and *P. yoelii*) which show a marked preference for young red blood cells (reticulocytes) (Garnham 1966). *Plasmodium falciparum* and *P. chabaudi* also both display cyto-adherence: the sticking of parasitized cells to other host cells during the last half of the replication cycle. Cytoadherence takes several forms. One is sequestration: the withdrawal of parasites from the peripheral circulation to the microvasculature where they adhere to endothelial cells. Another is rosetting: the adherence of uninfected red blood cells to infected cells to form small clusters of cells. A third is adherence of infected cells to infected cells. All three properties have been implicated as virulence factors in human malaria (David *et al.* 1983; Langreth & Peterson 1985; MacPherson *et al.* 1985; Carlson *et al.* 1990; Rowe *et al.* 1995; Roberts *et al.* 2000; Pain *et al.* 2001). Finally, both species provoke strain-specific immunity (Taliaferro 1949; Jeffery 1966; Jarra & Brown 1985; Snounou *et al.* 1989; Mota *et al.* 1998), and both species undergo clonal antigenic variation (McLean *et al.* 1982; Biggs *et al.* 1991; Roberts *et al.* 1992).

However, there are some marked differences between *P. falciparum* in the field and *P. chabaudi* in laboratory mice. The maximum parasitaemia reached in *P. falciparum* typically does not exceed 5% or 10^8 parasites ml^{-1} (e.g. Field & Niven 1937; Collins & Jeffery 1999) and all-case fatality rates are *ca.* 0.1–1% (Molineaux & Gramiccia 1980; Greenwood *et al.* 1987; Alles *et al.* 1998; Snow *et al.* 1999). In *P. chabaudi,* peak parasitaemia typically

reaches 30% or 10^9 ml^{-1} in laboratory mice (e.g. Jarra & Brown 1985) and mortality rates are typically *ca.* 5–20% in C57Bl/6J mice and much higher in less resistant mouse genotypes such as CBA/Ca and DBA (Stevenson *et al.* 1982). Levels of peak parasitaemia of *P. chabaudi* in its natural host, *Thamnomys rutilans*, are undocumented, though infections can be severe (Landau 1965, 1966), and in *Grammomys surdaster*, a very closely related and sympatric species to *T. rutilans* (Ellerman 1940), infections are similar to those in laboratory mice and often cause death (D. Walliker and R. Carter, personal communication). In *P. falciparum*, infections usually last less than six months (Diebner *et al.* 2000; Collins & Jeffery 2003) and almost always less than a year (Dietz *et al.* 1980). Infections of *P. chabaudi* in their natural host in its natural environment are known to occur throughout this host's life (*ca.* 2 years (Landau & Chabaud 1965)) probably because of the existence of dormant forms in the liver (Landau & Chabaud 1965; Landau & Killick-Kendrick 1966; Landau & Chabaud 1968) or other latent forms of the parasite in the lymph system (Landau *et al.* 1999). Chronic *P. chabaudi* infections lasting up to at least 1 year, though more typically two to three months, are also observed in mice (D. Walliker and R. Carter, personal communication). Cerebral malaria, a syndrome of *P. falciparum* in which parasites become lodged in the brain as a result of cytoadherence, and the form of disease responsible for most malaria-related deaths in Africa (Marsh & Snow 1997), is not an obvious feature of *P. chabaudi*. Lesions (Vuong *et al.* 1999) and parasites (Mota *et al.* 2000) have
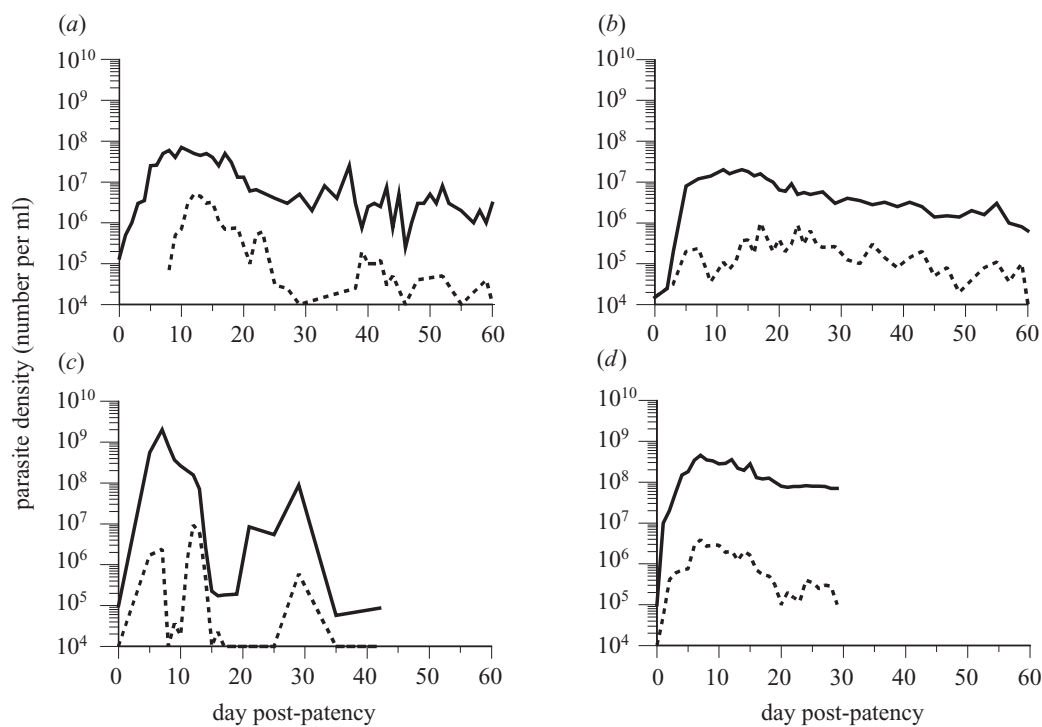
Figure 2. Typical patterns of asexual parasite and gametocyte densities through time for four species of malaria parasites: (*a*) *Plasmodium falciparum*; (*b*) *P. vivax*; (*c*) *P. chabaudi*; and (*d*) *P. berghei*. Data for *P. falciparum* and *P. vivax* are from Boyd (1949) and come from one host individual. Data for *P. berghei* (from Dearlsy *et al.* 1990) and *P. chabaudi* (Mackinnon & Read 2003) are the average patterns over three and five mice infected with one parasite clone (234L and CW, respectively). Data are reproduced with permission.

been found in the brain of *P. chabaudi*-infected mice, but most sequestration takes place in other vital organs such as the liver (Cox *et al.* 1987; Mota *et al.* 2000). Brain involvement similar to cerebral malaria does occur in related rodent species (*P. berghei*, Rest (1982) which also sequesters in other organs (Alger 1963), and in *P. yoelii*, Yoeli & Hargreaves 1974; Kaul *et al.* 1994), but this appears to be only after adaptation to the novel host (mice) through serial passage.

In addition to its similarities with *P. falciparum*, the *P. chabaudi* system is attractive as a model because of the availability of diverse parasite strains cryopreserved shortly after they were derived from the wild (Beale *et al.* 1978), the availability of host genotypes with well-characterized genetics, the ability to measure virulence, multiplication rate, gametocyte production, transmission to mosquitoes, cytoadherence and antigenic variation *in vivo*, and the ability to manipulate immunity and other factors that moderate virulence.

4. VIRULENCE–TRANSMISSIBILITY RELATIONSHIPS IN *PLASMODIUM CHABAUDI*

There are four main traits relevant to the evolution of virulence that we have quantified in the *P. chabaudi* system. Parasite multiplication ability, or 'growth', was measured as either the rate at which the asexual parasite population increased during the early stage of the infection, or as the maximum asexual parasite density or proportion of red blood cells infected (parasitaemia). Note that although these measures focus on multiplication rate during the acute phase, we are interested in effective multiplication rates throughout the infection. Virulence was

measured as the maximum or total amount of red blood cells destroyed by the infection, or the maximum or total amount of liveweight lost by the mouse, or less commonly, by death of the mouse. Note that, strictly speaking, virulence in the theoretical models is defined as the rate of host death, whereas in these experiments, we mainly measured morbidity. However, because of the positive relationship between host mortality and morbidity (Timms *et al.* 2001; Mackinnon *et al.* 2002a; Ferguson *et al.* 2003b), we use the term virulence to encompass both morbidity and mortality. Transmission rate was measured as the gametocyte density or proportion of cells infected with gametocytes (gametocytaemia): when averaged over time, this provided an indicator of lifetime transmission potential. Sometimes, actual transmission to mosquitoes was measured as the proportion of mosquitoes that became infected when allowed to feed on the mouse on a limited number of days around peak gametocytaemia (Mackinnon & Read 1999a; Ferguson *et al.* 2003b). Gametocyte density is a good predictor of transmission to mosquitoes in *P. chabaudi* (Taylor & Read 1997; Taylor *et al.* 1997; Mackinnon & Read 1999a; Buckling & Read 2001). Transmission back to the host has not been quantified in this system. Clearance rate was measured as the rate at which log parasite density decreased from peak parasitaemia to sub-detectable levels. As there are secondary peaks of parasitaemia during this time, a straight line was fitted through either all points or all peaks, and its slope used to indicate clearance rate.

Using a panel of 10 cloned parasite lines recently isolated from the wild, we showed that parasite clones that have high multiplication rate produce more asexual parasites, generate more gametocytes, infect more mosquitoes
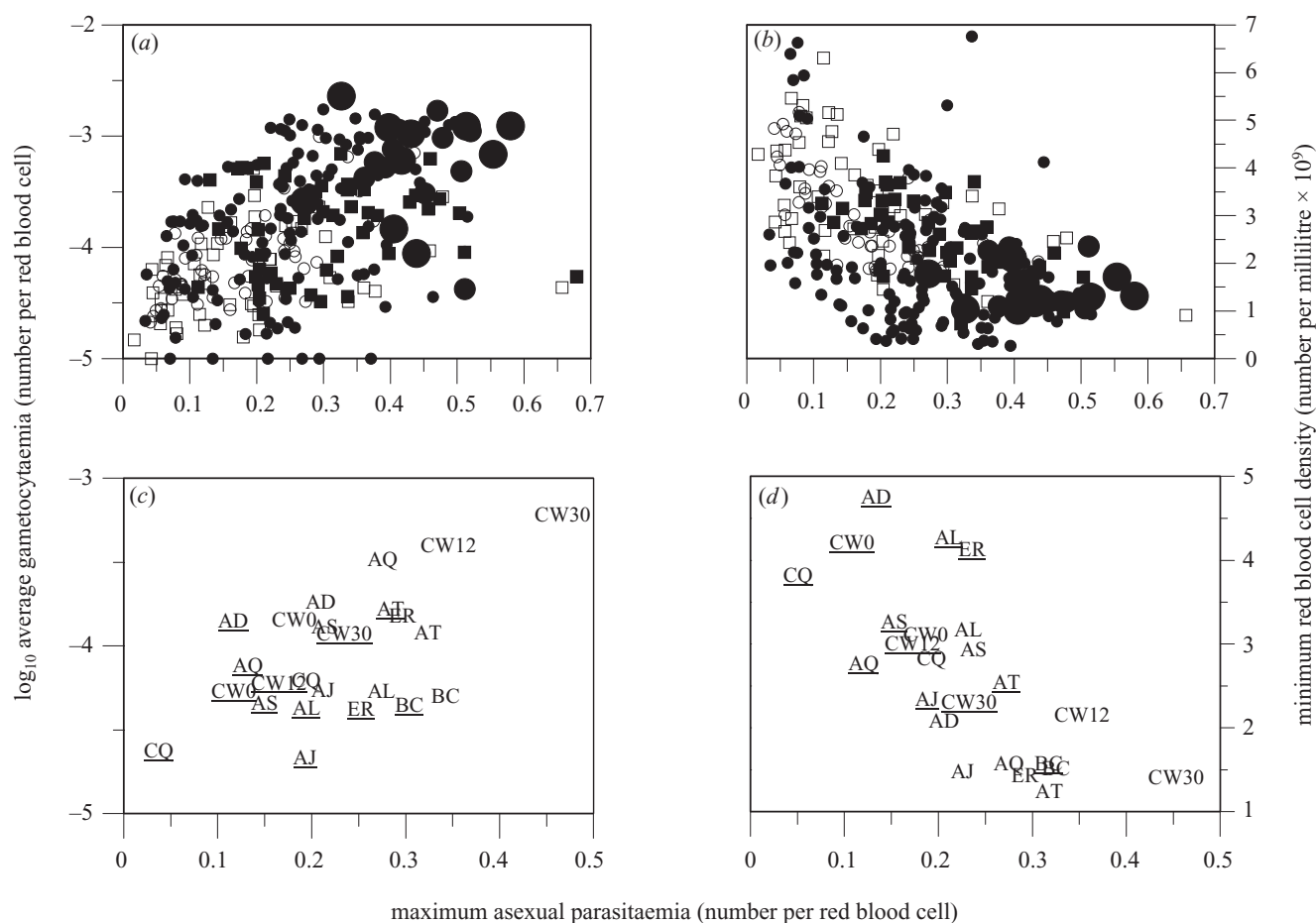
Figure 3. Phenotypic (i.e. across mice (*a*) and (*b*)) and genetic (across clone (*c*) and (*d*)) relationships between maximum asexual parasitaemia (*x*-axis), average gametocytaemia (*y*-axis (*a*) and (*c*)) and minimum red blood cell density (*y*-axis (*b*) and (*d*)) pooled over four studies of *P. chabaudi* infection in inbred laboratory mice. In (*a*) and (*b*), measurements made in individual female and male mice are shown as circles and squares, respectively. Circles of different sizes represent data from different host genotypes (smallest, C57Bl/6J; medium, CBA/Ca; largest, DBA). Closed and open symbols represent measurements in naive and immunized mice, respectively. In (*c*) and (*d*), lettered symbols represent the mean of the labelled clone and are underlined when measured in immunized mice. CW0, CW12 and CW30 denote clone CW after 0, 12 and 30 serial passages. Data taken from Mackinnon & Read (1999*a*, 2003; M. J. Mackinnon and A. F. Read, unpublished data) and Mackinnon *et al.* (2002*a*) with permission.

and have longer infections than clones with low multiplication rates (Mackinnon & Read 1999*a,b*, 2002*a*, 2003; Ferguson *et al.* 2003*b*). They also cause more anaemia and weight loss (morbidity) (Mackinnon & Read 1999*a,b*, 2002*a*, 2003; Ferguson *et al.* 2003*b*), and cause more mortality (Timms *et al.* 2001; Mackinnon *et al.* 2002*a*). Mortality reduced total lifetime gametocyte production in one experiment by 74% (Mackinnon *et al.* 2002*a*), but not in another experiment (Ferguson *et al.* 2003*b*). When the same parasite clones were injected into immunized as well as naive mice, it was found that immunity reduced growth rate, virulence, transmission rate and infection length, and that the positive relationships between these traits found in naive mice were also found in immunized mice (Mackinnon & Read 2003). Within a small range of conditions typical of one experiment, these relationships are quite noisy though generally statistically significant. Figure 3 shows these relationships in data pooled across four separate experiments that included 13 different parasite lines, three host genotypes, naive and immunized mice, and both host sexes. Taking this broader view, the phenotypic relationships among traits are very strong, and consistent

across, as well as within, experimental groups (figure 3*a,b*). When data were averaged over each parasite clone, these relationships were shown to have a parasite genetic basis (figure 3*c,d*). Thus the assumptions of the adaptive trade-off virulence model (figure 1) are clearly supported by the experimental data from *P. chabaudi*.

We also found that growth rate early in the infection and late in the infection are both correlated to maximum parasitaemia (figure 4). Thus despite the difference in factors thought to limit growth early versus late in the infection, it appears that successful multiplication is at least partly determined by a common mechanism that operates at all stages of the infection.

