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Host–parasite interactions for virulence and resistance in a malaria model system

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Abstract

A rich body of theory on the evolution of virulence (disease severity) attempts to predict the conditions that cause parasites to harm their hosts, and a central assumption to many of these models is that the relative virulence of pathogen strains is stable across a range of host types. In contrast, a largely nonoverlapping body of theory on coevolution assumes that the fitness effects of parasites on hosts is not stable across host genotype, but instead depends on host genotype by parasite genotype interactions. If such genetic interactions largely determine virulence, it becomes difficult to predict the strength and direction of selection on virulence. In this study, we tested for host-by-parasite interactions in a medically relevant vertebrate disease model: the rodent malaria parasite *Plasmodium chabaudi* in laboratory mice. We found that parasite and particularly host main effects explained most of the variance in virulence (anaemia and weight loss), resistance (parasite burden) and transmission potential. Host-by-parasite interactions were of limited influence, but nevertheless had significant effects. This raises the possibility that host heterogeneity may affect the rate of any parasite response to selection on virulence. This study of rodent malaria is one of the first tests for host-by-parasite interactions in any vertebrate disease; host-by-parasite interactions typical of those assumed in coevolutionary models were present, but were by no means pervasive.

Introduction

Pathogen evolution can be a key obstacle in the development of effective disease control programmes. Drug resistance is the most obvious example (e.g. Nkengasong *et al.*, 2004; Talisuna *et al.*, 2004; Olliaro, 2005; Tripathi *et al.*, 2005), but the possibility that pathogen virulence (here defined as harm to the host following infection) could also evolve is beginning to attract the attention of evolutionary biologists (e.g. Ewald, 1994; Gandon *et al.*, 2001; Dieckmann *et al.*, 2002). For the most part, virulence evolution has been studied using parasite-centred models, where the direction of selection acting on parasite-encoded virulence is modelled as an optimality problem (e.g. Bremermann & Pickering, 1983; May & Anderson, 1983; Frank, 1996; Andre *et al.*, 2003; Choo *et al.*, 2003; Day & Proulx, 2004). This has led to ideas of virulence management (e.g. Dieckmann *et al.*, 2002) and specific predictions about, for instance, public health strategies which could prompt the evolution of benign parasites (e.g. Ewald, 1994) or create the conditions which would favour more virulent pathogens (Gandon *et al.*, 2001).

This parasite-centric approach to virulence evolution, which has attracted some controversy (e.g. Soubeyrand & Plotkin, 2002; Ebert & Bull, 2003), coexists uneasily alongside a largely independent body of work on host–parasite coevolution which emphasizes that parasite and host genotypes together determine virulence (e.g. Ebert & Hamilton, 1996; Woolhouse *et al.*, 2002; Lambrechts *et al.*, 2005). Part of the unease is semantic: the term virulence is used in some of the coevolutionary literature (e.g. gene-for-gene and matching allele models) to mean the ability to infect a host, rather than as harm *per se*. However, even with these models, and certainly for

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more general notions of on-going host–parasite arms races, the harm done to hosts following infection (the definition of virulence we use here) is a key source of selection. A more important difference between the two literatures is the nature of the genetic control of disease severity. Coevolutionary arguments necessarily posit that harm is determined by interactions between parasite and host genotypes, with particular parasite strains being harmful on some host genotypes and benign on others. The specificity of these host–parasite interactions provides the genetic basis of alleged ongoing coevolutionary dynamics. The evolution of virulence literature ignores such genotype-by-genotype specificity and emphasizes instead parasite-encoded virulence.

The parasite-centric and coevolutionary views, which emphasize, respectively, parasite main effects and host genotype by parasite genotype interactions are not necessarily mutually exclusive. Optimality models typically assume that a given pathogen strain has a virulence phenotype that is stable across a range of host genotypes (e.g. Andre *et al.*, 2003; Choo *et al.*, 2003; Day & Proulx, 2004) (Fig. 1a). Assuming that virulence and transmission are positively correlated, as is often the case (e.g. Lipsitch & Moxon, 1997; Mackinnon & Read, 1999), the transmission success and virulence of each parasite genotype could still vary, depending on for example, host genetic background or immune status (Fig. 1b). However, the relative impact of the different pathogen strains remains constant across host types. In contrast, a variety of nonadditive (host) genotype × (parasite) genotype interactions are also possible. In some cases, some pathogen strains may cause more harm than others, with this effect more pronounced in certain host genotypes than others (Fig. 1c). Host-by-parasite interactions in coevolutionary models are such that a pathogen strain that imposes high virulence in a particular host genotype could actually be the more benign strain in another genotype, commonly known as crossing of reaction norms (Fig. 1d).

