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12

Kin-selection Models as Evolutionary Explanations of Malaria

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12.1 Introduction

Malaria, a disease caused by protozoan parasites of the genus *Plasmodium*, can substantially reduce host fitness in wild animals (Atkinson and Van Riper 1991; Schall 1996). In humans, the major disease syndromes – severe anemia, coma, and organ failure, as well as general pathology such as respiratory distress, aches, and nausea – cause considerable mortality and morbidity (Marsh and Snow 1997).

Biomedical research attributes malaria to red cell destruction, infected cell sequestration in vital organs, and the parasite-induced release of cytokines (Marsh and Snow 1997). But mechanistic explanations are just one type of explanation for any biological phenomenon, and, in recent years, evolutionary biologists have become interested in offering evolutionary explanations of infectious disease virulence. This is entirely appropriate (Read 1994). In the context of malaria, for example, the clinical outcome of infection has an important impact on parasite and host fitness and is – at least in part – determined by heritable variation in host and parasite factors (Greenwood *et al.* 1991). Yet in the recent rush to provide evolutionary explanations of disease, there has been, in our view, too little interaction between the models built by evolutionary biologists and reality. There is unlikely to be a simple, general model of virulence: the causes of disease and the fitness consequences for host and parasite are too variable. Instead, different models, and even different frameworks, will be relevant in different contexts. Only by evaluating specific models in the context of specific diseases will sensible evolutionary explanations of virulence be realized. Such evaluations seem to us an essential step if one aim of an evolutionary explanation is to contribute to virulence management. An evolutionary explanation of malaria would answer the question "Why has natural selection not eliminated the disease?" and would perhaps contribute to answering the question "Why is the clinical outcome of infection so variable?"

One evolutionary explanation, for instance, postulates that malaria is maintained by natural selection because it enhances the fitness of the parasite that causes it, since sick hosts have reduced antivector behavior (Day and Edman 1983; Ewald 1994a). Rather than evaluate that idea, we instead examine an idea that has attracted more attention from theorists. Kin-selection models of virulence represent an important component in the evolution of virulence literature. They postulate

that the genetic relatedness of parasites within hosts affects the outcome of virulence. In this chapter we attempt to evaluate the relevance of these models to malaria.

12.2 Kin-selection Models of Virulence

Most evolutionary models consider disease virulence to be a consequence of selection acting on parasite life history. The most frequently espoused view is that virulence is an incidental and unavoidable consequence of parasites extracting resources from hosts to maximize the production of effective transmission stages. Virulence *per se* is seen as detrimental to parasite fitness (it increases the risk of death of the hosts and, hence, of the parasites), but host damage is necessary for transmission. Thus, observed levels of virulence are said to represent schedules of host exploitation that optimize some measure of parasite fitness by balancing the risk of death with the need to maximize transmission-stage output. This idea has been much reviewed (Bull 1994; Read 1994, Frank 1996c; Ebert 1998b) and we hope to evaluate the relevance of it to malaria in due course. Here, we assume that this idea is applicable, and, therefore, we evaluate the relevance of an important development of the idea.

Many authors have pointed out that where mixed-genotype infections are common, levels of virulence greater than those optimal for single-genotype infections are favored by natural selection. This is because optimal rates of host exploitation are altered when unrelated parasite genotypes compete (Hamilton 1972; Eshel 1977; Axelrod and Hamilton 1981; Levin and Pimentel 1981; Bremermann and Pickering 1983; May and Anderson 1983a; Knolle 1989; Bremermann and Thieme 1989; Sasaki and Iwasa 1991; Frank 1992a, 1996c; Herre 1993, 1995; Nowak and May 1994; May and Nowak 1995; Van Baalen and Sabelis 1995a, 1995b; Ebert and Mangin 1997; Leung and Forbes 1998; and see Chapters 5 and 9). Parasites that slowly exploit hosts are outcompeted by those that exploit hosts more rapidly. Even if host life expectancy is reduced so that all parasites do worse, the prudent parasites do disproportionately badly, and are thus eliminated by natural selection. This "tragedy of the commons" appears in many areas of evolutionary biology (e.g., social evolution; Trivers 1985); the common link is relatedness. Here, prudent exploitation of hosts is favored when relatedness within an infection is high (e.g., all parasites are members of the same clone). But the kin-selective fitness benefits of prudence are reduced when within-host relatedness is lowered – that is, more selfish genotypes win.