The positive virulence–transmissibility relationships are also borne out when parasites undergo selection and adaptation. When parasite lines of various species are serially passaged (i.e. by transfer of asexual blood stages to new hosts by syringe rather than sexual stages by the mosquito), they often become more virulent, and generate higher parasite densities (James *et al.* 1936; Greenberg & Kendrick 1956; Sergent & Poncet 1959; Galli & Brambilla 1967; Hartley 1969; Dearsly *et al.* 1990; Mackinnon &
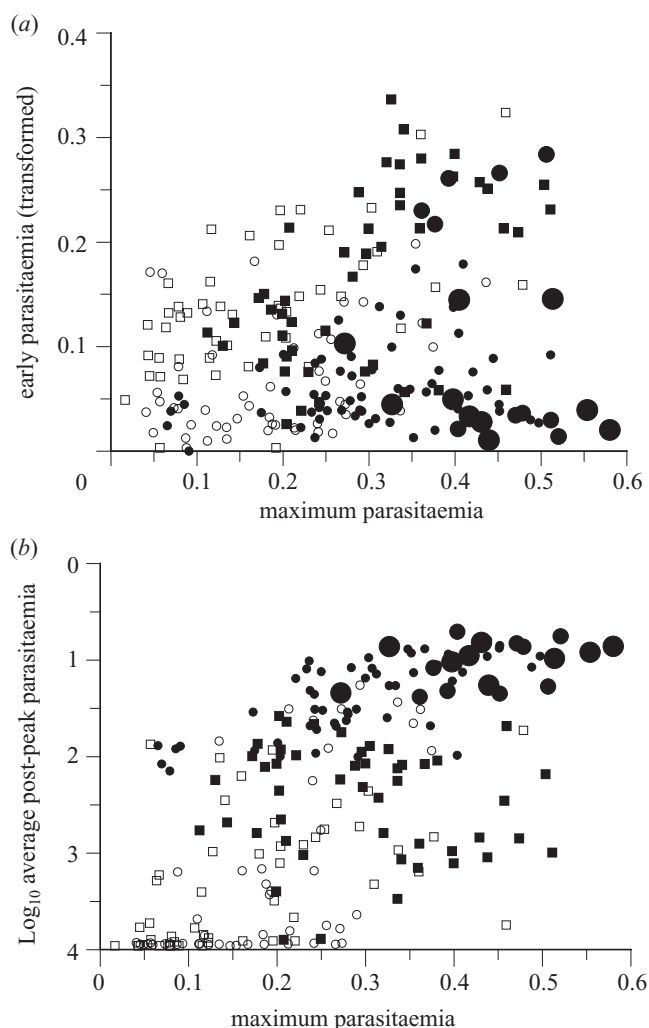
Figure 4. Relationships between (*a*) early and (*b*) late asexual multiplication (*y*-axes) and maximum parasitaemia (*x*-axes). Early multiplication was measured as parasitaemia on day 4 or 5 post-infection and was arcsine square-root transformed for plotting. Late multiplication was measured as the average parasitaemia from days 12–19 post-infection and was log_{10} transformed for plotting. Symbol definitions are as for figure 3. Data taken from Mackinnon & Read (2003; M. J. Mackinnon and A. F. Read, unpublished data) and Mackinnon *et al.* (2002*a*) with permission.

Read 1999*b*). In some cases this virulence increase has been accompanied by an increase in transmission potential (James *et al.* 1936; Mackinnon & Read 1999*b*). However, there are other examples where virulence has decreased with serial passage (Greenberg & Kendrick 1956; Carrescia & Arcolea 1957; Rest 1982) and where gametocyte production has been reduced by passage *in vivo* (Dearsly *et al.* 1990) or lost *in vitro* (Day *et al.* 1993). In the absence of regular passage through mosquitoes and hence maintenance of selection for the ability to sexually reproduce, results from serial passage experiments must be treated with caution. What these experiments do clearly show, however, is that malaria parasites can adapt at a remarkable rate. Within 150 replication cycles, a parasite line that caused virtually no weight loss and had a peak parasitaemia of 10% changed to cause 10% of body weight loss and a peak parasitaemia of 25% (Mackinnon & Read 1999*b*). In less resistant mouse genotypes, this line killed

70% of mice whereas the ancestral line incurred zero mortality (Mackinnon *et al.* 2002*a*). Undoubtedly, this reflects adaptation of *P. chabaudi* to its novel laboratory host, the mouse. Regardless of the cause, the fact that these parasites can adapt so quickly is vitally important to the question of whether noticeable virulence evolution will occur in response to vaccination programmes or other interventions in human malaria.

5. VIRULENCE–TRANSMISSIBILITY RELATIONSHIPS IN *PLASMODIUM FALCIPARUM*

Do these virulence–transmissibility relationships hold for *P. falciparum*? The best source of *in vivo* data in humans comes from the treatment of patients with neurosyphilis by infection with malaria. Between-strain variation in parasite growth rate and virulence has been demonstrated in these data from *P. falciparum* (James *et al.* 1932; Jeffery & Eyles 1955; Gravenor *et al.* 1995; Simpson *et al.* 2002; Read *et al.* 2003) as have differences in infection length (Jeffery & Eyles 1954, 1955), infectivity to mosquitoes (Jeffery & Eyles 1955) and gametocyte circulation time (Eichner *et al.* 2001). Variation in asexual growth rate *in vitro* of wild-caught *P. falciparum* isolates exists and, importantly, this correlates positively to *in vivo* virulence (Chotivanich *et al.* 2000). Between-strain variation in gametocyte production *in vitro* in *P. falciparum* has also been demonstrated (Graves *et al.* 1984). There is also evidence of between-strain variation for some of these characteristics in *P. vivax* (reviewed in McKenzie *et al.* 2002). However, there appear to be only two published studies, one in *P. falciparum* and one in *P. vivax*, that have examined the relationships among virulence, transmission, growth rate and infection length. The study in *P. falciparum* compared the El Limon strain from a high-transmission area in Central America (Panama) with the Santee–Cooper strain from a low-transmission area in North America (South Carolina) and found that the El Limon strain was more virulent, more persistent and had more transmission to mosquitoes than the Santee–Cooper strain (Jeffery & Eyles 1955), consistent with the virulence evolution hypothesis. A subsequent analysis of these data found no differences between these strains in parasite multiplication rate early in the infection (Simpson *et al.* 1999). Within strains, relationships between virulence and transmissibility have not yet been reported from these data. Another study in *P. vivax* found that repeated passage through humans resulted in a parasite line that had higher asexual parasitaemias, fevers, mortality and transmission to mosquitoes than its ancestral form (James *et al.* 1936).

In the absence of systematic studies of virulence–transmissibility relationships in human malaria parasites, we resorted to an analysis of field data from a large epidemiological survey in a malaria-endemic area in Nigeria where *P. falciparum* predominates (the Garki Project (Molineaux & Gramiccia 1980)). Such data introduce many factors other than parasite genetics that might impact on the growth-rate–virulence–clearance-rate–transmissibility relationships, such as host age, immune status and force of infection. Whereas it is only the genetic relationships among parasite traits that determine the direction of virulence evolution, which cannot be extracted from these data, the analysis revealed broad
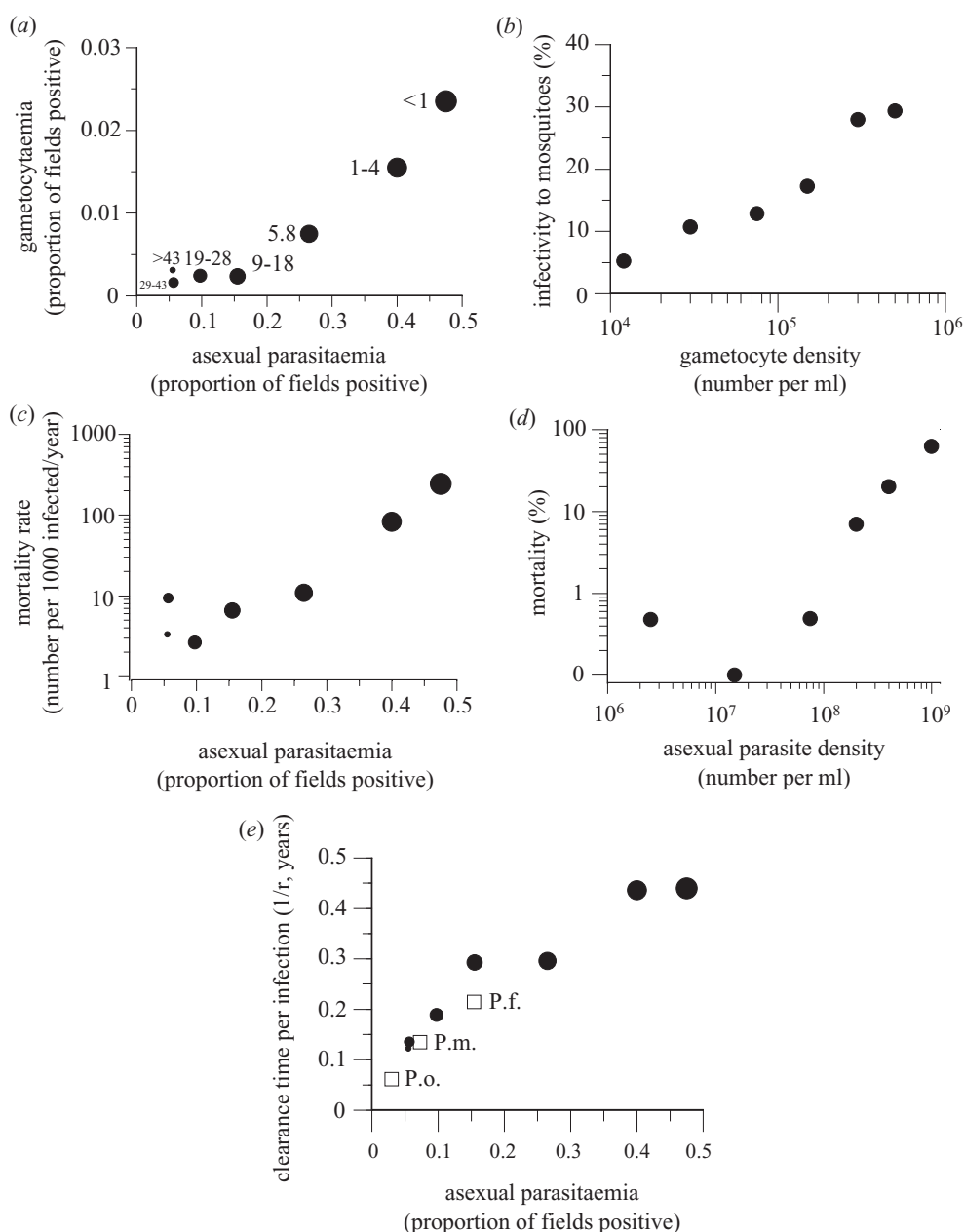
Figure 5. Relationships between asexual parasitaemia, virulence and transmission in human malaria in the field. Each point represents the average for an age-class, with symbols of decreasing size representing younger than 1, 1–4, 5–8, 9–18, 19–28, 29–43 and older than 43-year-olds, respectively, as labelled in (*a*). Data in (*a*), (*c*) and (*e*) are from the Garki project[1] and are derived from eight cross-sectional epidemiological surveys performed every 10 weeks during the baseline phase (Molineaux & Gramiccia 1980). Average gametocytaemia[2] (*a*), mortality rate[3] (*c*) and clearance time for each new infection allowing for superinfection[4] (*e*) increase with asexual parasitaemias[2]. In (*e*), mean values for the three parasite species in this area across all ages are also shown by the open square symbols (P.f., *Plasmodium falciparum*; P.m., *P. malariae*; P.o., *P. ovale*). Data in (*b*) are from Tchuinkam *et al.* (1993)[5] and illustrate that the relationship between infectivity to mosquitoes and log gametocyte density is positive and approximately linear. Data in (*d*) are from Field & Niven (1937) and, as in (*c*), illustrate that mortality increases with log asexual parasite density in a linear fashion (see endnotes for details). Data are reproduced with permission.

biological relationships between virulence and transmissibility consistent with the assumptions of the virulence evolution theory (figure 1). Figure 5*a* shows that asexual parasite density and gametocyte density among infected hosts are highly correlated across age groups and across seasons. Data from a separate study (Tchuinkam *et al.* 1993) illustrate how transmission of *P. falciparum* to mosquitoes increases with the log of gametocyte density, this in a linear way (figure 5*b*), consistent with other field studies on this relationship (reviewed in Carter & Graves

(1988); see also Collins & Jeffery (2002) and McKenzie *et al.* (2002) for data on *P. ovale* and *P. vivax*). Thus it is reasonable to assume that transmission to mosquitoes in the Garki Project was a log-linear function of the average gametocyte densities reported, further assuming that host age does not alter the infectivity per gametocyte, as supported by most studies where this has been examined (Rutledge & Gould 1969; Graves *et al.* 1988; Tchuinkam *et al.* 1993). Figure 5*c,d* shows that mortality rate closely correlates to asexual parasite densities across ages and
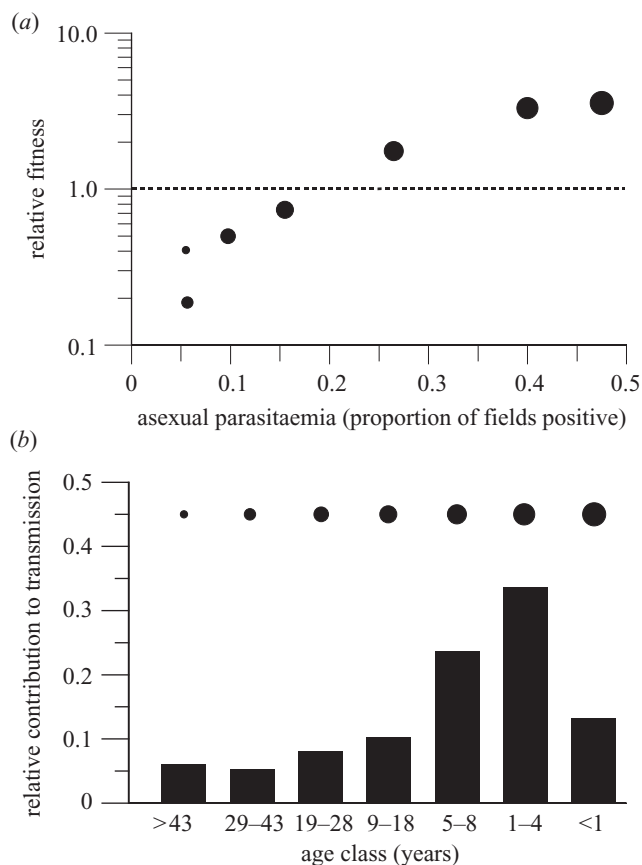
Figure 6. Relative fitness[6] of *Plasmodium falciparum* parasites infecting different age classes of host as a function of asexual parasitaemia based on data from the Garki malaria field project (see figure 5). Maximum fitness occurs at the upper end of the spectrum of asexual parasitaemias[7] (which we use here as a virulence indicator) that arise in children. Parasite fitness is about one-tenth of this in older hosts. The upper panel (*a*) shows fitness relative to the parasite population average (indicated by the horizontal line). The lower panel (*b*) shows the relative contribution of each age class to the total transmission in the population[8]. Different sized dots above the bars correspond to different age-classes and correspond to those in the upper panel and in figure 5. These two panels thus distinguish between the effects of host quality and host quantity on parasite fitness. The analysis suggests that evolution has maximized parasite fitness for host types that contribute most to transmission, i.e. children under 9 years of age who account for 71% of the total transmission.

seasons. Figure 5*e* shows that clearance rates decrease with asexual densities. It also shows that two other species, *P. ovale* and *P. malariae*, fit in well with the curves for *P. falciparum*: thus these relationships may hold across, as well as within, *Plasmodium* species. Indeed, data from malaria therapy infections with *P. ovale* (Collins & Jeffery 2002), *P. malariae* (McKenzie *et al.* 2001) and *P. vivax* (McKenzie *et al.* 2002) confirm the lower average asexual parasitaemias, lower gametocytaemias, lower virulence and faster clearance rates than for *P. falciparum*, thus further supporting this argument.

The data in figure 5 can be combined to calculate the expected lifetime transmission (i.e. fitness) of the parasite as a function of asexual multiplication. When this is done, the hypothesized virulence trade-off from evolutionary theory is revealed (figure 6*a*). Parasite fitness reached its maximum in 1-year-olds or younger and was almost equal to that in the 1–4-year-olds, despite the latter group having lower transmission rates (figure 5*a*). This is because of the higher mortality rates in under-1-year-olds or younger (figure 5*c*), which is balanced by their higher transmission rates. In adults, fitness was much less than that in children (by about 10-fold): this was because of lower gametocyte densities and shorter infections, thereby clearly illustrating the fitness benefits associated with virulence. Thus the benefits and costs of virulence assumed by theory are supported by these data. Note that these data, unlike those from *P. chabaudi*, do not directly support the trade-off proposed by the evolutionary models, which require that virulence variation is generated by parasite genetics rather than host factors: they are nonetheless consistent with the theory. They also provide an excellent example of how parasite fitness is maximized at intermediate levels of virulence: this is for the same reasons assumed by the evolutionary hypothesis described above.

This example from the field is also useful for demonstrating the main sources of selection on the parasite population. Calculating the relative contribution to total transmission in the population reveals that children under 9 years of age, in addition to having near-maximal per-host fitness, are also the class that contribute most to the fitness of the total population (figure 6*b*). Thus natural selection appears to have optimized fitness to suit the most abundant host type. This is just as expected from evolutionary theory, and has also been observed in other natural systems (e.g. Herre 1987; West & Herre 1998). Our overall conclusion from this analysis is that, in the field, too-virulent malaria parasites are maintained in the population by selection pressure for high transmission from semi-immune hosts, and that this is at the expense of young children who lack immune protection. Assuming that parasite strain differences in virulence are fixed (i.e. parasites do not modify their virulence according to which sort of host they are in), the virulence trade-off hypothesis can thus explain why malaria parasites kill some of their human hosts.

6. IMPLICATIONS FOR VACCINES AND OTHER CONTROL MEASURES

Immunity to malaria in humans is almost never perfect (i.e. infection-blocking), but does protect against disease. We reasoned that if, as the data suggest, asexual multiplication was really the key to transmission and virulence, then immunity that reduced asexual multiplication would also reduce both these fitness traits according to the general biological relationship between them. To test this hypothesis, we tested 10 clones of *P. chabaudi* for their growth–virulence–transmissibility relationships in naive and immunized mice. As expected, immunity reduced all three traits and hence overall parasite fitness, and the positive genetic relationships between multiplication rate, morbidity, infection length and transmission previously demonstrated in naive mice were also maintained in immunized mice. Parasite clones also maintained their virulence ranking across naive and immune hosts: thus clones were consistent in their relative fitness across immune environments (Mackinnon & Read 2003).