If such genotype-by-genotype interactions are widespread, it becomes increasingly difficult to predict evolutionary responses to selection on pathogen-encoded virulence determinants. If virulence in nature is largely a consequence of the genotype-by-genotype specificity of host–parasite interactions, it is difficult to imagine that parasite-centred optimality models of pathogen virulence could provide much insight into evolutionary trajectories. Precisely analogous arguments can be made for host resistance (ability to control parasites). If genetic variation for resistance is largely due to a main effect of host, then resistance evolution can be modelled as a simple host-centred optimality problem. If control of parasites is predominately a consequence of the specificity of host-by-parasite interactions, as in Fig. 1d, it cannot.

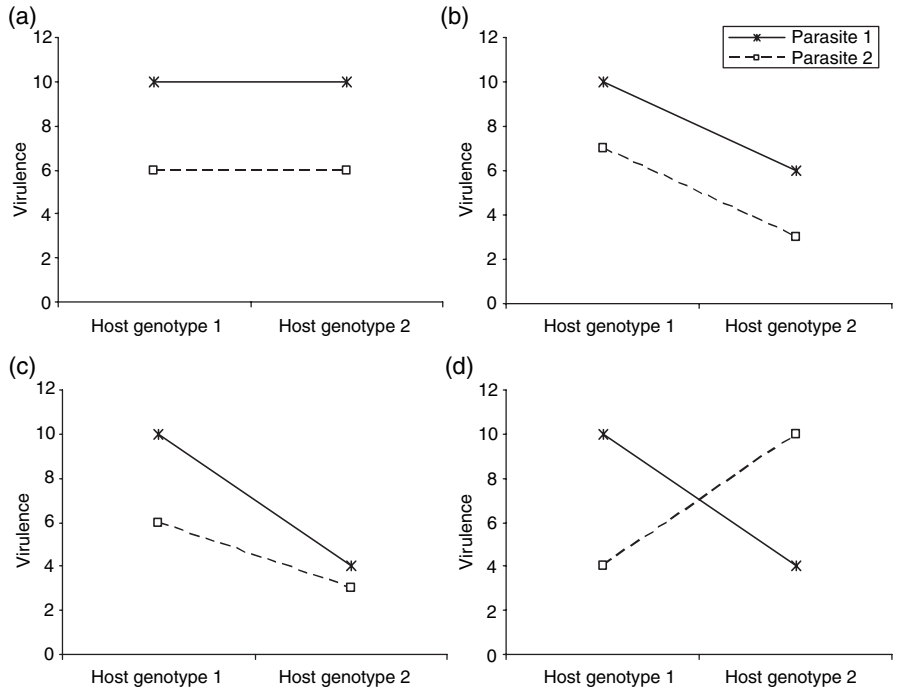

Fig. 1 A schematic representation of host, parasite and host-by-parasite interaction effects, showing a parasite main effect only (a), additive parasite and host main effects (b), nonadditive host and parasite interactions without crossing reaction norms where pathogen differences are more apparent in one of the host genotypes (c) and host-by-parasite interactions with crossing reaction norms of the sort assumed in most coevolutionary models (d). In this latter case, parasite genotypes virulent in one host genotype are less virulent in the other and vice versa.

There has been some work on specificity in host–pathogen interactions for plant and invertebrate hosts (e.g. Lively, 1996; Carius *et al*., 2001; Ferrari & Godfray, 2003; Little *et al*., 2006), but most studies on medically relevant diseases in vertebrates have concentrated on either host variation (e.g. Abel & Dessein, 1997; Amarante & Oliveira-Sequeira, 2002) or pathogen variation (e.g. Appleford & Smith, 1997; Macedo & Pena, 1998; Mackinnon & Read, 1999), but not both. Yet, in both clinical and animal model studies, host and parasite control of disease severity are well known. In malaria, for instance, it has long been recognized that both host factors (e.g. Stevenson *et al*., 1982; Burt, 1999; Troye-Blomberg, 2002) and parasite factors (e.g. Ariey *et al*., 2001; Read & Taylor, 2001; Mackinnon & Read, 2004a; Read *et al*., 2004; Kirchgatter & Del Portillo, 2005) can affect disease outcome. Indeed, studies on human malaria have given us some of the best examples of host factors associated with resistance/susceptibility, including sickle cell anaemia (e.g. Williams *et al*., 2005) and particular MHC alleles (e.g. Segal & Hill, 2003). Likewise, parasite-encoded phenotypes such as cytoadherence (e.g. Sherman *et al*., 2003) and rosetting (e.g. Rowe *et al*., 2002) are recognized to contribute to malaria severity. What is fundamentally lacking is any real understanding of the interactions between these host and parasite effects.