It follows from these ideas that where mixed-genotype infections occur, levels of virulence favored by natural selection are greater. There are two mechanisms by which natural selection acting on parasites could match virulence to within-host relatedness. Schedules of host exploitation could have become genetically fixed at levels that are evolutionarily stable for the average frequency of mixed-genotype infections found in a population. Alternatively, conditional strategies might have evolved, whereby parasites alter their exploitation schedules, and hence virulence, according to the type of infection they find themselves in (Sasaki and Iwasa 1991;

Frank 1992a; Van Baalen and Sabelis 1995a). Facultative life-history strategies are a common feature in many taxa (e.g., Wrensch and Ebbert 1993; Godfray 1994; Via *et al.* 1995). If conditional virulence strategies exist, there should be an association between within-host genetic diversity and virulence within a host population; if only genetically fixed strategies are possible, there will be no such association within populations, but there should be across them.

Are these ideas applicable to malaria, as several evolutionary biologists have suggested (Pickering 1980; Bremermann and Pickering 1983; Frank 1992a; Ewald 1994a)? *Plasmodium* infections consist of asexually replicating genotypes, which transmit to mosquitoes by producing gametocytes – terminal forms that are incapable of further replication in the vertebrate host. Natural infections often consist of unrelated genotypes, acquired from either the same or different infectious bites. Multiplicity of infection (the frequency of mixed infections, or the number of clones per host) is variable within populations and on average higher in areas where transmission rates are high (Day *et al.* 1992; Babiker and Walliker 1997; Paul and Day 1998; Arnot 1999). The potential for kin selection to affect the outcome of virulence evolution thus exists.

But does it? We begin by asking whether the multiplicity of infection correlates with disease outcome within populations, as would be expected if there are conditional virulence strategies. We then consider the issue of genetically fixed strategies, before summarizing results from our experimental work, which address some assumptions implicit in the foregoing arguments. We end by discussing the management implications of these ideas and data.

12.3 Conditional Virulence Strategies

In this section, we discuss field correlations and data from laboratory experiments concerning conditional virulence strategies.

Field correlations

Direct measurements of the genetic composition of infections that differ in clinical status are increasingly available from human populations afflicted by malaria. Genetic diversity can be assayed using monoclonal antibody analysis, isoenzyme analysis, and, most recently, polymerase chain reaction (PCR) amplification of highly polymorphic loci. This has made it possible to ask whether infections that consist of more than one genotype are more virulent, as would be expected if parasites are facultatively increasing rates of host exploitation in the presence of coinfecting competitors.

Although such studies are in their infancy, available data are summarized in Table 12.1. Care is needed in the interpretation of such data. Many estimates of the multiplicity of infection are almost certainly underestimates (Arnot 1999), and comparisons across studies are of limited value because the loci under study and clinical definitions vary. Nevertheless, within-study comparisons probably are meaningful, and here the picture that emerges is, if anything, opposite to that expected from kin-selection models of virulence. In the majority of studies, the

Table 12.1 Multiplicity of infection and disease status in field studies of humans infected with *Plasmodium falciparum*. *n* = number of people who are PCR-positive for parasites in the respective groups; ns = between-group difference not significant; s = between-group difference significant (significance as presented by author or from appropriate tests based on data presented); nt = between-group significance not tested and not possible to test from presented data. Standard errors given where reported or could be calculated from reported data.

			Average number of clones/person in people with			Proportion single clone infections in people with		
Location	Ref.	*n*	Asymptomatic infections	Mild malaria[a]	Severe malaria[b]	Asymptomatic infections	Mild malaria[a]	Severe malaria[b]
Senegal	[1]	30 and 56		2.3 ← ns →	2.4		0.29 ← s →	0.65
Senegal	[2–4]	24 and 10	4.0 ← nt →	1.4				
Senegal	[5]	166 and 25	1.65 ← s →	2.3		0.52 ← s →	0.20	
Gabon	[6]	99 and 99		1.3±0.1 ← ns →	1.2±0.4		0.50 ← ns →	0.70
Tanzania	[7]	76 and 71	5.0±0.25 ← s →	3.4±0.3				
The Gambia	[8]	118 and 35		2.0±0.1 ← ns →	2.1±0.2		0.45 ← ns →	0.46
Kenya	[9]	c.172 and 25	2.0 ← ns →	2.2		0.33 ← ns →	0.25	
Papua New Guinea	[10]	116 and 111	1.3±0.1 ← ns →	1.2±.04		0.74 ← ns →	0.82	
Sudan	[11]	160 in longitudinal study	c.1.39 ← s →	c.1.59		0.62 ← s →	0.49	
Papua New Guinea	[12]	82 single and 53 multiple infections	Prospective study: children infected with multiple clones had significantly lower risk of subsequent clinical attack					

[a] Febrile and parasite positive.