Given these findings, we built a model to predict the impact of anti-growth rate (blood-stage) vaccines on the

evolution of virulence in microparasites in general, which we applied to the problem of endemic malaria area (Gandon *et al.* 2001). We predicted that intrinsic virulence would increase as the degree of vaccine coverage and efficacy increased. This is because immunity protects the parasite from the fitness cost of virulence (host death) while still putting pressure on higher transmission. The consequences of this virulence evolution would be realized at two levels. Individual hosts would now be exposed to more dangerous parasites so that those hosts unfortunate enough to be unvaccinated (there would always be some) would suffer higher mortality. At the population level, there might be lower total mortality if vaccination coverage was high, but this benefit would be less than if parasite evolution had not occurred. Thus evolution would erode the benefits of vaccination. Retreating from a failing vaccination programme would lead to more unvaccinated people being at risk from these vaccine-generated virulent strains, and thus would be highly undesirable.

In the same theoretical framework, we also examined the impact of other types of vaccine currently under development for malaria: anti-toxin vaccines, anti-infection vaccines and transmission-blocking vaccines (reviewed in Hoffman 1996). We found that anti-toxin vaccines, like anti-growth-rate vaccines, would select for higher virulence because they also release the parasite from the fitness constraint of host death. However, anti-infection and transmission-blocking vaccines would have no such effects. This is because their transmission-blocking effects are decoupled from their virulence, and therefore they do not alter the costs and benefits of virulence (Gandon *et al.* 2001). These predictions for vaccines can be applied more generally to other control measures such as drugs and bednets. For example, bednets may act like infection-blocking or transmission-blocking vaccines, whereas drugs may act like anti-growth rate vaccines. Therefore, if this theory is relevant to field malaria, there are clear choices to be made among control strategies that will yield benefits in the long term as well as the short term. Fortunately, the most desirable approaches to control from an evolutionary and epidemiological point of view—bednets and other transmission-reducing measures—are also the cheapest, the easiest to implement and the most socially acceptable of the current options.

What is the long term in this context? The rate of adaptation of malaria parasite populations can be remarkably high in the laboratory (Greenberg & Kendrick 1956; Dearsly *et al.* 1990; Mackinnon & Read 1999*b*) but we do not know the rates of change in the field. The fact that drug resistance becomes a problem within 5–30 years of first using a drug is indicative of their potential to evolve rapidly (Peters 1987). Changes in population frequencies of antigenic types after a field vaccine trial (Genton *et al.* 2002) have already been documented, and escape mutants under vaccine pressure in experimental systems have also been observed (David *et al.* 1985). Our theoretical model, which was based on well-known parameter values derived from field data (Dietz *et al.* 1980; Aron & May 1982; Nedelman 1984; Struchiner *et al.* 1989), predicts that a new virulent mutant will reach a frequency of 50% in less than 40 years (Gandon *et al.* 2001). Moreover, malaria parasites have a well-known capacity for rapid phenotypic adaptation without genomic alteration. If vaccine-driven

adaptation was purely phenotypic and these changes were carried over to new hosts, i.e. stable, increases in virulence may be more easily reversible than if the change was genetic. However, reversal would necessarily come at the cost of host mortality unless an alternative form of selection pressure could be found.

7. MOLECULAR MECHANISMS FOR VIRULENCE IN RELATION TO PARASITE FITNESS

This evolutionary perspective differs from, although is not at odds with, the clinical and molecular explanations of malaria virulence which focus on 'how' rather than 'why' parasites cause damage to their hosts. Clinicians explain severe disease in terms of three main factors: anaemia, parasite toxins and cytoadherence (White & Ho 1992; Marsh & Snow 1997; Newbold *et al.* 1997). All of these share the same root cause: unrestrained asexual parasite multiplication (Field & Niven 1937; Kitchen 1949; Molineaux *et al.* 2001). What we are proposing here is that high multiplication ability is the key to parasite fitness and that virulence is an unfortunate side-effect of this. To illustrate how these two perspectives can be merged, we have modified the diagram of Marsh & Snow (1997) explaining the clinical basis of severe malarial disease to incorporate the transmission consequences of high multiplication rate and virulence (figure 7).

If asexual multiplication really is the Achilles' heel of parasite fitness, what are the mechanisms by which this is achieved? Three parasite properties stand out as candidates for virulence and fitness promoters: cytoadherence, antigenic variation and red cell invasibility. Whereas the molecular bases of these phenotypes have been reviewed recently (Ho & White 1999; Kyes *et al.* 2001), our purpose here is to relate this knowledge to the question of parasite fitness.

(a) *Cytoadherence*

Cytoadherence refers to a group of related phenomena in which parasite-infected cells adhere to other host cells during the last half of the parasite's replication cycle: these host cells include endothelial cells in the post-capillary venules ('sequestering'), uninfected red blood cells ('rosetting'), infected red blood cells ('autoagglutination' or 'clumping') and leucocytes. In isolation or by interacting (Handunetti *et al.* 1992; Ho & White 1999), and perhaps exacerbated by reduced red cell deformability (Dondorp *et al.* 1997), these cytoadherence phenotypes lead to a slowing and obstruction of blood flow in the post-capillary venules (MacPherson *et al.* 1985; Raventos-Suarez *et al.* 1985; Kaul *et al.* 1991, 1994; Nash *et al.* 1992) and lead to hypoxia and nitric oxide-related pathology (Clark & Rockett 1996; Dondorp *et al.* 1998). Together, these can lead to coma, organ failure and death. Many studies, both *in vitro* and *in vivo*, have shown associations between binding ability to at least some host cell receptors (principally CD36, ICAM-1 and CR1) and the occurrence of the most severe forms of disease (MacPherson *et al.* 1985; Carlson *et al.* 1990; Ho *et al.* 1991; Pongponratn *et al.* 1991; Treutiger *et al.* 1992; Ringwald *et al.* 1993; Rowe *et al.* 1995; Newbold *et al.* 1997, 1999; Kun *et al.* 1998; Roberts *et al.* 2000; Pain *et al.* 2001), whereas a few studies have found no such
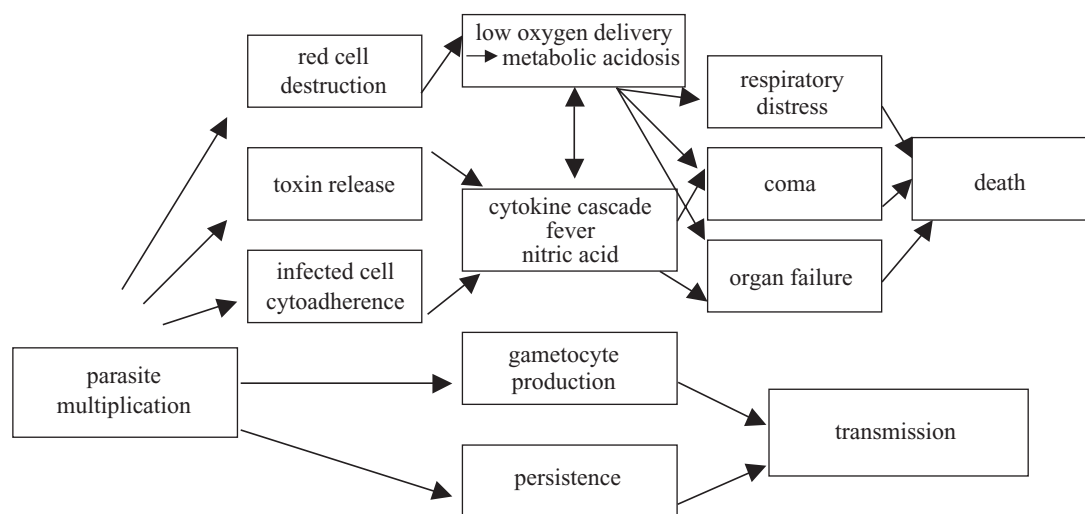
Figure 7. A model to explain why malaria parasites cause severe disease, which incorporates the evolutionary point of view. Adapted from Marsh & Snow (1997) with permission.

relationship (Ho *et al.* 1991; al-Yaman *et al.* 1995; Angkasekwinai *et al.* 1998; Rogerson *et al.* 1999) or a reverse one (Rogerson *et al.* 1999).

However, there is a lot to understand about how exactly cytoadherence leads to severe pathology (Berendt *et al.* 1994; Clark & Rockett 1994; Clark & Schofield 2000; Cooke *et al.* 2000). Moreover, and particularly important for the discussion here, these severe forms of disease are rare, and associations between cytoadherence and disease severity among most malaria patients who experience relatively mild, non-life-threatening forms of the disease have not been found. Thus, given the fitness cost to the parasite of host death (immediate cessation of transmission), it is difficult to explain why cytoadherence is so common in nature. Rosetting has been found in all *Plasmodium* species studied (Wahlgren 1986; David *et al.* 1988; Handunetti *et al.* 1989; Udomsangpetch *et al.* 1991, 1995; Angus *et al.* 1996) and varies between parasite strains within species (Wahlgren *et al.* 1990; Reeder *et al.* 1994; al-Yaman *et al.* 1995; Rowe *et al.* 1995; Mackinnon *et al.* 2002*b*). Sequestering does not occur in all species, appears to be a property of all parasite strains within species (although quantitative data are lacking on this point), and varies both between species (Alger 1963; Garnham 1966; Miller 1969; Desowitz *et al.* 1969; Yoeli & Hargreaves 1974; Cox *et al.* 1987; David *et al.* 1988; Gilks *et al.* 1990; Mota *et al.* 2000) and within species (reviewed in Newbold *et al.* 1997, 1999) in the degree and sites of sequestration. Autoagglutination has only been looked for and found in *P. falciparum* (Roberts *et al.* 1992) and *P. knowlesi* (Knisely *et al.* 1941) and has been shown to vary between isolates in *P. falciparum* (Roberts *et al.* 1992; Pain *et al.* 2001). The ubiquity of cytoadherence in the *Plasmodium* genus suggests that there is a beneficial as well as costly role of cytoadherence in parasite fitness. Alternatives to this adaptive explanation of cytoadherence are that cytoadherence-related death is just a rare aberration arising from excesses of within-host, short-sighted evolution (Levin & Bull 1994), or is some marker or by-product of another disease-related process (Newbold *et al.* 1997; Clough *et al.* 1998*a*) such as tumour necrosis factor production (Allan *et al.* 1993; Ringwald *et al.* 1993) or fever

(Udomsangpetch *et al.* 2002) or malaria infection-induced structural changes to infected and uninfected red blood cells (Dondorp *et al.* 1997; Chotivanich *et al.* 1998). If cytoadherence is an epiphenomenon of some other disease-causing process, we would expect our evolutionary analysis above to apply to that other process.

If cytoadherence does confer a fitness benefit, then how does it work? The most plausible argument is that it promotes asexual parasite population growth and hence production of gametocytes. Cytoadherence has been mooted to be a growth factor for two different, not mutually exclusive, reasons. Either it could increase the parasite's 'birth rate', or decrease the parasite's 'death rate', both of which have an impact on parasite population density. Hypotheses that have been proposed about how cytoadherence increases birth rates are as follows: (i) rosetting and/or sequestering increases the contact between uninfected cells and newly released merozoites, thus promoting invasion efficiency (Handunetti *et al.* 1989; Wahlgren *et al.* 1989); (ii) rosetting attracts the bursting schizont young cells that are more favourable for parasite growth (Clough *et al.* 1998*a*), or that have receptors suitable for merozoite invasion; (iii) sequestering provides a more favourable environment for parasite maturation (Ho & White 1999); and (iv) rosetting and/or sequestering promotes synchrony which in turn maximizes the number of viable merozoites per infected cell. The first two hypotheses have been directly tested only once by using an *in vitro* system and were rejected (Clough *et al.* 1998*a*). However, one study has shown an association between rosetting capacity *in vitro* and the asexual parasite density of the host at the time of blood donation (Rowe *et al.* 2002). Further indirect evidence in support of the birth-rate hypotheses comes from field data on severe disease: these are the positive link in *P. falciparum* between severe malaria and parasite multiplication potential measured *in vitro* (Chotivanich *et al.* 2000), and the correlation between cytoadherence *in vitro* and the incidence of severe anaemia (Newbold *et al.* 1997) and therefore perhaps asexual multiplication rate. Laboratory studies that also support the birth-rate argument are the higher growth rate and higher sequestration in a highly virulent mutant clone

of *P. yoelii* compared with avirulent clones (Yoeli & Hargreaves 1974; Yoeli *et al.* 1975), and the lower acute phase growth rate and virulence in a line of *P. falciparum* that had lost the ability to sequester (Langreth & Peterson 1985). However, in another study of a non-sequestering line in *P. chabaudi*, the rate of acute phase growth was similar to that of sequestering lines (Gilks *et al.* 1990).

Arguments for how cytoadherence reduces parasite death rates revolve around immune-clearance mechanisms. Specific hypotheses that have been proposed include the following: (i) parasites sequester to avoid being circulated through the spleen where immune-mediated killing of infected cells takes place (Barnwell *et al.* 1983*a*,*b*); (ii) rosetting protects infected cells from opsonization and phagocytosis and hence immune clearance, or from antibody interference with recognition of red blood cell ligands; (iii) immediately after schizont rupture, rosetting protects the body of merozoites from being coated with antibodies that either inhibit reinvasion (reviewed in Anders & Brown 1990) or cause multiple merozoites to invade the same cell (Lyon *et al.* 1986; Ramasamy *et al.* 1999), thereby avoiding a possible compromise to parasite viability; and (iv) binding of parasitized red blood cells to dendritic cells and macrophages protects the parasite from immune clearance by impairing their immune function (reviewed in Urban & Roberts 2002). Only the first of these hypotheses is supported by experimental evidence, and this is largely indirect. The main source of evidence is the observation from animal models that the parasite's expression of cytoadherence and/or associated variant antigens is downregulated in splenectomized animals (see Hommel *et al.* 1983; David *et al.* 1983; Barnwell *et al.* 1983*a*,*b*; Langreth & Peterson 1985; Handunetti *et al.* 1987; Gilks *et al.* 1990). This suggests that in the absence of the spleen, expression of these proteins on the surface of the red blood cell is costly to the parasite, but is maintained in spleen-intact animals because of the fitness advantage that it incurs. However, evidence on the consequences to parasite multiplication rate of the loss of these phenotypes is confusing. In one study in *P. falciparum*, there was a reduction in acute phase growth rate in a parasite line that had lost cytoadherence (Langreth & Peterson 1985), whereas another study in *P. knowlesi* showed a loss of acute phase growth rate and virulence when surface antigen expression was lost (Barnwell *et al.* 1983*a*). However, in a study on *P. chabaudi*, simultaneous loss of both cytoadherence and surface antigen expression had no effect on acute phase growth rate, but did show loss of ability to maintain a chronic infection (Gilks *et al.* 1990). Furthermore, in the *P. chabaudi* study, strain-specific immune protection during the acute growth phase required the presence of a spleen (Gilks *et al.* 1990). The transmission consequences of losing cytoadherence or variant antigen expression have never been quantified. The other death-rate hypotheses have yet to be tested.

Finally, cytoadherence may relate to parasite fitness directly through its effects on transmission stage densities, instead of indirectly through asexual parasite growth rate. It is known that in *P. falciparum*, immature gametocytes sequester (Thomson & Robertson 1935; Smalley *et al.* 1980). Molecular data indicate that gametocyte sequestration is mediated through PfEMP-1 (Rogers *et al.* 1996; Day *et al.* 1998; Hayward *et al.* 1999) but *in vivo* studies have yet to confirm this. If so, PfEMP-1-mediated cytoadherence of gametocyte-infected cells may promote gametocyte survival by allowing them to sequester and hide from the immune system and hence increase parasite fitness.

(b) *Antigenic variation*

The immune-based (death rate) hypotheses for how cytoadherence might promote fitness are attractive because the same protein that mediates all three cytoadherence phenotypes in *P. falciparum* (Baruch *et al.* 1995; Gardner *et al.* 1996; Rowe *et al.* 1997; Chen *et al.* 1998)—known as PfEMP-1 (Leech *et al.* 1984)—also expresses antigens on the surface of the infected red cell. These antigens are highly variable both between parasite clones and within clones owing to an antigenic switching mechanism. This variability is afforded by the multiple copies in the genome of their coding gene, denoted *var* (Baruch *et al.* 1995; Smith *et al.* 1995; Su *et al.* 1995). These antigens are recognized by the immune system in a highly variant-specific way (Marsh & Howard 1986; Forsyth *et al.* 1989; Newbold *et al.* 1992; Iqbal *et al.* 1993; Reeder *et al.* 1994; Giha *et al.* 1998; Bull *et al.* 1999; Giha *et al.* 1999; Nielsen *et al.* 2002; Ofori *et al.* 2002) although there appears to be some cross-reactivity between variants (Aguiar *et al.* 1992; Chattopadhyay *et al.* 2003). Low titres (Marsh *et al.* 1989) and lack of recognition by antibody to the variant expressed by the infecting type has been associated with disease severity in field studies (Bull *et al.* 1998, 1999, 2000; Giha *et al.* 2000; Nielsen *et al.* 2002).