To address this, we performed a fully cross-factored experiment, using four parasite genotypes of *Plasmodium chabaudi* and four inbred mouse strains as hosts. We measured the virulence of the subsequent infections as well as within-host parasite densities, which is a standard measure of host resistance to malaria parasites (e.g. Fortin *et al*., 2001). So far as we are aware, this is one of the few times host-by-parasite interactions have been tested for in a medically relevant vertebrate system (see also Mackinnon *et al*., 2002, de Roode et al. 2004, discussed below). Our results showed a combination of all possible scenarios (Fig. 1a–d), depending on the measure of virulence or parasite success.

Materials and methods

Parasites and hosts

Plasmodium chabaudi isolates were collected from *Thamnomys rutilans* in the Central African Republic in 1969 and 1970. Genetically distinct parasite clones (Carter, 1978), representing a range of virulence levels were then obtained from different isolates as described by Mackinnon & Read (1999). These clones are maintained as frozen stabilates, with the precise point in the clonal histories from which they come denoted with a subscript code. Below, we refer to them only using their letter codes for simplicity, but the clones were as follows: AJ_{4607}, AS_{11918}, CW_{512} and ER_{577}.

Hosts were female mice of inbred strains CBA/CaOlaHsd, DBA/2OlaHsd, C57BL/6JolaHsd and NIH/OlaHsd (Harlan, Bichester, UK) aged 6–8 weeks. These hosts were chosen as they differ at the MHC (Lyon & Searle, 1989) and control densities of *P. chabaudi* (clone AS) to varying degrees and are therefore known as more or less 'resistant' strains (Stevenson *et al*., 1982; Stevenson & Skamene, 1986). Mice were fed on 41B maintenance diet (Harlan England) and drinking water was supplemented with 0.05% *p*-amino benzoic acid to aid parasite growth. Artificial light was provided from 05:30 to 17:30 hours. From hereon, hosts will be referred to as C57, CBA, DBA and NIH, with the term 'strain' used to denote mouse genotype and 'clone' to refer parasite genotype.

Experimental design and inoculation of mice with parasites

The experiment was conducted in two replicate blocks 4 weeks apart. Both blocks consisted of 16 infected treatment groups (four clones × four strains), each with three replicate mice. Infections were initiated with an intra-peritoneal injection of 1×10^6 parasitized red blood cells. Inoculations were prepared by diluting infected blood from donor mice in a calf-serum solution [50% heat inactivated Calf Serum; 45% Ringer solution (27 mM KCL, 27 mM$CaCl_2$ and 0.15 M NaCl) with 20 units of heparin mL^{-1}]. Control mice received the same volume of uninfected red blood cells in calf-serum solution.

Mice were sampled daily between days 3 and 21 post-injection. Sampling involved determining mouse weight to an accuracy of 0.1 g and red blood cell density using flow cytometry (Beckman Coulter, High Wycombe, UK). Thin smears from tail blood were fixed in methanol and stained in Giemsa to determine levels of asexual parasitaemia and gametocytaemia using 1000× microscopy.

Trait definition and statistical analysis

Prior to analysis we defined and constructed the following traits to describe part or all of the infection. To determine the total number of asexual parasites and gametocytes produced during the infection, we calculated the area under the curves for parasite and gametocyte density through time. Parasite densities and gametocyte densities are the products of the parasitaemia or gametocytaemia multiplied by red blood cell density. All density data were transformed using [$\log_{10}$ (density +10)] to normalize the residuals. Only mice surviving until the end of the experiment were used in analysis of these data. We also determined the maximum parasite density and the number of days taken to reach the maximum. As all mice that died had declining parasite densities when they died, they were included in the

maximum parasite density analysis. Three mice had standard asexual infection profiles, but produced no detectable gametocytes. We had never previously observed infections with no gametocytes, and as these three zero values generated enormous residuals, these mice were removed from the gametocyte analysis so as to not violate statistical assumptions.

As measures of virulence, we determined the 'minimum weight' and the 'minimum red blood cell density' reached during the experiment. Most models of virulence focus on the risk of death as a virulence measure, for which minimum weight and minimum red blood cell density are correlates (Mackinnon & Read, 2004a). It was assumed that all mice reach these minima prior to death. Therefore, even mice that died were included in the virulence analysis. Initial weight and initial RBC density were included as covariates where applicable and the density data were transformed using [$\log_{10}$] to normalize the residuals.