[b] Parasite positive and severe anemia, altered consciousness, convulsions, or at least one other symptom of severe malaria (Warrell *et al.* 1990).

Sources: [1] Robert *et al.* (1996a), [2] Ntoumi *et al.* (1995), [3] Contamin *et al.* (1996), [4] Mercereau-Puijalon (1996), [5] Zwetyenga *et al.* (1998), [6] Kun *et al.* (1998), [7] Beck *et al.* (1997), [8] Conway *et al.* (1991), [9] Kyes *et al.* (1997), [10] Engelbrecht *et al.* (1995), [11] Roper *et al.* (1998), [12] Al-Yaman *et al.* (1997).

number of clones in an infection is unrelated to the severity of clinical symptoms. At least three studies provide evidence of an association between genetic diversity in an infection and disease severity (Robert *et al.* 1996a, Mercereau-Puijalon 1996, Beck *et al.* 1997, Al-Yaman *et al.* 1997), but it is the less diverse infections that are the more virulent. Only two studies show evidence that symptomatic infections – those detected when sick people report to clinics – contain more genotypes than infections discovered by random sampling of asymptomatic people (Roper *et al.* 1998, Zwetyenga *et al.* 1998).

A major problem in the interpretation of these studies is the (almost scandalous) lack of understanding of naturally acquired immunity against malaria. To the extent that there is a consensus view on the immunoepidemiology of malaria, it might be summarized as follows. Immunity is of two sorts: antiparasite and antidisease. The precise nature of either, or of the link between them, is unknown, but they are certainly not two sides of the same coin. For example, semi-immune people can often harbor high densities of parasites without any obvious effect on the host. Antiparasite immunity has a large strain-specific component. Effective protection may require multiple exposures to the same genotype and/or rapidly decay. Memory of recent or low-grade concurrent infections thus determines specificity of effective responses against new infections. Clinical disease is caused by antigenic types not previously seen by that individual. As children in malaria-endemic regions age, the repertoire of genotypes to which the immune system has been exposed increases, and they become protected against progressively more parasite genotypes. A variety of indirect immunological and epidemiological evidence is consistent with this view (Day and Marsh 1991; Gupta *et al.* 1994c; Mendis and Carter 1995; Mercereau-Puijalon 1996), but the evidence is far from definitive.

If this view is even approximately correct, an important implication is that the effects of previous exposure and genotype-specific immune responses are a major – perhaps *the* major – proximate factor to determine disease outcome. If so, any effect of conditional host exploitation strategies may be hard to detect. It may also explain why in some studies lower genetic diversity is associated with greater virulence. Genotypes not previously seen by a host may grow unchecked to high densities and trigger nonspecific effectors [tumor necrosis factor (TNF), fever, nitrous oxide, oxygen radicals] which eliminate other genotypes or suppress them below PCR-detection thresholds. Alternatively, high multiplicity of infection may indicate recent exposure to more genotypes, which reduces the chances of encountering a previously unseen genotype in the near future.

In light of these complexities, it may be possible to reconcile the data summarized in Table 12.1 with the existence of conditional host-exploitation strategies. Indeed, it is intriguing that both places where higher multiplicity of infection is associated with disease are areas with low year-round transmission (Roper *et al.* 1998, Zwetyenga *et al.* 1998), and so immunity against previously experienced genotypes may have time to wane. Ideally, what is required are comparisons of the severity of disease following infection with one or more previously unseen genotypes in hosts with identical exposure histories. In the uncontrolled world of

field correlations, such data are unlikely to be forthcoming. In this respect, animal models can play an important role.

Laboratory experiments

Using the rodent malaria *Plasmodium chabaudi* in laboratory mice, we compared the virulence of mixed clone and single clone infections (Taylor *et al.* 1998b). We used anemia and weight loss as virulence measures, because these measures are correlated with mortality rates (Mackinnon and Read 1999a). All mice were infected with the same number of parasites; mixed clone infections were initiated with varying ratios of the two clones. We found that mixed clone infections were more virulent. Mice infected with two clones lost about 30% more weight than those infected with one; mice with mixed clone infections were also more anemic. These findings are certainly consistent with the theory that parasites conditionally alter host exploitation strategies in response to the presence of competing clones. However, parasite densities were no higher in mixed clone infections. The rate of parasite proliferation correlated with virulence across all mice, but for a given rate of proliferation, mixed clone infections were still more virulent. If the parasites employed conditional host-exploitation strategies, the effects were not detectable in terms of parasite replication, as is conventionally assumed in models of virulence.