A possible mechanism by which variant-specific antibodies provide protection is through disrupting the cytoadherence properties of the PfEMP-1 molecule. Evidence in support of this hypothesis is the strain-specific blocking of binding to endothelial cells *in vitro* (Udeinya *et al.* 1983) and *in vivo* (David *et al.* 1983, 1988; Mota *et al.* 2000). Further, antibody-mediated abrogation of rosetting has been observed many times (David *et al.* 1988; Carlson *et al.* 1990; Wahlgren *et al.* 1990; Treutiger *et al.* 1992; Tourneur *et al.* 1992; Helmby *et al.* 1993; Rogerson *et al.* 1996; Barragan *et al.* 1998; Kun *et al.* 1998; but see al-Yaman *et al.* 1995; Rowe *et al.* 1995; Clough *et al.* 1998*b*) though this has not always been associated with severe disease. Also there may be other parasite molecules besides PfEMP-1 that are involved in cytoadherence (Chaiyaroj *et al.* 1994; Crabb *et al.* 1997; Ho & White 1999; Pouvelle *et al.* 2000; Trenholme *et al.* 2000), as well as other variable antigens expressed on the surface of the infected cell in *P. falciparum* (Fernandez *et al.* 1999; Kyes *et al.* 1999), some of which are known to be recognized by the immune system (Abdel-Latif *et al.* 2002). It is therefore not clear whether immune protection is a direct consequence of blocking cytoadherence, nor whether this is mediated through antibody recognition of PfEMP-1 epitopes. Associations between disease and the expression of specific PfEMP-1 ligands and the presence of antibodies to them have been found in only a few of the extensive field surveys (Ho *et al.* 1991; Ockenhouse *et al.* 1991; Newbold *et al.* 1997; Fried *et al.* 1998; Ricke *et al.* 2000). Moreover, although associations have been found between the incidence of disease and host genetic polymorphisms in cell receptors known to bind malaria parasites through PfEMP-1, these have been in the

unexpected direction, i.e. the more common variants in malaria-endemic areas are associated with severe malaria (Fernandez-Reyes *et al.* 1997; Aitman *et al.* 2000). The relationship between PfEMP-1, cytoadherence-related disease and strain-specific immunity is clearly complex.

Interestingly, antigenic types associated with severe disease tend to be more commonly recognized by the population at large than types from patients with mild disease (Bull *et al.* 1999, 2000; Nielsen *et al.* 2002). Antigenic types from older children are also less commonly recognized than types from younger children (Bull *et al.* 2000; Nielsen *et al.* 2002). These observations strongly suggest that immune protection is obtained by acquiring cumulative exposure to the diverse antigenic types of PfEMP-1 circulating in the parasite population, with disease resulting from infection with antigenic types to which the host has not been previously exposed (reviewed in Bull & Marsh 2002). We further suggest here that the association between common types and disease might indicate a higher fitness (mediated by cytoadherence?), and hence higher population frequency, of the more virulent types.

Cytoadherence aside, there is strong evidence that antigenic variation *per se* allows the parasite to persist in the face of immunity (reviewed in Brown *et al.* 1986; Phillips *et al.* 1997). By expressing a novel antigen, the immune system is unable to recognize and destroy the parasite. This advantage is most obvious after the acute phase during chronic infection. Although variant antigens are present early in the infection (McLean *et al.* 1990; Brannan *et al.* 1994; Peters *et al.* 2002), the advantage to acute phase multiplication appears to be zero or small (Voller & Rossan 1969; Brown 1973; Barnwell *et al.* 1983*a*; Gilks *et al.* 1990). Like cytoadherence, perhaps the most compelling evidence that antigenic variation confers a fitness advantage is its prevalence throughout the *Plasmodium* genus. Evidence for antigenic variation exists in all species of parasite studied (*P. berghei* (Cox 1962); *P. knowlesi* (Brown & Brown 1965); *P. cynomolgi* (Voller & Rossan 1969); *P. falciparum* (Hommel *et al.* 1983; Biggs *et al.* 1991; Roberts *et al.* 1992); *P. chabaudi* (McLean *et al.* 1982); *P. fragile* (Handunetti *et al.* 1987); and probably *P. vivax* (Mendis *et al.* 1988; del Portillo *et al.* 2001)). Different sets of genes are responsible in different species (Howard & Barnwell 1985; al-Khedery *et al.* 1999; del Portillo *et al.* 2001; Janssen *et al.* 2001, 2002), suggesting convergent evolution for this phenotype. Interestingly, like *P. falciparum*, the molecule responsible for both cytoadherence and antigenic variation in *P. chabaudi* appears to be one and the same, as loss of one of these phenotypes is accompanied by loss of the other (Gilks *et al.* 1990). Although a surface antigen of the approximate size of PfEMP-1 has been identified in *P. chabaudi* schizonts (Newbold *et al.* 1984), searches of the *P. chabaudi* genome for *var*-like genes have revealed no such homologues (Janssen *et al.* 2001). Instead, another multigene family, putatively coding for variant surface antigens has been identified in *P. chabaudi*, with homologues in *P. vivax* (del Portillo *et al.* 2001), *P. yoelii* and *P. berghei* (Janssen *et al.* 2001, 2002). If these new putative antigenic variation genes do turn out to also code for cytoadherence, this would suggest convergent evolution for joint control of cytoadherence and antigenic variation, thus strengthening the argument that these phenotypes promote parasite fitness.

(c) *Red cell invasion and selectivity*

The other prime molecular candidates for virulence and fitness promoter are the proteins on the merozoite that mediate red cell invasion. Variation in red cell invasion rates may be attributable to differences in the efficiency of red cell invasion by merozoites (invasion efficiency), or to variation in the proportion of red cells that are invadable (selectivity). There are two forms of evidence in relation to the importance of selectivity in invasion rates: one in species with very high red cell selectivity, and the other in species with low selectivity. In *P. yoelii*, which has a strong predilection for reticulocytes, a mutation that allows parasites to also infect mature red cells leads to very rapid multiplication rate and hypervirulence (Yoeli & Hargreaves 1974; Yoeli *et al.* 1975). This has been linked to invasion proteins located in the apex of the merozoite (Freeman *et al.* 1980; Holder & Freeman 1981; Ogun & Holder 1996; Preiser & Jarra 1998). In *P. falciparum*, which invades both immature and mature red cells, strains with lower selectivity (as indicated by a lower proportion of cells infected with more than one parasite) were found to have higher virulence *in vivo* and growth rate *in vitro* (Simpson *et al.* 1999; Chotivanich *et al.* 2000). This link between cell selectivity and virulence is further corroborated by evidence that parasites infecting hosts with a genetically defective form of haemoglobin—haemoglobin E— have high red blood cell selectivity (Chotivanich *et al.* 2002) and are protected against severe malaria (Hutagalung *et al.* 1999). Across-species comparisons, at least between *P. falciparum* and *P. vivax*, also support a link between higher cell selectivity and lower virulence (e.g. Miller *et al.* 2002) but there are multiple possible explanations for these species differences (e.g. see Clark & Cowden 1999). The lower selectivity of *P. falciparum* may be due to its ability to use several alternative invasion pathways (Mitchell *et al.* 1986; Hadley *et al.* 1987; Perkins & Holt 1988; Soubes *et al.* 1997; Okoyeh *et al.* 1999) compared with the unique receptor (the Duffy blood group antigen) used by *P. vivax* (Miller & Carter 1976). However, as for cytoadherence, the effects of parasite ligand diversity and host cell receptor diversity in invasion success are still very unclear. It is also unclear as to how much of the variation in invasion rates is attributable to red cell selectivity compared with invasion efficiency, and therefore how much ligand diversity matters. As for cytoadherence, there is evidence for phenotypic switching mechanisms in invasion pathways (Dolan *et al.* 1990; Soubes *et al.* 1997; Preiser *et al.* 1999) and, once invaded, parasites can rapidly adapt to achieve normal maturation in host cells that normally prevent this (Luzzatto *et al.* 1983). Thus there is much complexity in red cell invasion rates yet to be unravelled. The transmission consequences of altered red cell selectivity have not been documented. Thus there is little doubt that invasion success is an important component of parasite survival, but the key properties and their consequences to asexual and gametocyte densities are not yet known.

8. FUTURE CHALLENGES

Within the context of the evolutionary framework summarized above, we see five areas for further theoretical and empirical work.

(a) The fitness advantages of cytoadherence, antigenic variation and red cell invasion rates

As seen above, there are strong, but mostly indirect, arguments that cytoadherence, surface antigens and red cell invasion properties link virulence with asexual multiplication. However, there is little direct evidence so far that these properties do, in fact, promote parasite transmission and fitness. The *P. chabaudi* model is the ideal system in which to test the birth-rate and death-rate hypotheses outlined above on how these mechanisms might promote replication or transmission. The replication pattern and fitness of parasite lines that vary in rosetting and other cytoadherence, cell selectivity or immune evasion characteristics can be compared, both with and without immune manipulation. In addition, whole genome and proteome screens will soon be able to be performed on parasite material from isogenic lines of *P. chabaudi* selected for different virulence properties in order to identify relevant molecules.

(b) Mechanisms of multiplication early and late in the infection

In the above, we used maximum or average asexual parasitaemia as measures of parasite multiplication and showed them to correlate to virulence, transmission and persistence. Implicit in our argument is the assumption that what maintains population growth during the early stage of the infection is the same or correlated to that which maintains growth later in the infection. Although our studies in *P. chabaudi* (figure 4) and others in *P. falciparum* (Gravenor *et al.* 1995; Simpson *et al.* 1999) also show that early growth rate and maximum parasitaemia are correlated, it is likely that different growth-determining mechanisms operate more strongly at different stages of the infection. What is needed now is a dissection of the processes that lead to successful multiplication at different times in the infection. In addition to cytoadherence, other possible growth mechanisms, such as the number of merozoites per schizont, degree of synchrony, cycle length and merozoite invasion success, need to be studied. Such information may provide new targets for therapy.

(c) The fitness advantages of virulence *per se*

The view we have espoused is the conventional view in the contemporary evolution of virulence literature: virulence is an unavoidable side-effect of fitness-enhancing traits. It is, however, possible that virulence itself brings direct transmission advantages. For instance, some authors have advocated the view that *Plasmodium* virulence is maintained by natural selection because it directly enhances parasite fitness because sick hosts have reduced anti-vector behaviour (e.g. Day & Edman 1983; Ewald 1994) or attract more mosquitoes to feed when hosts have high gametocyte densities (Ferguson *et al.* 2003*a*). This idea that sickness *per se* enhances transmission is amenable to direct testing with experimental model systems like *P. chabaudi*. Although we do not doubt that extremely sick hosts will be less likely to prevent vectors feeding, the key issue is the extent to which transmission is enhanced by such behavioural changes, and this in relation to the growth-related advantages we have described above. It is our experience with *P. chabaudi* that gametocyte densities are at their lowest at the point in the infection when mice

appear behaviourally unresponsive to stimuli. And in the context of *P. falciparum*, given the extremely low gametocyte densities at which patients infect mosquitoes (Boyd *et al.* 1935; Robertson 1945; Muirhead-Thomson & Mercier 1952; Muirhead-Thomson 1954; Rutledge & Gould 1969) and the long asymptomatic periods of infectivity to mosquitoes (Collins & Jeffery 2003) compared with the short period of sickness, it seems unlikely to us that any changes in host behaviour or attractiveness during the period of acute disease would have a large impact on malaria transmission. Nevertheless, this requires quantitative analysis.

(d) The selective consequences of mixed infections

Mixed genotype infections are the rule in *P. falciparum* populations (Day *et al.* 1992). This can add an extra layer of complexity to virulence evolution: within-host selection between competing genotypes. This complexity has been of substantial interest among theoreticians (reviewed by Read & Taylor 2001) with the most common conclusion being that in-host selection will favour the evolution of more virulent parasites. This is because optimal rates of virulence are altered where competition occurs. In the simplest cases, competing strains reduce the duration of infection for a clone (for instance, by killing the host or prompting a more potent immune response). Thus more virulent clones are favoured by selection because the fitness cost of self-induced host death is reduced: there is no point in saving the golden goose if a competitor will kill it anyway. However, it is now being realized that this theoretical conclusion depends very much on the biological details of the competition (Chao *et al.* 2000; Brown *et al.* 2002). In mixed infections, selection might favour direct chemical attack between combatants, with the result that hosts are less damaged by parasite populations that detrimentally affect each other. Several interventions are likely to reduce the number of competing clones in infections (e.g. bednets) and so it would be highly desirable to understand the fitness consequences of competition in malaria. For instance, transmission-blocking and infection-blocking vaccines could work to reduce virulence if competition does reduce parasite transmission (Gandon *et al.* 2001). Competition certainly occurs in rodent models of malaria (reviewed in Read & Taylor 2001; see also de Roode *et al.* 2003), and probably in *P. falciparum* (Bruce *et al.* 2000). However, the transmission and virulence consequences of this competition are unclear (Read & Taylor 2001; Read *et al.* 2002). In some cases, competitively suppressed clones transmitted as well or even better than they would have done from single clone infections; in other cases, clonal transmission was almost certainly reduced (Read *et al.* 2003). In *P. chabaudi*, genetically diverse infections are more virulent (Taylor *et al.* 1998; Read *et al.* 2003), but in *P. falciparum*, virulence can be increased, decreased or unaltered by diversity (reviewed in Read & Taylor 2001). Substantially more work is required to elucidate any generalities for virulence in mixed-clone infections.

In addition to the question of how same-species mixed infections affect virulence evolution, there is also the question of how mixed infections with other pathogenic organisms affect virulence. Interactions between malaria virulence and other organisms such as bacteria and HIV

can have profound influences on mortality, morbidity and infection rates (Berkley *et al.* 1999; Holmes *et al.* 2003) and will therefore affect the optimal level of malaria parasite virulence that evolves. So far, these indirect effects from other diseases have been ignored in theoretical models of virulence evolution and disease epidemiology, and relevant data for building such models are very scarce.

(e) *Limits to virulence*

Most models of virulence evolution have assumed that host death is the constraint that sets the upper limit to virulence. In the *P. chabaudi* system, we rarely (by intention) encountered host mortality because most of our experiments were performed in the most resistant mouse genotype available, the C57Bl/6J. Thus we did not measure virulence directly, but instead measured morbidity (weight loss and anaemia). Although this correlates to the probability of mortality (Timms *et al.* 2001; Mackinnon *et al.* 2002a; Ferguson *et al.* 2003b), we do not yet know whether mortality is the primary source of back-selection on virulence. In human malaria, case mortality rarely reaches 1%, but this seems to be sufficient to impose at least some non-negligible cost to lifetime transmission (figure 6). Note that it is not the absolute mortality rates that determine the upper limit to virulence. What matters is the marginal cost versus the marginal gain to lifetime transmission of rising virulence: even if these are slight, the trade-off theory still applies. Nevertheless, there may be sources of selection for lower virulence other than mortality in the vertebrate host. These include reduced infectivity to mosquitoes as a result of a poor-quality blood meal or mortality-driven selection by the mosquito against heavy oocyst loads and hence gametocyte production and virulence in the vertebrate host (Ferguson & Read 2002). However, so far our data do not provide evidence that virulence in the vertebrate host has an impact on virulence in the vector (Ferguson *et al.* 2003b). In addition, it is possible that the mosquito may play a role in limiting the spread of a virulent (or any) mutant through population bottlenecking (Bergstrom *et al.* 1999; Elena *et al.* 2001; Wahl *et al.* 2002) or through resetting of gene expression during meiosis. Studies on the maintenance of virulence phenotypes through population bottlenecks and mosquitoes so far suggest that the vector is not an important constraint on virulence evolution in malaria (M. J. Mackinnon and A. F. Read, unpublished data).

(f) *Application to other human malaria species*

Most of the data on malaria virulence pertain to *P. falciparum* because this is the species that causes most damage. However, *P. vivax* also contributes significantly to the global burden of morbidity and may well supplant *P. falciparum* if control of the latter is successful (Mendis *et al.* 2001). An understanding of the forces driving *P. vivax* virulence and transmission, as well as its ecological interactions with *P. falciparum*, are therefore required, but at this stage, few relevant data are available. We see no reason why the same sort of virulence–transmissibility relationships as in *P. falciparum* should not also apply to *P. vivax*. The fact that mortality due to *P. vivax* is very low does not preclude such relationships: it is the marginal costs and benefits of virulence that dictate the level of virulence that evolves, not their absolute values. One possible

reason why *P. vivax* maintains lower virulence than *P. falciparum* is that it can generate life-long relapsing infections through reactivation of parasite forms that survive very long periods in the liver or other tissues. Evolutionary theory predicts that the longer the average length of infection, the lower that virulence will evolve (Van Baalen & Sabelis 1995; Day 2003). Interestingly, the other less virulent human-infecting species, *P. ovale* and *P. malariae*, can also generate life-long infections (Garnham 1966). Nevertheless, there are alternative explanations for why these other species are so much less virulent than *P. falciparum*, and investigation of why this is the case may yield further leads into malaria virulence factors.