We investigated the effect of host strain (four levels), parasite clone (four levels) and a host-by-parasite interaction on these variables using General Linear Models (Minitab 14, Minitab Inc., State College, PA, USA) or Proportional Hazards (JMP 5.1, SAS Institute Inc., Cary, NC, USA). For all our models we first fitted the maximal models including the main effects of host strain, parasite clone and experimental block, a covariate (when relevant) and all two- and three-way interactions. We then minimized the models by removing nonsignificant terms ($P > 0.05$), beginning with the highest-level interaction. As 'block' main effects are of little biological interest in their own right, we reported them only if they interacted significantly with the host-by-parasite interaction. We also investigated the relationships between asexual parasites, transmission stages and virulence, which are key relationships in evolutionary theory (Frank, 1996), with regression analysis of the variables of 'total parasite density', 'total gametocyte density' and 'weight loss'.

Results

The kinetics of parasite and gametocyte densities are illustrated in Fig. 2 and virulence in Fig. 3. Within all host strains, parasites increased in density followed by a dramatic reduction and then recrudescence (Fig. 2). Patterns of virulence were largely the inverse of the parasite densities, with an initial reduction in both weight and red blood cell densities, followed by either a full or partial return to preinfection levels (Fig. 3). Nine of 96 mice died during the course of infection. Five of these were of the NIH strain, with three infected with the AJ parasite clone and two with ER clone. Three further deaths occurred in CBA hosts, with two of these infected with AS and the other with the AJ. One further death occurred in host strain DBA with an AJ clone infection.

Parasite dynamics

Total parasite density in an infection was significantly affected by host and parasite main effects, as well as by a host-by-parasite interaction (Fig. 4a; Table 1a; $P = 0.029$). However, across both blocks, clone AJ in host strain CBA displayed highly unusual dynamics, reaching its peak parasite density late in the infection (Fig. 2a). On its removal, the interaction term was no longer significant (Table 1b; $P = 0.37$). In this and all further analyses of parasite dynamics, if any other single group was removed, the host-by-parasite interaction remained significant or marginally significant. We therefore report all results with and without the AJ–CBA treatment group (Table 1).

Within each host strain, a clone effect on parasite density could be detected only in strains CBA and DBA ($F_{3,16} = 11.6$, $P < 0.01$; $F_{3,16} = 3.5$, $P = 0.037$ respectively; C57: $F_{3,16} = 1.9$, $P = 0.16$; NIH: $F_{3,16} = 0.97$, $P = 0.43$). In host strain CBA, total parasite densities of parasite clones AJ and ER were lower than densities of AS and CW, while in host strain DBA, total parasite density of clone AJ was greater than the others.

The average time taken to reach maximum parasite density ranged between 5 and 11 days. This timing was influenced by main effects and a host-by-parasite interaction (Fig. 4b, $\chi^2_9 = 19.04$,$P = 0.024$). On removal of the AJ–CBA treatment group, timing of the peak parasite density was not affected by host or parasite main effects or their interaction (host: $\chi^2_3 = 2.7$,$P = 0.47$; parasite: $\chi^2_3 = 6.7$ $P = 0.08$; host-by-parasite: $\chi^2_9 = 14.8$, $P = 0.09$). Total gametocyte density was also determined by a host-by-parasite interaction (Fig. 4c; Table 1a; $P = 0.04$). Removing the AJ–CBA treatment group, the interaction was no longer significant (Table 1b; $P = 0.29$). An effect of parasite clone on gametocyte density was detected within all host strains (C57: $F_{3,17} = 7.8$, $P < 0.01$; CBA: $F_{3,16} = 72.8$, $P < 0.001$; DBA: $F_{3,15} = 6.7$, $P < 0.001$; NIH: $F_{3,14} = 0.1.9$, $P = 0.025$).