We believe our data are most parsimoniously explained not by conditional virulence strategies, but instead by the additional costs to hosts of mounting a response against genetically diverse parasites, in terms of both consumption of host resources and immunopathology. Diverse parasite populations may, for example, stimulate a larger number of T- or B-cell clones or stimulate a greater immune cascade, causing the destruction of more red blood cells (RBCs), or trigger increased production of self-damaging effectors such as TNF and fever. Direct evidence for any of this is currently lacking, but the idea is amenable to experimental testing. What we do know is that infections with genetically diverse parasites take longer to clear (Taylor *et al.* 1997a, 1997b, 1998b; Read and Anwar, unpublished) and that prolonged infection results in prolonged anemia (Read and Anwar, unpublished). Longer clearance times do not, however, explain the greater weight loss induced by mixed clone infections: maximum weight loss occurs during "crisis" (well before clearance) when there is a rapid reduction in parasite numbers associated with low RBC densities and strong nonspecific immune activity (Jarra and Brown 1989).

In sum, then, field data from *P. falciparum* provide, with two exceptions, either no evidence of conditional virulence strategies, or evidence against them. Uncontrolled field correlations are hard to interpret, especially in the face of strain-specific immunity, but controlled experiments with *P. chabaudi* in mice also fail to show any evidence of facultative alterations in growth strategies in response to the presence of coinfecting genotypes. A suggestion of conditional virulence strategies in lizard malaria (Pickering *et al.* 2000) is based on a correlation between surrogate measures of virulence and genetic relatedness. There is no evidence of a correlation between the same surrogates in other lizard malarias (Schall 1989),

P. falciparum in humans (Robert *et al.* 1996b), *P. chabaudi* in rodents (Taylor 1997), or in *Haemoproteus*, a related genus of avian blood parasites (Shutler *et al.* 1995).

12.4 Genetically Fixed Virulence Strategies

Conditional virulence strategies require the ability of a clonal lineage to recognize the presence of nonkin and modify host exploitation strategies accordingly. It may be that such sophistication is beyond what is, after all, just a single-celled protozoan (however, this "simple" organism is sufficiently sophisticated to outwit a century of biomedical science). If so, kin-selection models of virulence predict that host exploitation strategies appropriate for some average level of within-host competition in a population should be favored by selection.

This idea requires heritable variation in the levels of virulence induced by malaria parasites on which selection acts. Moreover, this variation should be positively and genetically correlated with replication rates within hosts and, in the absence of host death, with transmission rates between hosts. Theoreticians have suggested that various epidemiological patterns are consistent with the existence of virulent genotypes or strains of *P. falciparum* circulating within human populations (Gupta *et al.* 1994c), but the issue is contentious (Marsh and Snow 1997). The only parasite phenotype that has been found to correlate consistently with disease outcome is rosetting, whereby uninfected erythrocytes become stuck to infected cells (Carlson *et al.* 1990). The ability to rosette is under parasite genetic control, being encoded by specific variant types of the *var* multigene family (Rowe *et al.* 1997; Chen *et al.* 1998). In the laboratory, rapid increases in the virulence of rodent malaria have been attributed to point mutations (Yoeli *et al.* 1975). In controlled laboratory infections of single clones of *P. chabaudi* in a single mouse genotype, we found substantial differences between clones in virulence. These differences were repeatable over successive passages. Moreover, the genetic architecture was as assumed by parasite-centered models of virulence: virulence and rates of within-host replication were genetically correlated, as were virulence and infectivity to mosquitoes (Mackinnon and Read 1999a). Insofar as these results are generalizable, there appears to be the necessary raw material for natural selection on virulence to act in accordance with the evolutionary models and generate genetically fixed virulence strategies.

Are these strategies fixed as we would expect from the kin-selection models? In areas with high transmission, where there is a high multiplicity of infection (e.g., *P. falciparum* in Tanzania; Babiker *et al.* 1994), levels of parasite virulence should be higher than in areas where the force of infection is lower, so that the majority of hosts are infected with a single clone (e.g., *P. falciparum* in Papua New Guinea; Paul *et al.* 1995). Testing that prediction is unfortunately fraught with difficulties. Levels of host immunity are also likely to vary with transmission rates and, hence, the multiplicity of infection, which confounds cross-community correlations between average levels of within-host diversity and morbidity and mortality measures. Genetic differences between host populations may also confound any

such tests. Direct comparisons of virulence of isolates from different populations grown in a "common garden" would resolve that difficulty; the problem is to find an ethical or biologically realistic garden. Of the strains used for malaria therapy of neurosyphilis in nonimmune Europeans in the first half of the 20th century, a number of geographically distinct races were recognized that differed in their clinical virulence. Recent analysis of data gathered at that time reveals repeatable strain differences in within-host growth rates, but comparable data on virulence seems to lacking (Gravenor *et al.* 1995).