9. CONCLUSION

The laboratory and field data are overwhelmingly in favour of the hypothesis that virulence in malaria is an unavoidable consequence of natural selection maintaining the transmission-related advantages of high asexual multiplication. This broad-brush, whole-organism view fits well with the current molecular and clinical data on the mechanistic causes of virulence, but a full understanding of how natural selection is acting on specific virulence mechanisms is not yet available. A reductionist approach to biology emphasizes complexity over generality. Although we do not underestimate the obvious complexity of the mechanisms underpinning malaria pathogenesis, we emphasize that what matters to virulence evolution are the fitness consequences of virulence variation at the level of the whole parasite, and their impact on the whole parasite population. Irrespective of the molecular basis, evolutionary analysis of virulence brings us to the conclusion that particular attention should be paid to the transmission consequences of interventions such as vaccines and drugs, as transmission success will ultimately determine how the parasite population will respond to these measures.

ENDNOTES

[1]Data came from figs 20, 22, 30, 31, 35 and 65–71 of the Garki Project Report (Molineaux & Gramiccia 1980).

[2]Asexual parasitaemia and gametocytaemia were calculated only from people positive for *Plasmodium falciparum* and thus do not directly reflect parasite prevalence.

[3]Mortality in (c) is the malaria-induced mortality rate among infected people. This was estimated from the all-cause mortality rates (M) from the Garki report by using the age-specific values of mortality rate due to non-malaria causes (δ) as 100, 80, 5, 5, 5, 10 and 31 for age-classes younger than 1, 1–4, 5–8, 9–18, 29–43 and older than 43 years old, respectively, and then estimating the malaria-induced mortality rate α as $\alpha = (M - \delta)/Y$, where Y is the proportion of parasite positive people in the age-class in question (values of 0.6, 0.90, 0.92, 0.76, 0.38, 0.32 and 0.3 for the increasing age-classes). These values of δ and α correspond to malaria-attributable fractions of all deaths of 59% in younger than 1-year-olds, 48% in 1–4-year-olds and 39% in the remaining population. Note that the higher values of malaria-induced mortality in infants is consistent with the higher frequency of the *HbS* allele among infants than older children and adults (Molineaux & Gramiccia 1980). The *HbS* allele confers sickle cell anaemia in the homozygous state but has a malaria-protective effect.

[4]Clearance time for each new infection is the inverse of clearance rate for each new infection, r. This was estimated from the formula $r = h/\ln(h/R + 1)$ to allow for superinfection (Dietz *et al.* 1980), where R is the actual clearance rate based on the rate at which people converted from positive to negative from one survey to the next (i.e. cleared all infections), and h is the 'conversion rate' from negative to positive estimated from the longitudinal survey data. Note that the values of R used here were those given as $\hat{r}$ in the Garki report, as obtained by the method of Bekessy *et al.* (1976). As these were based on being slide-positive versus negative for asexual parasites, these were probably overestimates of the true value of R and hence r.

[5]Data were taken from table 3*b* in Tchuinkam *et al.* (1993) where 86 experimental mosquito feeds were performed on gametocyte carriers. Each point represents the mean of groups classified according to gametocyte density. Data do not include feeds in which zero mosquitoes became infected.

[6]Fitness was calculated as $W = \beta/(\delta + \alpha + \chi)$, where β is the log average gametocyte density, δ is the background (non-malaria) mortality rate, α is the malaria-induced mortality rate and χ is the rate of recovery from the infection. This expression is analogous to the R_0 of the parasite typically used to represent parasite fitness, but is not equivalent because it does not include the effects of mosquito density and biting rate. Gametocyte density was log-transformed because infectivity to mosquitoes linearly increases with log gametocyte density (figure 5*b*). Here, we used clearance rate from each individual infection ($\chi = r$) to allow for superinfection (see figure 5*e* and endnote 4) as we are interested in the fitness of a parasite per new infection. α was not similarly adjusted but its small value relative to χ meant that adjustment would have little influence on W. Relative fitness was calculated as W divided by the population weighted average fitness where the weights were the proportion of people in each age class.

[7]The near-equal fitness of age-classes at the upper end of the spectrum arises because of the balance between higher gametocyte densities and lower clearance rates in the youngest age-class against their higher mortality rate as compared with older children. Thus the nearly flat fitness function at this end of the spectrum provides support for the virulence trade-off proposed by evolutionary theory. Note that small alterations in values for malaria-induced mortality rate and clearance rate (both of which are estimated with considerable uncertainty from these data) can render the fitness in younger than 1-year-olds slightly less than that in 1–4-year-olds.

[8]This was calculated as relative parasite fitness (as shown in figure 6(*a*)) multiplied by the proportion of the population in each age-class.

REFERENCES

Abdel-Latif, M. S., Khattab, A., Lindenthal, C., Kremsner, P. G. & Klinkert, M. Q. 2002 Recognition of variant *rifin* antigens by human antibodies induced during natural *Plasmodium falciparum* infections. *Infect. Immun.* **70**, 7013–7021.

Aguiar, J. C. (and 12 others) 1992 Agglutination of *Plasmodium falciparum*-infected erythrocytes from east and west African isolates by human sera from distinct geographic regions. *Am. J. Trop. Med. Hyg.* **47**, 621–632.

Aitman, T. J. (and 12 others) 2000 Population genetics: malaria susceptibility and CD36 mutation. *Nature* **405**, 1015–1016.

Alger, N. E. 1963 Distribution of schizonts of *Plasmodium berghei* in tissues of rats, mice and hamsters. *J. Protozool.* **10**, 6–10.

al-Khedery, B., Barnwell, J. W. & Galinski, M. R. 1999 Antigenic variation in malaria: a 3′ genomic alteration associated with the expression of a *P. knowlesi* variant antigen. *Mol. Cell* **3**, 131–141.

Allan, R. J., Rowe, J. A. & Kwiatkowski, D. 1993 *Plasmodium falciparum* varies in its ability to induce tumor necrosis factor. *Infect. Immun.* **61**, 4772–4776.

Alles, H. K., Mendis, K. N. & Carter, R. 1998 Malaria mortality rates in South Asia and in Africa: implications for malaria control. *Parasitol. Today* **14**, 369–375.

al-Yaman, F., Genton, B., Mokela, D., Raiko, A., Kati, S., Rogerson, S. J., Reeder, J. & Alpers, M. P. 1995 Human cerebral malaria: lack of significant association between erythrocyte rosetting and disease severity. *Trans. R. Soc. Trop. Med. Hyg.* **89**, 55–58.

Anders, R. F. & Brown, G. V. 1990 Vaccines against asexual blood stages of *Plasmodium falciparum*. In *New generation vaccines* (ed. G. C. Woodrow & M. M. Levine), pp. 491–512. New York: Marcel Dekker.

Angkasekwinai, P., Looareesuwan, S. & Chaiyaroj, S. C. 1998 Lack of significant association between rosette formation and parasitized erythrocyte adherence to purified CD36. *SE Asian J. Trop. Med. Public Health* **29**, 41–45.

Angus, B. J., Thanikkul, K., Silamut, K., White, N. J. & Udomsangpetch, R. 1996 Rosette formation in *Plasmodium ovale* infection. *Am. J. Trop. Med. Hyg.* **55**, 560–561.

Aron, J. L. & May, R. M. 1982 The population dynamics of malaria. In *Population dynamics of infectious diseases: theory and applications* (ed. R. M. Anderson), pp. 139–179. London: Chapman & Hall.

Baird, J. K. 1998 Age-dependent characteristics of protection v. susceptibility to *Plasmodium falciparum*. *Ann. Trop. Med. Parasitol.* **92**, 367–390.

Barnwell, J. W., Howard, R. J. & Miller, L. H. 1983*a* Influence of the spleen on the expression of surface antigens on parasitized erythrocytes. In *Ciba Foundation Symposium on Malaria and the Red Cell* (ed. D. Evered & J. Whelan), pp. 117–132. London: Pitman.

Barnwell, J. W., Howard, R. J., Coon, H. G. & Miller, L. H. 1983*b* Splenic requirement for antigenic variation and expression of the variant antigen on the erythrocyte membrane in cloned *Plasmodium knowlesi* malaria. *Infect. Immun.* **40**, 985–994.

Barragan, A., Kremsner, P. G., Weiss, W., Wahlgren, M. & Carlson, J. 1998 Age-related buildup of humoral immunity against epitopes for rosette formation and agglutination in African areas of malaria endemicity. *Infect. Immun.* **66**, 4783–4787.

Baruch, D. I., Pasloske, B. L., Singh, H. B., Bi, X., Ma, X. C., Feldman, M., Taraschi, T. F. & Howard, R. J. 1995 Cloning the *P. falciparum* gene encoding PfEMP1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. *Cell* **82**, 77–87.

Beale, G. H., Carter, R. & Walliker, D. 1978 Genetics. In *Rodent malaria* (ed. R. Killick-Kendrick & W. Peters), pp. 213–245. London: Academic.

Bekessy, A., Molineaux, L. & Storey, J. 1976 Estimation of incidence and recovery rates of *Plasmodium falciparum* parasitaemia from longitudinal data. *Bull. World Hlth Org.* **54**, 685–693.

Berendt, A. R. (and 10 others) 1994 Molecular mechanisms of sequestration in malaria. *Parasitology* **108**(Suppl. 1), S19–S28.

Bergstrom, C. T., McElhaney, P. & Real, L. A. 1999 Transmission bottlenecks as determinants of virulence in rapidly evolving pathogens. *Proc. Natl Acad. Sci. USA* **96**, 5095–5100.

Berkley, J., Mwarumba, S., Bramham, K., Lowe, B. S. & Marsh, K. 1999 Bacteraemia complicating severe malaria in children. *Trans. R. Soc. Trop. Med. Hyg.* **93**, 283–286.

Biggs, B.-A., Gooze, L., Wycherley, K., Wollish, W., Southwell, B., Leech, J. H. & Brown, G. V. 1991 Antigenic variation in *Plasmodium falciparum*. *Proc. Natl Acad. Sci. USA* **88**, 9171–9174.

Boyd, M. F. 1949 *Malariology*. London: W. B. Saunders.

Boyd, M. F., Stratman-Thomas, W. K. & Kitchen, S. F. 1935 On the relative susceptibility of *Anopheles quadrimaculatus* to *Plasmodium vivax* and *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* **15**, 485–493.

Brannan, L. R., Turner, C. M. R. & Phillips, R. S. 1994 Malaria parasites undergo antigenic variation at high rates *in vivo*. *Proc. R. Soc. Lond.* B **256**, 71–75.

Brown, K. N. 1973 Antibody induced variation in malaria parasites. *Nature* **242**, 49–50.

Brown, K. N. & Brown, I. N. 1965 Immunity to malaria: antigenic variation in chronic infections of *Plasmodium knowlesi*. *Nature* **208**, 1286–1288.

Brown, K. N., Berzins, K., Jarra, W. & Schetters, T. 1986 Immune responses to erythrocytic malaria. *Clin. Immunol. Allergy* **6**, 227–249.

Brown, S. P., Hochberg, M. E. & Grenfell, B. T. 2002 Does multiple infection select for raised virulence? *Trends Microbiol.* **10**, 401–405.

Bruce, M. C., Donnelly, C. A., Alpers, M. P., Galinski, M. R., Barnwell, J. W., Walliker, D. & Day, K. P. 2000 Cross-species interactions between malaria parasites in humans. *Science* **287**, 845–848.

Buckling, A. G. L. & Read, A. F. 2001 The effect of partial host immunity on the transmission of malaria parasites. *Proc. R. Soc. Lond.* B **268**, 2325–2330. (DOI 10.1098/rspb.2001.1808.)

Bull, J. J., Molineux, I. J. & Rice, W. R. 1991 Selection of benevolence in a host–parasite system. *Evolution* **45**, 875–882.

Bull, P. C. & Marsh, K. 2002 The role of antibodies to *Plasmodium falciparum*-infected-erythrocyte surface antigens in naturally acquired immunity to malaria. *Trends Microbiol.* **10**, 55–58.

Bull, P. C., Lowe, B. S., Kortok, M., Molyneux, C. S., Newbold, C. I. & Marsh, K. 1998 Parasite antigens on the infected red cell are targets for naturally acquired immunity to malaria. *Nature Med.* **4**, 358–360.

Bull, P. C., Lowe, B. S., Kortok, M. & Marsh, K. 1999 Antibody recognition of *Plasmodium falciparum* erythrocyte surface antigens in Kenya: evidence for rare and prevalent variants. *Infect. Immun.* **67**, 733–739.

Bull, P. C., Kortok, M., Kai, O., Ndungu, F., Ross, A., Lowe, B. S., Newbold, C. I. & Marsh, K. 2000 *Plasmodium falciparum*-infected erythrocytes: agglutination by diverse Kenyan plasma is associated with severe disease and young host age. *J. Infect. Dis.* **182**, 252–259.

Carlson, J., Helmby, H., Hill, A. V. S., Brewster, D., Greenwood, B. M. & Wahlgren, M. 1990 Human cerebral malaria: association with erythrocyte rosetting and lack of anti-rosetting antibodies. *Lancet* **336**, 1457–1460.

Carrescia, P. M. & Arcolea, G. 1957 Importanza della virulenza del ceppo di *Plasmodium berghei* nel determinare infezioni ad andamento rapido nei topi albini. *Riv. Malariol.* **36**, 65–72.

Carter, R. & Graves, P. M. 1988 Gametocytes. In *Principles and practice of malariology* (ed. W. H. Wernsdorfer & I. McGregor), pp. 253–305. Edinburgh: Churchill Livingstone.

Chaiyaroj, S. C., Coppel, R. L., Magowan, C. & Brown, G. V. 1994 A *Plasmodium falciparum* isolate with a chromosome 9 deletion expresses a trypsin-resistant cytoadherence molecule. *Mol. Biochem. Parasitol.* **67**, 21–30.

Chao, L., Hanley, K. A., Burch, C. L., Dahlberg, C. & Turner, P. E. 2000 Kin selection and parasite evolution: higher and lower virulence with hard and soft selection. *Q. Rev. Biol.* **75**, 261–275.

Chattopadhyay, R., Sharma, A., Srivastava, V. K., Pati, S. S., Sharma, S. K., Bhabani, S. D. & Chitnis, C. E. 2003 *Plasmodium falciparum* infection elicits both variant-specific and cross-reactive antibodies against variant surface antigens. *Infect. Immun.* **71**, 597–604.

Chen, Q. J., Fernandez, V., Sundstrom, A., Schlichtherle, M., Datta, S., Hagblom, P. & Wahlgren, M. 1998 Developmental selection of *var* gene expression in *Plasmodium falciparum*. *Nature* **394**, 392–395.

Chotivanich, K. T., Udomsangpetch, R., Pipitaporn, B., Angus, B. J., Suputtamongkol, Y., Pukrittayakamee, S. & White, N. J. 1998 Rosetting characteristics of uninfected erythrocytes from healthy individuals and malaria patients. *Ann. Trop. Med. Parasitol.* **92**, 45–56.

Chotivanich, K. T., Udomsangpetch, R., Simpson, J. A., Newton, P., Pukrittayakamee, S., Looareesuwan, S. & White, N. J. 2000 Parasite multiplication potential and the severity of *falciparum* malaria. *J. Infect. Dis.* **181**, 1206–1209.

Chotivanich, K., Udomsangpetch, R., Pattanapanyasat, K., Chierakul, W., Simpson, J. A., Looareesuwan, S. & White, N. J. 2002 Hemoglobin E: a balanced polymorphism protective against high parasitemias and thus severe *P. falciparum* malaria. *Blood* **100**, 1172–1176.

Clark, I. A. & Cowden, W. B. 1999 Why is the pathology of *falciparum* worse than that of *vivax* malaria? *Parasitol. Today* **15**, 458–461.

Clark, I. A. & Rockett, K. A. 1994 The cytokine theory of human malaria. *Parasitol. Today* **10**, 410–412.

Clark, I. A. & Rockett, K. A. 1996 Nitric oxide and parasitic disease. *Adv. Parasitol.* **37**, 1–56.

Clark, I. A. & Schofield, L. 2000 Pathogenesis of malaria. *Parasitol. Today* **16**, 451–454.

Clayton, D. A. & Tompkins, D. M. 1994 Ectoparasite virulence is linked to mode of transmission. *Proc. R. Soc. Lond.* B **56**, 211–217.

Clough, B., Atilola, F. A. & Pasvol, G. 1998*a* The role of rosetting in the multiplication of *Plasmodium falciparum*: rosette formation neither enhances nor targets parasite invasion into uninfected red cells. *Br. J. Haematol.* **100**, 99–104.

Clough, B., Atilola, F. A., Black, B. & Pasvol, G. 1998*b* *Plasmodium falciparum*: the importance of IgM in the rosetting of parasite-infected erythrocytes. *Exp. Parasitol.* **89**, 129–132.

Collins, W. E. & Jeffery, G. M. 1999 A retrospective examination of sporozoite- and trophozoite-induced infections with *Plasmodium falciparum*: development of parasitologic and clinical immunity during primary infection. *Am. J. Trop. Med. Hyg.* **61**(Suppl. 1), 4–19.

Collins, W. E. & Jeffery, G. M. 2002 A retrospective examination of sporozoite-induced and trophozoite-induced infections with *Plasmodium ovale*: development of parasitologic and clinical immunity during primary infection. *Am. J. Trop. Med. Hyg.* **66**, 492–502.

Collins, W. E. & Jeffery, G. M. 2003 A retrospective examination of mosquito infection on humans infected with *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* **68**, 366–371.

Cooke, B. M., Wahlgren, M. & Coppel, R. L. 2000 *Falciparum* malaria: sticking up, standing out and out-standing. *Parasitol. Today* **16**, 416–420.