Once virulence factors and the parasite genes that encode them have been identified, informative field data should be forthcoming. It would be of considerable interest, for example, to determine whether mean rosetting rates correlate with multiplicity of infection across populations.

12.5 Within-host Competition and Between-host Fitness

If the predictions of the kin-selection models are currently hard to test in the malaria context, what of the models' assumptions? Two distinct sources of selection for increased virulence when mixed infections occur can be identified in the theoretical work to date. The first arises when the presence of "competing" parasites increases the likelihood of host death. Even if the transmission rates of individual clones are otherwise unaffected by coinfecting parasites, this situation favors higher levels of virulence (May and Nowak 1995; Leung and Forbes 1998). The other source of selection arises from exploitation or interference competition, in which the population sizes and/or transmission rates of clones that proliferate within a host are reduced by the presence of competitors (e.g., Frank 1992a; Van Baalen and Sabelis 1995a; Herre 1995). This could occur through conventional resource competition or through apparent competition (*sensu* Holt 1977), with the immune response triggered by one population having a detrimental effect on the other. The most mathematically tractable case (or at least the most frequently modeled), is of competition so severe that less virulent parasites do not transmit at all from mixed infections (e.g., Levin and Pimentel 1981; Bremermann and Pickering 1983; Knolle 1989; Bremermann and Thieme 1989; Nowak and May 1994; Leung and Forbes 1998) – what Van Baalen and Sabelis (1995a) term superseding infections.

We do not know if the presence of coinfecting malaria clones increases the probability of host death. As described above, one experimental study (Taylor *et al.* 1998b) and two field studies (Roper *et al.* 1998, Zwetyenga *et al.* 1998) suggest virulence increases with the multiplicity of infection; a number of other field studies suggest that it does not (Table 12.1). As well as the attendant ambiguities associated with this data, we do not know how disease levels translate into mortality rates, or even whether observed mortality rates are sufficiently high to impose selection on virulence; case fatality rates may be as low as 2.5 per 1 000 (Greenwood *et al.* 1991).

On the other hand, it seems highly likely that resource and/or apparent competition affect within-host population sizes. We are unaware of any direct evidence

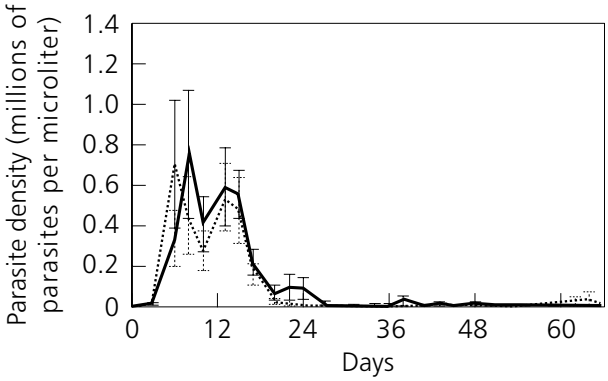

Figure 12.1 Parasite density during the course of infections in mice consisting of one (dotted curve) or two (continuous curve) clones of *Plasmodium chabaudi* (vertical lines are ± standard errors). $n = 9$ single clone infections; $n = 11$ two-clone infections. *Source*: Read and Anwar (unpublished).

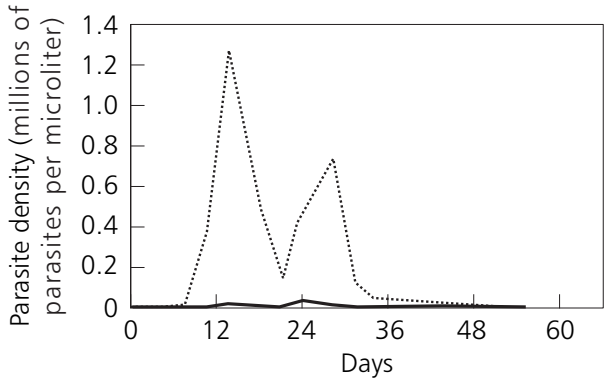

Figure 12.2 Density of two clones of *Plasmodium chabaudi*, AS (continuous curve) and CB (dotted curve), in a single mouse. Clone AS was inoculated 3 days after clone CB (day 0). Clones were distinguished by monoclonal antibody labeling. *Source*: Read and Anwar (unpublished).

from humans, but we have found in the rodent malaria *P. chabaudi* in laboratory mice that the total number of blood-stage parasites produced during an infection is unaffected by the genetic diversity of the inoculum, implying a cap on total densities (Figure 12.1). Depending on initial conditions, clonal populations can be reduced to <10% of that achieved in a single clone infection by the presence of coinfecting genotypes (Figures 12.2 and 12.3; Taylor *et al.* 1997a, 1997b, 1998b; Taylor and Read 1998; Read and Anwar, unpublished).

However, for within-host competition to have any long-term evolutionary consequences, it has to affect the transmission success of individual clones. None of our experimental data are consistent with the idea of superseding infections: in all the infections we examined, all the clones present successfully infected mosquitoes. Moreover, and quite unexpectedly, we found that, despite comparable parasite densities in infections consisting of one or two clones, mixed infections had higher gametocyte densities and were more infectious to mosquitoes

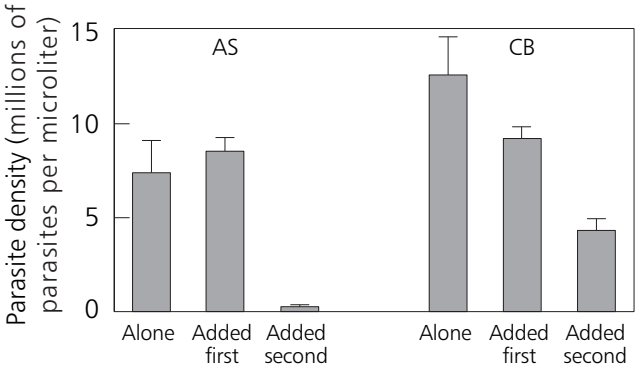

Figure 12.3 Total parasite densities in mice inoculated with either AS alone, CB alone, or with clones added sequentially. When added first, clone AS does as well as it does on its own; when added second (3 days after CB), it does substantially worse. CB always does better on its own, does somewhat worse when AS is added 3 days later, and does even worse if added second. Clones were distinguished by monoclonal antibody labeling; each bar is the mean of 4–6 infections. *Source*: Read and Anwar (unpublished).

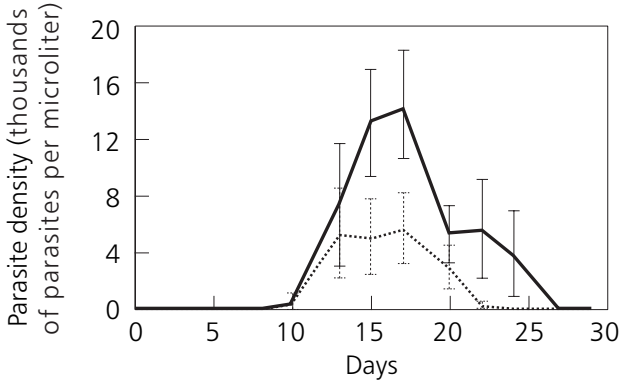

Figure 12.4 Gametocyte (transmission stage) densities in peripheral blood of mice infected with one (dotted curve) or two (continuous curve) clones (vertical lines are ± standard error). Same infections as for Figure 12.1; total gametocyte density is greater in mixed clone infections ($p = 0.034$). *Source*: Read and Anwar (unpublished).

(Figure 12.4; Taylor *et al.* 1997a; Read and Anwar, unpublished). Molecular genetic analysis of the parasites that successfully infected the mosquitoes showed that clones in mixed infections transmitted at least as well as they did from single clone infections, and often did substantially better (Figure 12.5; Taylor *et al.* 1997b). This is because, in some cases, competitively suppressed clones are able to achieve higher densities toward the end of the infection when the transmission stages are being produced (Figure 12.6, Taylor and Read 1998). We hypothesize this occurs because the clone that dominates the bulk of the infection also dominates the attention of the specific component of the host immune response, so that in effect the "successful" competitor shields the "suppressed" genotype from immune clearance. This theory is amenable to experimental testing; it would also