Cox, H. W. 1962 The behavior of *Plasmodium berghei* strains isolated from relapsed infections of white mice. *J. Protozool.* **9**, 114–118.

Cox, J., Semoff, S. & Hommel, M. 1987 *Plasmodium chabaudi*: a rodent malaria model for *in vivo* and *in vitro* cytoadherence of malaria parasites in the absence of knobs. *Parasite Immunol.* **9**, 543–561.

Crabb, B. S., Cooke, B. M., Reeder, J. C., Waller, R. F., Caruana, S. R., Davern, K. M., Wickham, M. E., Brown, G. V., Coppel, R. L. & Cowman, A. F. 1997 Targeted gene disruption shows that knobs enable malaria-infected red cells to cytoadhere under physiological shear stress. *Cell* **89**, 287–296.

David, P. H., Hommel, M., Miller, L. H., Udeinya, I. J. & Oligino, L. D. 1983 Parasite sequestration in *Plasmodium falciparum* malaria: spleen and antibody modulation of cytoadherence of infected erythrocytes. *Proc. Natl Acad. Sci. USA* **80**, 5075–5079.

David, P. H., Hudson, D. E., Hadley, T. J., Klotz, F. W. & Miller, L. H. 1985 Immunization of monkeys with a 140 kildalton merozoite surface protein of *Plasmodium knowlesi* malaria: appearance of alternate forms of this protein. *J. Immunol.* **134**, 4146–4152.

David, P. H., Handunetti, S. M., Leech, J. H., Gamage, P. & Mendis, K. N. 1988 Rosetting: a new cytoadherence property of malaria-infected erythrocytes. *Am. J. Trop. Med. Hyg.* **38**, 289–297.

Day, T. 2003 Virulence evolution and the timing of disease life-history events. *Trends Ecol. Evol.* **18**, 113–118.

Day, J. F. & Edman, J. D. 1983 Malaria renders mice susceptible to mosquito feeding when gametocytes are most infective. *J. Parasitol.* **69**, 163–170.

Day, K. P. & Marsh, K. 1991 Naturally acquired-immunity to *Plasmodium falciparum*. *Immunoparasitol. Today* **1**, A68–A71.

Day, K. P., Koella, J. C., Nee, S., Gupta, S. & Read, A. F. 1992 Population genetics and dynamics of *Plasmodium falciparum*: an ecological view. *Parasitology* **104**(Suppl. 1), S35–S52.

Day, K. P., Karamalis, F., Thompson, J., Barnes, D., Brown, H., Brown, G. V. & Kemp, D. 1993 Virulence and transmissibility of *Plasmodium falciparum* map to chromosome 9. *Proc. Natl Acad. Sci. USA* **90**, 8292–8296.

Day, K. P., Hayward, R. E., Smith, D. & Culvenor, J. G. 1998 CD36-dependent adhesion and knob expression of the transmission stages of *Plasmodium falciparum* is stage-specific. *Mol. Biochem. Parasitol.* **93**, 167–177.

Dearsly, A. L., Sinden, R. E. & Self, I. A. 1990 Sexual development in malarial parasites: gametocyte production, fertility and infectivity to the mosquito vector. *Parasitology* **100**, 359–368.

del Portillo, H. A. (and 12 others) 2001 A superfamily of variant genes encoded in the subtelomeric region of *Plasmodium vivax*. *Nature* **410**, 839–842.

de Roode, J. C., Read, A. F., Chan, B. H. K. & Mackinnon, M. J. 2003 Infection dynamics and virulence of three-clone infections with the rodent malaria parasite *Plasmodium chabaudi*. *Parasitology* **127**, 411–418.

Desowitz, R. S., Miller, L. H., Buchanan, R. D. & Permpanicer, B. 1969 The sites of deep vascular schizogony in *P. coatneyi* malaria. *Trans. R. Soc. Trop. Med. Hyg.* **63**, 198–202.

Diebner, H. H., Eichner, M., Molineaux, L., Collins, W. E., Jeffery, G. M. & Dietz, K. 2000 Modelling the transition of asexual blood stages of *Plasmodium falciparum* to gametocytes. *J. Theor. Biol.* **202**, 113–127.

Dieckmann, U., Metz, J. A. J., Sabelis, M. W. & Sigmund, K. 2001 *Virulence management: the adaptive dynamics of pathogen–host interactions*. Cambridge University Press.

Dietz, K., Molineaux, L. & Thomas, A. 1980 The mathematical model of transmission. In *The Garki Project. Research on the epidemiology and control of malaria in the Sudan savanna of West Africa* (ed. L. Molineaux & G. Gramiccia), pp. 262–289. Geneva: World Health Organization.

Dolan, S. A., Miller, L. H. & Wellems, T. E. 1990 Evidence for a switching mechanism in the invasion of erythrocytes by *Plasmodium falciparum*. *J. Clin. Invest.* **86**, 618–624.

Dondorp, A. M., Angus, B. J., Hardeman, M. R., Chotivanich, K. T., Silamut, K., Ruangveerayuth, R., Kager, P. A., White, N. J. & Vreeken, J. 1997 Prognostic significance of reduced red cell deformability in severe *falciparum* malaria. *Am. J. Trop. Med. Hyg.* **57**, 507–511.

Dondorp, A. M. (and 11 others) 1998 Nitric oxides in plasma, urine, and cerebrospinal fluid in patients with severe *falciparum* malaria. *Am. J. Trop. Med. Hyg.* **59**, 497–502.

Ebert, D. 1994 Virulence and local adaptation of a horizontally transmitted parasite. *Science* **265**, 1084–1086.

Ebert, D. & Bull, J. J. 2003 Challenging the trade-off model for the evolution of virulence: is virulence management feasible? *Trends Microbiol.* **11**, 15–20.

Ebert, D. & Mangin, K. L. 1997 The influence of host demography on the evolution of virulence of a microsporidian gut parasite. *Evolution* **51**, 1828–1837.

Eichner, M., Diebner, H. H., Molineaux, L., Collins, W. E., Jeffery, G. M. & Dietz, K. 2001 Genesis, sequestration and survival of *Plasmodium falciparum* gametocytes: parameter estimates from fitting a model to malariatherapy data. *Trans. R. Soc. Trop. Med. Hyg.* **95**, 497–501.

Elena, S. F. 2001 Evolutionary history conditions the timing of transmission in vesicular stomatitis virus. *Infect. Genet. Evol.* **1**, 151–159.

Elena, S. F., Sanjuan, R., Borderia, A. V. & Turner, P. E. 2001 Transmission bottlenecks and the evolution of fitness in rapidly evolving RNA viruses. *Infect. Genet. Evol.* **1**, 41–48.

Ellerman, J. R., Hayman, R. W. & Holt, C. W. C. 1940 *The families and genera of living rodents with a list of named forms (1758–1936)*. London: British Museum.

Ewald, P. W. 1994 *Evolution of infectious disease*. Oxford University Press.

Fenner, F., Day, M. F. & Woodroofe, G. M. 1956 The epidemiological consequences of the mechanical transmission of myxomatosis by mosquitoes. *J. Hyg.* **54**, 284–303.

Ferguson, H. M. & Read, A. F. 2002 Why is the effect of malaria parasites on mosquito survival still unresolved? *Trends Parasitol.* **18**, 256–261.

Ferguson, H. M., Rivero, A. & Read, A. F. 2003*a* The influence of malaria parasite genetic diversity and anaemia on mosquito feeding and fecundity. *Parasitology* **127**, 9–19.

Ferguson, H. M., Mackinnon, M. J., Chan, B. H. K. & Read, A. F. 2003*b* Mosquito mortality and the evolution of malaria virulence. *Evolution* **57**, 2792–2804.

Fernandez, V., Hommel, M., Chen, Q. J., Hagblom, P. & Wahlgren, M. 1999 Small, clonally variant antigens expressed on the surface of the *Plasmodium falciparum*-infected erythrocyte are encoded by the *rif* gene family and are the target of human immune responses. *J. Exp. Med.* **190**, 1393–1403.

Fernandez-Reyes, D., Craig, A. G., Kyes, S. A., Peshu, N., Snow, R. W., Berendt, A. R., Marsh, K. & Newbold, C. I. 1997 A high frequency of Africans coding polymorphism in the N-terminal domain of ICAM-1 predisposing to cerebral malaria in Kenya. *Hum. Mol. Genet.* **6**, 1357–1360.

Field, J. W. & Niven, J. C. 1937 A note on prognosis in relation to parasite counts in acute subtertian malaria. *Trans. R. Soc. Trop. Med. Hyg.* **30**, 569–574.

Forsyth, K. P., Philip, G., Smith, T., Kum, E., Southwell, B. & Brown, G. V. 1989 Diversity of antigens expressed on the surface of erythrocytes infected with mature *Plasmodium falciparum* parasites in Papua New Guinea. *Am. J. Trop. Med. Hyg.* **41**, 259–265.

Frank, S. A. 1996 Models of parasite virulence. *Q. Rev. Biol.* **71**, 37–78.

Freeman, R. R., Trejdosiewicz, A. J. & Cross, G. A. 1980 Protective monoclonal antibodies recognising stage-specific merozoite antigens of a rodent malaria parasite. *Nature* **284**, 366–368.

Fried, M., Nosten, A., Brockman, A., Brabin, B. T. & Duffy, P. E. 1998 Maternal antibodies block malaria. *Nature* **395**, 851–852.

Galli, L. & Brambilla, E. 1967 Progressivo aumento della virulenza di un ceppo di *Plasmodium berghei*. *Riv. Parassit.* **28**, 173–176.

Gandon, S., Mackinnon, M. J., Nee, S. & Read, A. F. 2001 Imperfect vaccines and the evolution of parasite virulence. *Nature* **414**, 751–755.

Gardner, J. P., Pinches, R. A., Roberts, D. J. & Newbold, C. I. 1996 Variant antigens and endothelial receptor adhesion in *Plasmodium falciparum*. *Proc. Natl Acad. Sci. USA* **93**, 3503–3508.

Garnham, P. C. C. 1966 *Malaria parasites and other haemosporidia*. Oxford: Blackwell Scientific.

Genton, B. (and 14 others) 2002 A recombinant blood-stage malaria vaccine reduces *Plasmodium falciparum* density and exerts selective pressure on parasite populations in a phase 1-2b trial in Papua New Guinea. *J. Infect. Dis.* **185**, 820–827.

Giha, H. A., Theander, T. G., Staalsoe, T., Roper, C., Elhassan, I. M., Babiker, H. A., Satti, G. M. H., Arnot, D. E. & Hviid, L. 1998 Seasonal variation in agglutination of *Plasmodium falciparum*-infected erythrocytes. *Am. J. Trop. Med. Hyg.* **58**, 399–405.

Giha, H. A., Staalsoe, T., Dodoo, D., Elhassan, I. M., Roper, C., Satti, G. M. H., Arnot, D. E., Theander, T. G. & Hviid, L. 1999 Nine-year longitudinal study of antibodies to variant antigens on the surface of *Plasmodium falciparum*-infected erythrocytes. *Infect. Immun.* **67**, 4092–4098.

Giha, H. A., Staalsoe, T., Dodoo, D., Roper, C., Satti, G. M. H., Arnot, D. E., Hviid, L. & Theander, T. G. 2000 Antibodies to variable *Plasmodium*-infected erythrocyte surface antigens are associated with protection from novel malaria infections. *Immunol. Lett.* **71**, 117–126.

Gilks, C. F., Walliker, D. & Newbold, C. I. 1990 Relationships between sequestration, antigenic variation and chronic parasitism in *Plasmodium chabaudi chabaudi*: a rodent malaria model. *Parasite Immunol.* **12**, 45–64.

Gravenor, M. B., McLean, A. R. & Kwiatkowski, D. 1995 The regulation of malaria parasitaemia: parameter estimates for a population model. *Parasitology* **110**, 115–122.

Graves, P. M., Carter, R. & McNeill, K. M. 1984 Gametocyte production in cloned lines of *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* **33**, 1045–1050.

Graves, P. M., Burkot, T. R., Carter, R., Cattani, J. A., Lagog, M., Parker, J., Brabin, B. J., Gibson, F. D., Bradley, D. J. & Alpers, M. P. 1988 Measurement of malarial infectivity of human populations to mosquitoes in the Madang area, Papua New Guinea. *Parasitology* **96**, 251–263.

Greenberg, J. & Kendrick, L. P. 1956 Some characteristics of *Plasmodium berghei* passed within inbred strains of mice. *J. Parasitol.* **43**, 423–427.

Greenwood, B. M., Bradley, A. K., Greenwood, A. M., Byass, P., Jammeh, K., Marsh, K., Tulloch, S., Oldfield, S. J. & Hayes, R. 1987 Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. *Trans. R. Soc. Trop. Med. Hyg.* **81**, 478–486.

Greenwood, B. M., Marsh, K. & Snow, R. 1991 Why do some African children develop severe malaria? *Parasitol. Today* **7**, 277–281.

Greenwood, M., Bradford Hill, A., Topley, W. W. C. & Wilson, J. 1936 Experimental epidemiology. Special Report Series no. 209, Medical Research Council. London: HMSO.

Gupta, S. & Day, K. P. 1996 Virulence and transmissibility in *Plasmodium falciparum* malaria. In *Models for infectious human diseases: their structure and relation to data* (ed. V. Isham & G. Medley), pp. 160–180. Cambridge University Press.

Gupta, S., Hill, A. V. S., Kwiatkowski, D., Greenwood, A. M., Greenwood, B. M. & Day, K. P. 1994 Parasite virulence and disease patterns in *Plasmodium falciparum* malaria. *Proc. Natl Acad. Sci. USA* **91**, 3715–3719.

Hadley, T. J., Klotz, F. W., Pasvol, G., Haynes, J. D., McGinniss, M. H., Okubo, Y. & Miller, L. H. 1987 *Falciparum* malaria parasites invade erythrocytes that lack glycophorin A and B (MkMk). Strain differences indicate receptor heterogeneity and two pathways for invasion. *J. Clin. Invest.* **80**, 1190–1193.

Handunetti, S. M., Mendis, K. N. & David, P. H. 1987 Antigenic variation of cloned *Plasmodium fragile* in its natural host *Macaca sinica*. *J. Exp. Med.* **165**, 1269–1283.

Handunetti, S. M., David, P. H., Perera, K. L. R. L. & Mendis, K. N. 1989 Uninfected erythrocytes form 'rosettes' around *Plasmodium falciparum* infected erythrocytes. *Am. J. Trop. Med. Hyg.* **40**, 115–118.

Handunetti, S. M., Hasler, T. H. & Howard, R. J. 1992 *Plasmodium falciparum*-infected erythrocytes do not adhere well to C32 melanoma cells or CD36 unless rosettes with uninfected erythrocytes are first disrupted. *Infect. Immun.* **60**, 928–932.

Hartley, E. G. 1969 Increased virulence of *Plasmodium cynomolgi bastionelli* in the rhesus monkey. *Trans. R. Soc. Trop. Med. Hyg.* **63**, 411–412.

Hay, S. I., Rogers, D. J., Toomer, J. F. & Snow, R. W. 2000 Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: literature survey, internet access and review. *Trans. R. Soc. Trop. Med. Hyg.* **94**, 113–127.

Hayward, R. E., Tiwari, B., Piper, K. P., Baruch, D. I. & Day, K. P. 1999 Virulence and transmission success of the malarial parasite *Plasmodium falciparum*. *Proc. Natl Acad. Sci. USA* **96**, 4563–4568.

Helmby, H., Cavalier, L., Pettersson, U. & Wahlgren, M. 1993 Rosetting *Plasmodium falciparum*-infected erythrocytes express unique strain-specific antigens on their surface. *Infect. Immun.* **61**, 284–288.

Herre, E. A. 1987 Optimality, plasticity and selective regime in fig wasp sex ratios. *Nature* **1987**, 627–629.

Herre, E. A. 1993 Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* **259**, 1442–1445.

Ho, M. & White, N. J. 1999 Molecular mechanisms of cytoadherence in malaria. *Am. J. Physiol.* **276**, C1231–C1242.

Ho, M., Davis, T. M. E., Silamut, K., Bunnag, D. & White, N. J. 1991 Rosette formation of *Plasmodium falciparum*-infected erythrocytes from patients with acute malaria. *Infect. Immun.* **59**, 2135–2139.

Hoffman, S. L. 1996 *Malaria vaccine development: a multi-immune response approach*. Washington, DC: American Society for Microbiology Press.

Holder, A. A. & Freeman, R. R. 1981 Immunization against blood-stage rodent malaria using purified parasite antigens. *Nature* **294**, 361–364.

Holmes, C. B., Losina, E., Walensky, R., Yazdanpanah, Y. & Freedberg, K. A. 2003 Review of human immunodeficiency virus type 1-related opportunistic infections in sub-Saharan Africa. *Clin. Infect. Dis.* **36**, 652–662.

Hommel, M., David, P. H. & Oligino, L. D. 1983 Surface alterations of erythrocytes in *Plasmodium falciparum* malaria: antigenic variation, antigenic diversity and the role of the spleen. *J. Exp. Med.* **157**, 1137–1148.

Howard, R. J. & Barnwell, J. W. 1985 Immunochemical analysis of surface membrane antigens on erythrocytes infected with non-cloned SICA[+] or cloned SICA[−] *Plasmodium knowlesi*. *Parasitology* **91**, 245–261.