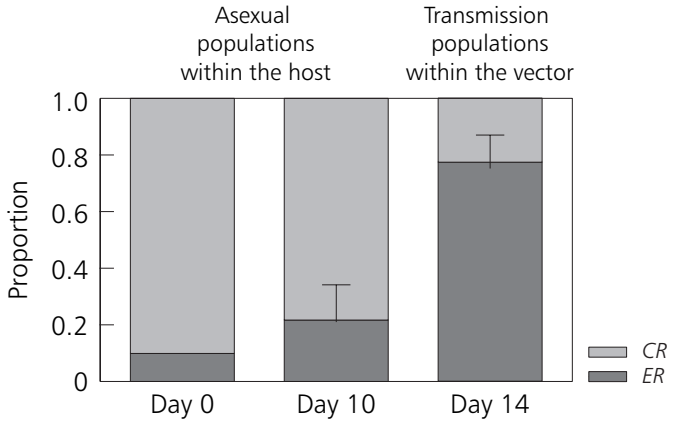

Figure 12.5 Relative frequencies of two *Plasmodium chabaudi* clones, ER and CR, in mixed infections in mice. On day 0, clones were inoculated at a 9:1 ratio, a difference that was maintained through the first 10 days of the infection, when the bulk of the parasites are present. Nevertheless, almost the opposite ratio was observed among parasites that successfully transmitted to mosquitoes. Clones in mice were distinguished by monoclonal antibody assays; genotype frequencies in mosquitoes were determined by PCR. *Source*: Taylor *et al.* (1997b).

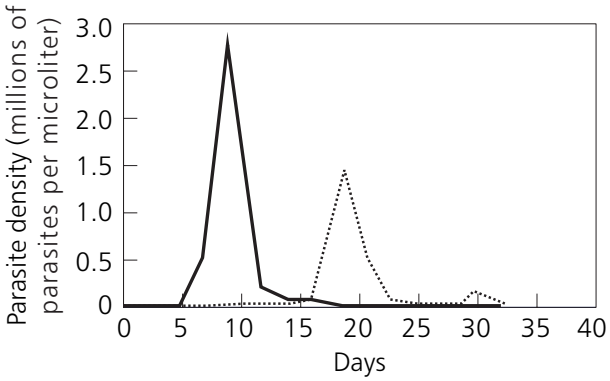

Figure 12.6 Density of two clones of *Plasmodium chabaudi*, AS (continuous curve) and CB (dotted curve), in a single mouse. Clone AS was inoculated 3 days before clone CB (day 0). Clones were distinguished by monoclonal antibody labeling. *Source*: Read and Anwar (unpublished).

benefit from theoretical work on within-host competition in the presence of strain-specific and nonspecific immunity. Such models are in their infancy and their very complexity may rule out simple generalizations (Box 12.1).

Whatever the mechanism, our data clearly demonstrate that despite often substantial competition within an infection, individual genotypes transmit at least as well from mixed infections. This is counter to the assumption of all current kin-selection models of virulence. Assuming that the patterns in mice generalize, it would be of substantial interest to understand the population level consequences of the *positive* feedback between the multiplicity of infection and infectiousness that we find, for both disease epidemiology and the evolution of virulence.

Box 12.1 Models of within-host competition between parasite strains

Within-host competition between parasite strains is the critical element of kin-selection models of the evolution of virulence. Yet, most theoretical work concerns population level (epidemiological) models with no attempt to model explicitly the within-host processes involved (e.g., Levin and Pimentel 1981; Nowak and May 1994; Van Baalen and Sabelis 1995a; see Boxes 5.1, 9.2, and 9.3). These models generally assume the outcome of within-host competition to be fixed in some mathematically tractable way (e.g., only the more virulent clone transmits from a mixed-clone infection, see Levin and Pimentel 1981). Models for the evolution of virulence that describe the outcome of competition between parasite strains as the emergent property of explicit within-host processes do not yet exist (but see Chapter 22 for such a model in a predator–prey metapopulation context).

Actually, explicit within-host models of competition in any context are relatively rare. A number of models of single genotype infections incorporate some sort of intra-clone competition, either with explicitly modeled limiting resources, or by including unspecified logistic constraints on growth (e.g., Anderson *et al.* 1989; Gravenor *et al.* 1995; Hetzel and Anderson 1996). While an important first step toward modeling the more-than-one strain case, single-strain models necessarily ignore parasite heterogeneity in competitive ability, immunogenicity, and susceptibility to immune clearance.

There are two published attempts to model explicitly within-host competition between strains. Smith and Holt (1996) argue that the machinery of mechanistic resource–consumer theory (Tilman 1982) provides a useful lens through which to view the internal struggle between pathogens. In their view, within-host dynamics can be seen as a consequence of competition for limited resources, such as glutamine or iron. The essential output of this approach is predictions about the ability of a pathogen to invade or exclude a competitor. The determinant of this is the critical resource concentration at which a strain's birth rate balances its death rate; that is, the strain with the lower critical resource concentration wins. This approach can be extended to incorporate competition for multiple resources and – by considering the effects of increasing pathogen mortality on critical resource concentration – also the immune pressure. However, we see two principal challenges. First, it is an equilibrium approach. In reality, equilibrium may not be achieved before the host clears the competitors. And even if competitive exclusion does occur, the excluded strain may achieve substantial transmission in the interim. Indeed, if there were a trade-off between persistence and rapid growth, it is possible to envisage situations whereby the excluded pathogen achieves higher total transmission stage densities than the eventual "winner." Second, the important complexities of strain-specific and strain-transcending immunity need to be incorporated. Resource limitation may be an important determinant of competition for only a minority of infections, if at all: host protective responses may halt population growth first.