Hutagalung, R., Wilairatana, P., Looareesuwan, S., Brittenham, G. M., Aikawa, M. & Gordeuk, V. R. 1999 Influence of hemoglobin E trait on the severity of *falciparum* malaria. *J. Infect. Dis.* **179**, 283–286.

Hviid, L. 1998 Clinical disease, immunity and protection against *Plasmodium falciparum* malaria in populations living in endemic areas. *Expert Rev. Mol. Med.* See http://www-ermm.cbcu.cam.ac.uk/lhc/txt001lhc.htm.

Iqbal, J., Perlmann, P. & Berzins, K. 1993 Serological diversity of antigens expressed on the surface of erythrocytes infected with *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg.* **87**, 583–588.

James, S. P., Nicol, W. D. & Shute, P. G. 1932 A study of induced malignant tertian malaria. *Proc. R. Soc. Lond.* B **25**, 1153–1181.

James, S. P., Nicol, W. D. & Shute, P. G. 1936 Clinical and parasitological observations on induced malaria. *Proc. R. Soc. Lond.* B **29**, 879–894.

Janssen, C. S., Barrett, M. P., Lawson, D., Quail, M. A., Harris, D., Bowman, S., Phillips, R. S. & Turner, C. M. R. 2001 Gene discovery in *Plasmodium chabaudi* by genome survey sequencing. *Mol. Biochem. Parasitol.* **113**, 251–260.

Janssen, C. S., Barrett, M. P., Turner, C. M. R. & Phillips, R. S. 2002 A large gene family for putative variant antigens shared by human and rodent malaria parasites. *Proc. R. Soc. Lond.* B **269**, 431–436. (DOI 10.1098/rspb.2001.1903.)

Jarra, W. & Brown, K. N. 1985 Protective immunity to malaria: studies with cloned lines of *Plasmodium chabaudi* and *P. berghei* in CBA/Ca mice. 1. The effectiveness and inter- and intra-species specificity of immunity induced by infection. *Parasite Immunol.* **7**, 585–606.

Jarra, W. & Brown, K. N. 1989 Invasion of mature and immature erythrocytes of CBA/Ca mice by a cloned line of *Plasmodium chabaudi chabaudi*. *Parasitology* **99**, 157–163.

Jeffery, G. M. 1966 Epidemiological significance of repeated infections with homologous and heterologous strains and species of *Plasmodium*. *Bull. World Hlth Org.* **35**, 873–882.

Jeffery, G. M. & Eyles, D. E. 1954 The duration in the human host of infections with a Panama strain of *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* **3**, 219–224.

Jeffery, G. M. & Eyles, D. E. 1955 Infectivity to mosquitoes of *Plasmodium falciparum* as related to gametocyte density and duration of infection. *Am. J. Trop. Med. Hyg.* **7**, 781–789.

Kaul, D. K., Roth, E. F., Nagel, R. L., Howard, R. J. & Handunetti, S. M. 1991 Rosetting of *Plasmodium falciparum*-infected red blood cells with uninfected red blood cells enhances microvascular obstruction under flow conditions. *Blood* **78**, 812–819.

Kaul, D. K., Nagel, R. L., Llena, J. F. & Shear, H. L. 1994 Cerebral malaria in mice: demonstration of cytoadherence of infected red blood cells and microrheologic correlates. *Am. J. Trop. Med. Hyg.* **50**, 512–521.

Kitchen, S. F. 1949 Symptomatology: general considerations. In *Malariology* (ed. M. F. Boyd), pp. 967–994. London: W. B. Saunders Co.

Knisely, M. H., Stratman-Thomas, W. K. & Eliot, T. S. 1941 *Anat. Rec.* **79**(Suppl. 1), 90.

Kun, J. F. J., Schmidt-Ott, R. J., Lehman, L. G., Lell, B., Luckner, D., Greve, B., Matousek, P. & Kremsner, P. G. 1998 Merozoite surface antigen 1 and 2 genotypes and rosetting of *Plasmodium falciparum* in severe and mild malaria in Lambarene, Gabon. *Trans. R. Soc. Trop. Med. Hyg.* **92**, 110–114.

Kyes, S. A., Rowe, J. A., Kriek, N. & Newbold, C. I. 1999 *Rifins*: a second family of clonally variant proteins expressed on the surface of red cells infected with *Plasmodium falciparum*. *Proc. Natl Acad. Sci. USA* **96**, 9333–9338.

Kyes, S. A., Horrocks, P. & Newbold, C. I. 2001 Antigenic variation at the infected red cell surface in malaria. *A. Rev. Microbiol.* **55**, 673–707.

Landau, I. 1965 Description de *Plasmodium chabaudi* n. sp., parasite de rongeurs africains. *C. R. Hebd. Seances Acad. Sci.* **260**, 3758–3761.

Landau, I. 1966 Comments on sporozoite-induced infections in rodent hosts. *Milit. Med.* **131**, 919–922.

Landau, I. & Chabaud, A. 1965 Infection naturelle par deux *Plasmodium* du rongeur *Thamnomys rutilans* en République Centre Africaine. *C. R. Hebd. Seances Acad. Sci.* **261**, 230–232.

Landau, I. & Chabaud, A. 1968 Schizogonie hépatique secondaire dans le paludisme spontané des rongeurs. *C. R. Hebd. Seances Acad. Sci. Ser.* D **266**, 1730–1733.

Landau, I. & Killick-Kendrick, R. 1966 Rodent plasmodia of the République Centrafricaine: the sporogony and tissue stages of *Plasmodium chabaudi* and *P. berghei yoelii*. *Trans. R. Soc. Trop. Med. Hyg.* **60**, 633–649.

Landau, I., Chabaud, A., Mora-Silvera, E., Coquelin, F., Boulard, Y. & Snounou, G. 1999 Survival of rodent malaria merozoites in the lymphatic network: potential role in chronicity of the infection. *Parasite* **6**, 311–322.

Langreth, S. G. & Peterson, E. 1985 Pathogenicity, stability and immunogenicity of a knobless clone of *Plasmodium falciparum* in Colombian owl monkeys. *Infect. Immun.* **47**, 760–766.

Leech, J. H., Barnwell, J. W., Aikawa, M., Miller, L. H. & Howard, R. J. 1984 *Plasmodium falciparum* malaria: association of knobs on the surface of infected erythrocytes with a histidine-rich protein and the erythrocyte skeleton. *J. Cell Biol.* **98**, 1256–1264.

Levin, B. R. & Bull, J. J. 1994 Short-sighted evolution and the virulence of pathogenic microorganisms. *Trends Microbiol.* **2**, 76–81.

Levin, B. R. & Svanborg-Eden, C. 1990 Selection and evolution of virulence in bacteria: an ecumenical excursion and modest suggestion. *Parasitology* **100**(Suppl. 1), S103–S115.

Lipsitch, M. & Moxon, E. R. 1997 Virulence and transmissibility of pathogens: what is the relationship? *Trends Microbiol.* **5**, 31–36.

Luzzatto, L., Sodeinde, O. & Martini, G. 1983 Genetic variation in the host and adaptive phenomena in *Plasmodium falciparum* infection. In *Ciba Foundation Symposium on Malaria and the Red Cell* (ed. D. Evered & J. Whelan), pp. 159–173. London: Pitman.

Lyon, J. A., Haynes, J. D., Diggs, C. L., Chulay, J. D. & Pratt-Rossiter, J. M. 1986 *Plasmodium falciparum* antigens synthesised by schizonts and stabilised at the merozoite surface by antibodies when schizonts mature in the presence of growth inhibitory serum. *J. Immunol.* **136**, 2252–2258.

McKenzie, F. E., Jeffery, G. M. & Collins, W. E. 2001 *Plasmodium malariae* blood-stage dynamics. *J. Parasitol.* **87**, 626–637.

McKenzie, F. E., Jeffery, G. M. & Collins, F. H. 2002 *Plasmodium vivax* blood-stage dynamics. *J. Parasitol.* **88**, 521–555.

Mackinnon, M. J. & Read, A. F. 1999a Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. *Evolution* **53**, 689–703.

Mackinnon, M. J. & Read, A. F. 1999b Selection for high and low virulence in the malaria parasite *Plasmodium chabaudi*. *Proc. R. Soc. Lond.* B **266**, 741–748. (DOI 10.1098/rspb.1999.0699.)

Mackinnon, M. J. & Read, A. F. 2003 Effects of immunity on relationships between growth rate, virulence and transmission in semi-immune hosts. *Parasitology* **126**, 103–112.

Mackinnon, M. J., Gunawardena, D. M., Rajakaruna, J., Weerasinghe, S., Mendis, K. N. & Carter, R. 2000 Quantifying genetic and nongenetic contributions to malarial infection in a Sri Lankan population. *Proc. Natl Acad. Sci. USA* **97**, 12 661–12 666.

Mackinnon, M. J., Gaffney, D. J. & Read, A. F. 2002*a* Virulence in malaria parasites: host genotype by parasite genotype interactions. *Infect. Genet. Evol.* **1**, 287–296.

Mackinnon, M. J., Walker, P. R. & Rowe, J. A. 2002*b* *Plasmodium chabaudi*: rosetting in a rodent malaria model. *Exp. Parasitol.* **101**, 121–128.

McLean, S. A., Pearson, C. D. & Phillips, R. S. 1982 *Plasmodium chabaudi*: evidence of antigenic variation during recrudescent parasitaemias in mice. *Exp. Parasitol.* **54**, 296–302.

McLean, S. A., MacDougall, L. M. & Phillips, R. S. 1990 Early appearance of variant parasites in *Plasmodium chabaudi* infections. *Parasite Immunol.* **12**, 97–103.

MacPherson, G. G., Warrell, M. J., White, N. J., Looareesuwan, S. & Warrell, D. A. 1985 Human cerebral malaria: a quantitative ultrastructural analysis of parasitized erythrocyte sequestration. *Am. J. Pathol.* **119**, 385–401.

Marsh, K. 1992 Malaria: a neglected disease? *Parasitology* **104**, 53–69.

Marsh, K. & Howard, R. J. 1986 Antigens induced on erythrocytes by *P. falciparum*: expression of diverse and conserved determinants. *Science* **231**, 150–153.

Marsh, K. & Snow, R. W. 1997 Host–parasite interaction and morbidity in malaria endemic areas. *Phil. Trans. R. Soc. Lond.* B **352**, 1385–1394. (DOI 10.1098/rstb.1997.0124.)

Marsh, K., Otoo, L. N., Hayes, R. J., Carson, D. C. & Greenwood, B. M. 1989 Antibodies to blood stage antigens of *Plasmodium falciparum* in rural Gambians and their relation to protection against infection. *Trans. R. Soc. Trop. Med. Hyg.* **83**, 293–303.

Mendis, K. N., Ihalamulla, R. I. & David, P. H. 1988 Diversity of *Plasmodium vivax*-induced antigens on the surface of infected human erythrocytes. *Am. J. Trop. Med. Hyg.* **38**, 42–46.

Mendis, K., Sina, B. J., Marchesini, P. & Carter, R. 2001 The neglected burden of *Plasmodium vivax* malaria. *Am. J. Trop. Med. Hyg.* **64**, 97–106.

Messenger, S. L., Molineux, I. J. & Bull, J. J. 1999 Virulence evolution in a virus obeys a trade-off. *Proc. R. Soc. Lond.* B **266**, 397–404. (DOI 10.1098/rspb.1999.0651.)

Miller, L. H. 1969 Distribution of mature trophozoites and schizonts of *Plasmodium falciparum* in the organs of *Aotus trivigatus*, the night monkey. *Am. J. Trop. Med. Hyg.* **18**, 860–865.

Miller, L. H. & Carter, R. 1976 Innate resistance in malaria. *Exp. Parasitol.* **40**, 132–146.

Miller, L. H., Baruch, D. I., Marsh, K. & Doumbo, O. K. 2002 The pathogenic basis of malaria. *Nature* **415**, 673–679.

Mitchell, G. H., Hadley, T. J., McGinniss, M. H., Klotz, F. W. & Miller, L. H. 1986 Invasion of erythrocytes by *Plasmodium falciparum* malaria parasites: evidence for receptor heterogeneity and two receptors. *Blood* **67**, 1519–1521.

Molineaux, L. & Gramiccia, G. 1980 *The Garki Project: research on the epidemiology and control of malaria in the Sudan savanna of West Africa*. Geneva, Switzerland: World Health Organization.

Molineaux, L., Diebner, H. H., Eichner, M., Collins, W. E., Jeffery, G. M. & Dietz, K. 2001 *Plasmodium falciparum* parasitaemia described by a new mathematical model. *Parasitology* **122**, 379–391.

Mota, M. M., Brown, K. N., Holder, A. A. & Jarra, W. 1998 Acute *Plasmodium chabaudi chabaudi* malaria infection induces antibodies which bind to the surfaces of parasitized erythrocytes and promote their phagocytosis by macrophages *in vitro*. *Infect. Immun.* **66**, 4080–4086.

Mota, M. M., Jarra, W., Hirst, E., Patnaik, P. K. & Holder, A. A. 2000 *Plasmodium chabaudi*-infected erythrocytes adhere to CD36 and bind to microvascular endothelial cells in an organ-specific way. *Infect. Immun.* **68**, 4135–4144.

Muirhead-Thomson, R. C. 1954 Factors determining the true reservoir of infection of *Plasmodium falciparum* and *Wuchereria bancrofti* in a West African village. *Trans. R. Soc. Trop. Med. Hyg.* **48**, 208–225.

Muirhead-Thomson, R. C. & Mercier, E. C. 1952 Factors in malaria transmission by *Anopheles albimanus* in Jamaica. Part I. *Ann. Trop. Med. Parasitol.* **25**, 103–116.

Nash, G. B., Cooke, B. M., Carlson, J. & Wahlgren, M. 1992 Rheological properties of rosettes formed by red blood cells parasitized by *Plasmodium falciparum*. *Br. J. Haematol.* **82**, 757–763.

Nedelman, J. 1984 Inoculation rate and recovery rates in the malaria model of Dietz, Molineaux and Thomas. *Math. Biosci.* **69**, 209–233.

Nesse, R. M. & Williams, G. 1994 *Why we get sick: the new science of Darwinian medicine*. New York: Times Books.

Newbold, C. I., Schryer, M., Boyle, D. B., McBride, J. S., McLean, A., Wilson, R. J. M. & Brown, K. N. 1984 A possible molecular basis for strain specific immunity to malaria. *Mol. Biochem. Parasitol.* **11**, 337–347.

Newbold, C. I., Pinches, R., Roberts, D. J. & Marsh, K. 1992 *Plasmodium falciparum*: the human agglutinating antibody response to the infected red cell surface is predominantly variant specific. *Exp. Parasitol.* **75**, 281–292.

Newbold, C. I., Warn, P., Black, G., Berendt, A. R., Craig, A. G., Snow, R. W., Msobo, M., Peshu, N. & Marsh, K. 1997 Receptor-specific adhesion and clinical disease in *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* **57**, 389–398.

Newbold, C. I., Craig, A., Kyes, S., Rowe, J. A., Fernandez-Reyes, D. & Fagan, T. 1999 Cytoadherence, pathogenesis and the infected red cell surface in *Plasmodium falciparum*. *Int. J. Parasitol.* **29**, 927–937.

Nielsen, M. A., Staalsoe, T., Kurtzhals, J. A. L., Goka, B. Q., Dodoo, D., Alifrangis, M., Theander, T. G., Akanmori, B. D. & Hviid, L. 2002 *Plasmodium falciparum* variant surface antigen expression varies between isolates causing severe and non-severe malaria and is modified by acquired immunity. *J. Immunol.* **168**, 3444–3450.

Ockenhouse, C. F., Ho, M., Tandon, N. N., Vanseventer, G. A., Shaw, S., White, N. J., Jamieson, G. A., Chulay, J. D. & Webster, H. K. 1991 Molecular basis of sequestration in severe and uncomplicated *Plasmodium falciparum* malaria: differential adhesion of infected erythrocytes to CD36 and ICAM-1. *J. Infect. Dis.* **164**, 163–169.

Ofori, M. F., Dodoo, D., Staalsoe, T., Kurtzhals, J. A. L., Koram, K., Theander, T. G., Akanmori, B. D. & Hviid, L. 2002 Malaria-induced acquisition of antibodies to *Plasmodium falciparum* variant surface antigens. *Infect. Immun.* **70**, 2982–2988.

Ogun, S. A. & Holder, A. A. 1996 A high molecular mass *Plasmodium yoelii* rhoptry protein binds to erythrocytes. *Mol. Biochem. Parasitol.* **76**, 321–324.

Okoyeh, J. N., Pillai, C. R. & Chitnis, C. E. 1999 *Plasmodium falciparum* field isolates commonly use erythrocyte invasion pathways that are independent of sialic acid residues of glycophorin A. *Infect. Immun.* **67**, 5784–5791.

Pain, A., Ferguson, D. J. P., Kai, O., Urban, B. C., Lowe, B. S., Marsh, K. & Roberts, D. J. 2001 Platelet-mediated clumping of *Plasmodium falciparum*-infected erythrocytes is a common adhesive phonotype and is associated with severe malaria. *Proc. Natl Acad. Sci. USA* **98**, 1805–1810.