Hellriegel (1992) explicitly incorporated those complexities. She extended the coupled ordinary differential equations of Anderson *et al.* (1989) to include two coinfecting malaria strains, competition for resources (erythrocytes), and specific and nonspecific immunity against different parasite stages. At its height, her

continued

Box 12.1 *continued*

model was not analytically tractable as it involved 15 equations with at least 21 parameters and variables. Assessment of equilibria provides some insight into long-term behavior, but does not necessarily reveal the most interesting dynamic features. Numerical simulations are the only way to explore nonequilibrium dynamics and these show that the population dynamics of a clone can be dramatically altered by the presence of a competitor, the order of infection of competitors, and the kind of immune response elicited.

Finally, models of within-host competition are somewhat analogous to models of within-host competition between antigenic variants and virulence mutants generated within an infection of a single strain (Bonhoeffer and Nowak 1994a, 1994b; Antia *et al.* 1996). Models such as those of Antia *et al.* (1996) incorporate variant-specific and cross-reactive immunity, as well as differences in growth and clearance rates of "competing" variants. Again, numerical simulation seems to be the only way forward: these models show that a hugely diverse range of outcomes is possible, and it is unclear what generalities might be revealed by further numerical exploration. And again, statements about who finally wins within a host may not be relevant; Bonhoeffer and Nowak (1994a) give an example in which strains that outperform their competitors in the long run nonetheless achieve small population sizes when summed over the whole infection.

12.6 Management Implications

Much of the motivation for thinking about the evolution of virulence (and the motivation for this volume) is that evolutionary models of virulence will contribute to disease management. It is certainly one of our interests. Yet we hope that the above summary cautions against the understandable urge to assume that elegant theory, even when relatively well-developed, is relevant to disease control in the field. We are not yet in a position to say even whether current kin-selection models are relevant to malaria. There is some evidence that the genetic architecture of malaria parasites is of the sort assumed by kin-selection models of virulence, but relatively little field evidence is in accord with the expectations of the models (indeed, the bulk of the evidence points to the reverse), and experimental evidence most likely to support the models has other explanations. Competition within hosts does occur, but our evidence to date suggests that within-host competition actually *enhances* the transmission success of individual clones. That it might actually be preferable to be competitively inferior within a host is an unexpected conclusion, and one that raises many new questions. If it proves to be a widespread phenomenon, it is difficult to see how kin-selection models of virulence, at least in their current form, can be profitably applied to malaria parasites. At the very least, these data demonstrate that unexpected phenomena may exist, which can confound theory based on intuitively appealing assumptions.

In light of this, we consider that the formulation of management advice from parasite-centered models of virulence currently is premature. The clinical outcome

of a malaria infection is undoubtedly affected by many things, including ecological factors such as inoculation dose and prior exposure (Box 12.1) and genetic factors in hosts as well as in parasites. All of these probably vary with the epidemiological situation. This makes it a challenge to assess the impact, if any, of evolutionary arguments that place parasite genetics center stage. As things stand, it is entirely conceivable that parasite adaptation may play, at best, a trivial role. Even if it is important, confounding factors may alter or even reverse the outcome of intervention strategies derived from evolutionary theory.

12.7 Discussion

We believe the data summarized above point to the need to understand both the epidemiological and evolutionary consequences of variation in the multiplicity of infection (see also Van Baalen and Sabelis 1995b). Intervention strategies designed to reduce exposure to infectious mosquitoes presumably reduce the average number of clones per host (data on that would be very interesting). What does this mean for average levels of infectiousness? Do multiple infections select for *reductions* in rates of host exploitation? How could that be stable? Experimentally, there are many challenges. When clones (which, on their own, are relatively virulent or avirulent) are in the same host, what happens to total virulence? In the absence of host death, can clones ever *reduce* the transmission success of others in the same host? Is the intrinsic virulence of a clone a more important determinant of the outcome than the initial conditions, such as size of inoculum, infection sequences, and inter-infection intervals? We hope this chapter has demonstrated how such questions are brought into sharp focus by trying to view the theoretical models in the context of particular disease realities. None of the issues are intractable; in the next few years it may be possible to evaluate more successfully the relevance of evolutionary theory for malaria control.

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