Pasvol, G., Weatherall, D. J. & Wilson, R. J. M. 1980 The increased susceptibility of young red cells to invasion by the malarial parasite *Plasmodium falciparum*. *Br. J. Haematol.* **45**, 285–295.

Perkins, M. E. & Holt, E. H. 1988 Erythrocyte receptor recognition varies in *Plasmodium falciparum* isolates. *Mol. Biochem. Parasitol.* **27**, 23–34.

Peters, J., Fowler, E., Gatton, M., Chen, N., Saul, A. & Cheng, Q. 2002 High diversity and rapid changeover of expressed *var* genes during the acute phase of *Plasmodium falciparum* infections in human volunteers. *Proc. Natl Acad. Sci. USA* **99**, 10 689–10 694.

Peters, W. 1987 *Chemotherapy and drug resistance in malaria.* London: Academic.

Phillips, R. S. 2001 Current status of malaria and potential for control. *Clin. Microbiol. Rev.* **14**, 208–226.

Phillips, R. S., Brannan, L. R., Balmer, P. & Neuville, P. 1997 Antigenic variation during malaria infection: the contribution from the murine parasite *Plasmodium chabaudi*. *Parasite Immunol.* **19**, 427–434.

Pongponratn, E., Riganti, M., Punpoowong, B. & Aikawa, M. 1991 Microvascular sequestration of parasitized erythrocytes in human *falciparum* malaria: a pathological study. *Am. J. Trop. Med. Hyg.* **44**, 168–175.

Pouvelle, B., Buffet, P. A., Lepolard, C., Scherf, A. & Gysin, J. 2000 Cytoadhesion of *Plasmodium falciparum* ring-stage-infected erythrocytes. *Nature Med.* **6**, 1264–1268.

Preiser, P. R. & Jarra, W. 1998 *Plasmodium yoelii*: differences in the transcription of the 235 kDa rhoptry protein multigene family in lethal and nonlethal lines. *Exp. Parasitol.* **89**, 50–57.

Preiser, P. R., Jarra, W., Capiod, T. & Snounou, G. 1999 A rhoptry-protein-associated mechanism of clonal phenotypic variation in rodent malaria. *Nature* **398**, 618–622.

Ramasamy, R., Yasawardena, S., Kanagaratnam, R., Buratti, E., Baralle, F. E. & Ramasamy, M. S. 1999 Antibodies to a merozoite surface protein promote multiple invasion of red blood cells by malaria parasites. *Parasite Immunol.* **21**, 397–407.

Raventos-Suarez, C., Kaul, D. K., Macaluso, F. & Nagel, R. L. 1985 Membrane knobs are required for the microcirculatory obstruction induced by *Plasmodium falciparum*-infected erythrocytes. *Proc. Natl Acad. Sci. USA* **82**, 3829–3833.

Read, A. F. & Taylor, L. H. 2001 The ecology of genetically diverse infections. *Science* **292**, 1099–1102.

Read, A. F., Mackinnon, M. J., Anwar, M. A. & Taylor, L. H. 2002 Kin selection models as evolutionary explanations of malaria. In *Virulence management: the adaptive dynamics of pathogen–host interactions* (ed. U. Dieckmann, J. A. J. Metz, M. W. Sabelis & K. Sigmund), pp. 165–178. Cambridge University Press.

Read, A. F., Gandon, S., Nee, S. & Mackinnon, M. J. 2003 The evolution of pathogen virulence in response to animal and public health interventions. In *Evolutionary aspects of infectious diseases* (ed. D. Dronamraj). Cambridge University Press. (In the press.)

Reeder, J. C., Rogerson, S. J., al-Yaman, F., Anders, R. F., Coppel, R. L., Novakovic, S., Alpers, M. P. & Brown, G. V. 1994 Diversity of agglutinating phenotype, cytoadherence and rosette-forming characteristics of *Plasmodium falciparum* isolates from Papua New Guinean children. *Am. J. Trop. Med. Hyg.* **51**, 45–55.

Rest, J. R. 1982 Cerebral malaria in inbred mice. I. A new model and its pathology. *Trans. R. Soc. Trop. Med. Hyg.* **76**, 410–415.

Richie, T. L. & Saul, A. 2002 Progress and challenges for malaria vaccines. *Nature* **415**, 694–701.

Ricke, C. H., Staalsoe, T., Koram, K., Akanmori, B. D., Riley, E. M., Theander, T. G. & Hviid, L. 2000 Plasma antibodies from malaria-exposed pregnant women recognize variant surface antigens on *Plasmodium falciparum*-infected erythrocytes in a parity-dependent manner and block parasite adhesion to chondroitin sulfate A. *J. Immunol.* **165**, 3309–3316.

Ringwald, P., Peyron, F., Lepers, J. P., Rabarison, P., Rakptpmalala, C., Razanamparany, M., Rabodonirina, M., Roux, J. & Lebras, J. 1993 Parasite virulence factors during *falciparum*-malaria: rosetting, cytoadherence, and modulation of cytoadherence by cytokines. *Infect. Immun.* **61**, 5198–5204.

Roberts, D. J., Craig, A. G., Berendt, A. R., Pinches, R., Nash, G., Marsh, K. & Newbold, C. I. 1992 Rapid switching to multiple antigenic and adhesive phenotypes in malaria. *Nature* **357**, 689–692.

Roberts, D. J., Pain, A., Kai, O., Kortok, M. & Marsh, K. 2000 Autoagglutination of malaria-infected red blood cells and malaria severity. *Lancet* **355**, 1427–1428.

Robertson, J. D. 1945 Notes on the gametocyte threshold for infection of *Anompheles gambiae* Giles, 1902, and *Anopheles melas* Theobald, 1903, in West Africa. *Ann. Trop. Med. Parasitol.* **39**, 8–10.

Rogers, N. J., Daramola, O., Targett, G. A. T. & Hall, B. S. 1996 CD36 and intercellular adhesion molecule 1 mediate adhesion of developing *Plasmodium falciparum* gametocytes. *Infect. Immun.* **64**, 1480–1483.

Rogerson, S. J., Beck, H.-P., al-Yaman, F., Currie, B., Alpers, M. P. & Brown, G. V. 1996 Disruption of erythrocyte rosettes and agglutination of erythrocytes infected with *Plasmodium falciparum* by the sera of Papua New Guineans. *Trans. R. Soc. Trop. Med. Hyg.* **90**, 80–84.

Rogerson, S. J., Tembenu, R., Dobano, C., Plitt, S., Taylor, T. E. & Molyneux, M. E. 1999 Cytoadherence characteristics of *Plasmodium falciparum*-infected erythrocytes from Malawian children with severe and uncomplicated malaria. *Am. J. Trop. Med. Hyg.* **61**, 467–472.

Rowe, J. A., Obeiro, J., Newbold, C. I. & Marsh, K. 1995 *Plasmodium falciparum* rosetting is associated with malaria severity in Kenya. *Infect. Immun.* **63**, 2323–2326.

Rowe, J. A., Moulds, J. M., Newbold, C. I. & Miller, L. H. 1997 *P. falciparum* rosetting mediated by a parasite-variant erythrocyte membrane protein and complement receptor 1. *Nature* **388**, 292–295.

Rowe, J. A., Obeiro, J., Marsh, K. & Raza, A. 2002 Positive correlation between rosetting and parasitemia in *Plasmodium falciparum* clinical isolates. *Am. J. Trop. Med. Hyg.* **66**, 458–460.

Rutledge, L. C. & Gould, D. J. 1969 Factors affecting the infection of anophelines with human malaria in Thailand. *Trans. R. Soc. Trop. Med. Hyg.* **63**, 613–619.

Sachs, J. & Malaney, P. 2002 The economic and social burden of malaria. *Nature* **415**, 680–685.

Sergent, E. & Poncet, A. 1959 Des variations experimentales de la virulence de *Plasmodium berghei*: exaltation-atténuation-mithridatisme. *Arch. Inst. Pasteur Alger.* **37**, 228–234.

Simpson, J. A., Silamut, K., Chotivanich, K., Pukrittayakamee, S. & White, N. J. 1999 Red cell selectivity in malaria: a study of multiple-infected erythrocytes. *Trans. R. Soc. Trop. Med. Hyg.* **93**, 165–168.

Simpson, J. A., Aarons, L., Collins, W. E., Jeffery, G. M. & White, N. J. 2002 Population dynamics of untreated *Plasmodium falciparum* malaria within the adult human host during the expansion phase of the infection. *Parasitology* **124**, 247–263.

Smalley, M. E., Abdalla, S. & Brown, J. 1980 The distribution of *Plasmodium falciparum* in the peripheral blood and bone marrow of Gambian children. *Trans. R. Soc. Trop. Med. Hyg.* **75**, 103–105.

Smith, J. D., Chitnis, C. E., Craig, A. G., Roberts, D. J., Hudson-Taylor, D. E., Peterson, D. S., Pinches, R., Newbold, C. I. & Miller, L. H. 1995 Switches in expression of *Plasmodium falciparum var* genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. *Cell* **82**, 101–110.

Snounou, G., Jarra, W., Viriyakosl, S., Wood, J. C. & Brown, K. N. 1989 Use of a DNA probe to analyse the dynamics of infection with rodent malaria parasites confirms that parasite clearance during crisis is predominantly strain- and species-specific. *Mol. Biochem. Parasitol.* **37**, 37–46.

Snow, R. W., Craig, M., Deichmann, U. & Marsh, K. 1999 Estimating mortality, morbidity, and disability due to malaria among Africa's non-pregnant population. *Bull. World Hlth Org.* **77**, 624–640.

Soubes, S. C., Wellems, T. E. & Miller, L. H. 1997 *Plasmodium falciparum*: a high proportion of parasites from a population of the Dd2 strain are able to invade erythrocytes by an alternative pathway. *Exp. Parasitol.* **86**, 79–83.

Stearns, S. C. 1999 *Evolution in health and disease*. Oxford University Press.

Stevenson, M. M., Lyanga, J. J. & Skamene, E. 1982 Murine malaria: genetic control of resistance to *Plasmodium chabaudi*. *Infect. Immun.* **38**, 80–88.

Struchiner, C. J., Halloran, M. E. & Spielman, A. 1989 Modeling malaria vaccines. I. New uses for old ideas. *Math. Biosci.* **94**, 87–113.

Su, X., Heatwole, V. M., Wertheimer, S. P., Guinet, F., Herrfeldt, J. A., Peterson, D. S., Ravetch, J. A. & Wellems, T. E. 1995 The large diverse gene family *var* encodes proteins involved in cytoadherence and antigenic variation of *Plasmodium falciparum*-infected erythrocytes. *Cell* **82**, 89–100.

Taliaferro, W. H. 1949 Immunity to the malaria infections. In *Malariology* (ed. M. F. Boyd), pp. 935–965. London: W. B. Saunders.

Taylor, L. H. & Read, A. F. 1997 Why so few transmission stages? Reproductive restraint by malaria parasites. *Parasitol. Today* **13**, 135–140.

Taylor, L. H., Walliker, D. & Read, A. F. 1997 Mixed-genotype infections of the rodent malaria *Plasmodium chabaudi* are more infectious to mosquitoes than single-genotype infections. *Parasitology* **115**, 121–132.

Taylor, L. H., Mackinnon, M. J. & Read, A. F. 1998 Virulence of mixed-clone and single-clone infections of the rodent malaria *Plasmodium chabaudi*. *Evolution* **52**, 583–591.

Tchuinkam, T., Mulder, B., Dechering, K., Stoffels, H., Verhave, J.-P., Cot, M., Carnevale, P., Meuwissen, J. H. E. T. & Robert, V. 1993 Experimental infections of *Anopheles gambiae* with *Plasmodium falciparum* of naturally infected gametocyte carriers in Cameroon: factors influencing the infectivity to mosquitoes. *Trop. Med. Parasitol.* **44**, 271–276.

Thomson, J. G. & Robertson, A. 1935 The structure and development of *Plasmodium falciparum* gametocytes in the internal organs and peripheral circulation. *Trans. R. Soc. Trop. Med. Hyg.* **29**, 31–40.

Timms, R., Colegrave, N., Chan, B. H. K. & Read, A. F. 2001 The effect of parasite dose on disease severity in the rodent malaria *Plasmodium chabaudi*. *Parasitology* **123**, 1–11.

Tourneur, N., Scherf, A., Wahlgren, M. & Gysin, J. 1992 The squirrel monkey as an experimental model for *Plasmodium falciparum* erythrocyte rosette formation. *Am. J. Trop. Med. Hyg.* **47**, 633–642.

Trenholme, K. R., Gardiner, D. L., Holt, D. C., Thomas, E. A., Cowman, A. F. & Kemp, D. J. 2000 clag9: a cytoadherence gene in *Plasmodium falciparum* essential for binding of parasitized erythrocytes to CD36. *Proc. Natl Acad. Sci. USA* **97**, 4029–4033.

Treutiger, C.-J., Hedlund, I., Helmby, H., Carlson, J., Jepson, A., Twumasi, P., Kwiatkowski, D., Greenwood, B. M. & Wahlgren, M. 1992 Rosette formation in *Plasmodium falciparum* isolates and anti-rosette activity of sera from Gambians with cerebral or uncomplicated malaria. *Am. J. Trop. Med. Hyg.* **46**, 503–510.

Turner, C. M. R., Aslam, N. & Dye, C. 1995 Replication, differentiation, growth and the virulence of *Trypanosoma brucei* infections. *Parasitology* **111**, 289–300.

Turner, P. E., Cooper, V. S. & Lenski, R. E. 1998 Tradeoff between horizontal and vertical modes of transmission in bacterial plasmids. *Evolution* **52**, 315–329.

Udeinya, I. J., Graves, P. M., Carter, R., Aikawa, M. & Miller, L. H. 1983 *Plasmodium falciparum*: effect of time in continuous culture on binding to human endothelial cells and amelanotic melanoma-cells. *Exp. Parasitol.* **56**, 207–214.

Udomsangpetch, R., Brown, A. E., Smith, C. D. & Webster, H. K. 1991 Rosette formation by *Plasmodium coatneyi*-infected red blood cells. *Am. J. Trop. Med. Hyg.* **44**, 399–401.

Udomsangpetch, R., Thanikkul, K., Pukrittayakamee, S. & White, N. J. 1995 Rosette formation by *Plasmodium vivax*. *Trans. R. Soc. Trop. Med. Hyg.* **89**, 635–637.

Udomsangpetch, R., Pipitaporn, B., Silamut, K., Pinches, R., Kyes, S., Looareesuwan, S., Newbold, C. I. & White, N. J. 2002 Febrile temperatures induce cytoadherence of ring-stage *Plasmodium*-infected erythrocytes. *Proc. Natl Acad. Sci. USA* **99**, 11 825–11 829.

Urban, B. C. & Roberts, D. J. 2002 Malaria, monocytes, macrophages and myeloid dendritic cells: sticking of infected erythrocytes switches off host cells. *Curr. Opin. Immunol.* **14**, 458–465.

Van Baalen, M. & Sabelis, M. W. 1995 The dynamics of multiple infection and the evolution of virulence. *Am. Nat.* **146**, 881–910.

Voller, A. & Rossan, R. N. 1969 Immunological studies with simian malarias. I. Antigenic variants of *Plasmodium cynomolgi bastianellii*. *Trans. R. Soc. Trop. Med. Hyg.* **63**, 46–56.

Vuong, P. N., Richard, F., Snounou, G., Coquelin, F., Chabaud, A., Gonnet, F., Chabaud, A. G. & Landau, I. 1999 Development of irreversible lesions in the brain, heart and kidney following acute and chronic murine malaria infection. *Parasitology* **119**, 543–553.

Wahl, L. M., Gerrish, P. J. & Saika-Voivod, I. 2002 Evaluating the impact of population bottlenecks in experimental evolution. *Genetics* **162**, 961–971.

Wahlgren, M. 1986 Antigens and antibodies involved in humoral immunity to *Plasmodium falciparum*. PhD thesis, University of Stockholm, Sweden.

Wahlgren, M., Carlson, J., Udomsangpetch, R. & Perlmann, P. 1989 Why do *Plasmodium falciparum*-infected erythrocytes form spontaneous erythrocyte rosettes? *Parasitol. Today* **5**, 183–185.

Wahlgren, M., Carlson, J., Ruanjurachuporn, W., Conway, D. J., Helmby, H., Martinez, A., Patarroyo, M. E. & Riley, E. M. 1990 Geographical distribution of *Plasmodium falciparum* erythrocyte rosetting and frequency of rosetting antibodies in human sera. *Am. J. Trop. Med. Hyg.* **43**, 333–338.

Weiss, R. A. 2002 Virulence and pathogenesis. *Trends Microbiol.* **10**, 314–317.

West, S. A. & Herre, E. A. 1998 Stabilizing selection and variance in fig wasp sex ratios. *Evolution* **52**, 475–485.

White, N. J. & Ho, M. 1992 The pathophysiology of malaria. *Adv. Parasitol.* **31**, 83–173.

Yoeli, M. & Hargreaves, B. 1974 Brain capillary blockage produced by a virulent strain of rodent malaria. *Science* **184**, 572–573.

Yoeli, M., Hargreaves, B., Carter, R. & Walliker, D. 1975 Sudden increase in virulence in a strain of *Plasmodium berghei yoelii*. *Ann. Trop. Med. Parasitol.* **69**, 173–178.