Virulence

Some of the virulence variation was due to host-by-parasite interactions (Table 1, Fig. 5; minimum weight; $P = 0.0008$, minimum RBC; $P = 0.048$). As with parasite densities, exclusion of the AJ–CBA treatment group resulted in a nonsignificant host-by-parasite interaction term for minimum RBC (Table 1b; $P = 0.83$), whereas with the removal of any other group, the interaction still remained significant or marginally significant. In contrast, no single group accounted for the host-by-parasite interaction in minimum weight, which remained significant despite the removal of clone of the AJ–CBA treatment group (Table 1b; $P = 0.002$). Within each host strain, the effect of parasite clone on minimum weight reached during infection could only be detected in strains C57 and NIH ($F_{3,16} = 4.9$, $P = 0.012$; $F_{3,16} =$

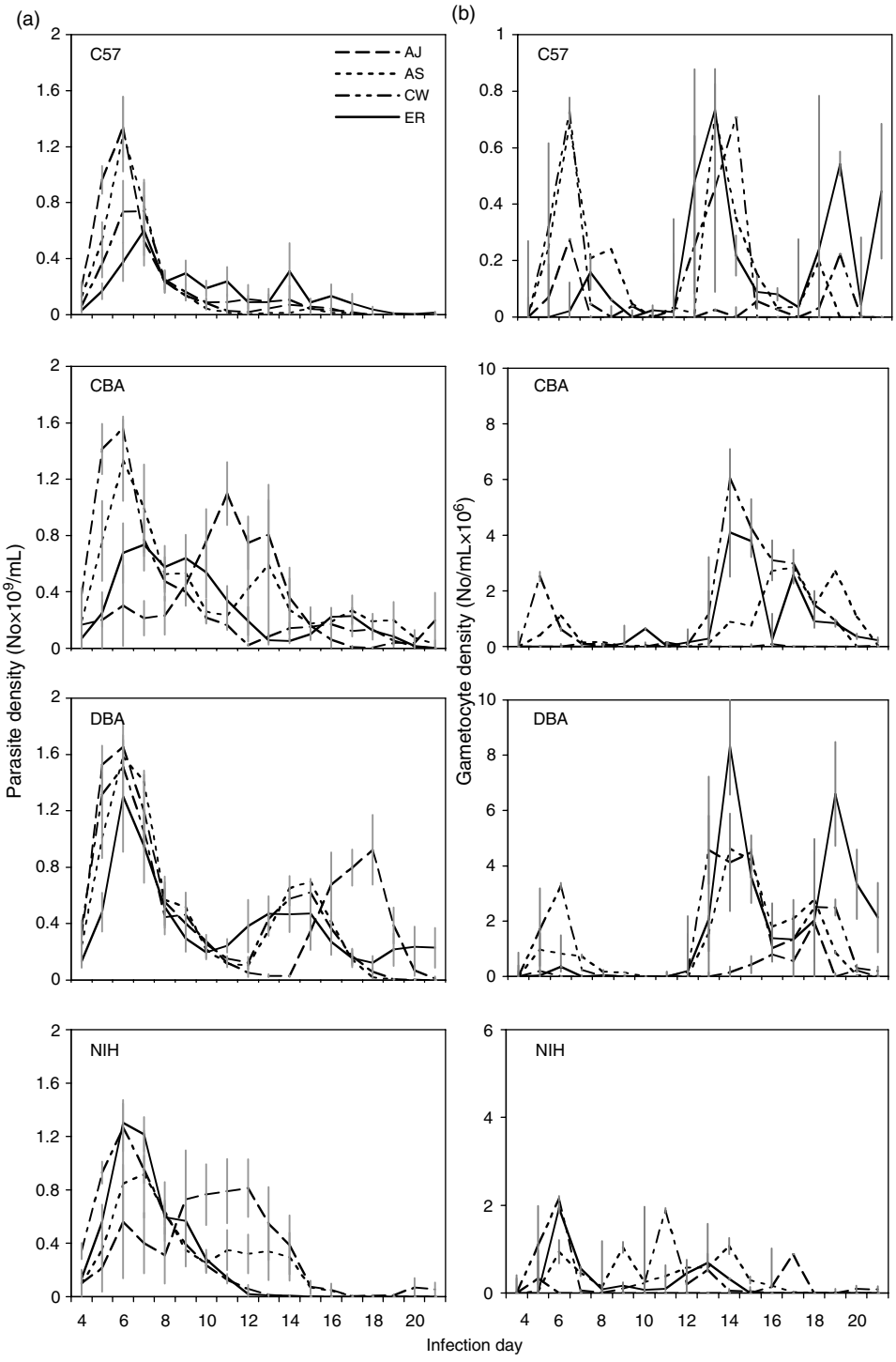

Fig. 2 Parasite (a) and gametocyte (b) densities through time, for each of the four *Plasmodium chabaudi* clones in each of the four host strains tested. Each line represents the mean density for each parasite clone (AJ, AS, CW or ER) in each host strain (C57, CBA, DBA and NIH) with the associated standard error, averaged across the two experimental blocks. Each data point is up to six mice. Note different *y*-axis for gametocyte densities, which usually constitute <1% of all parasites.

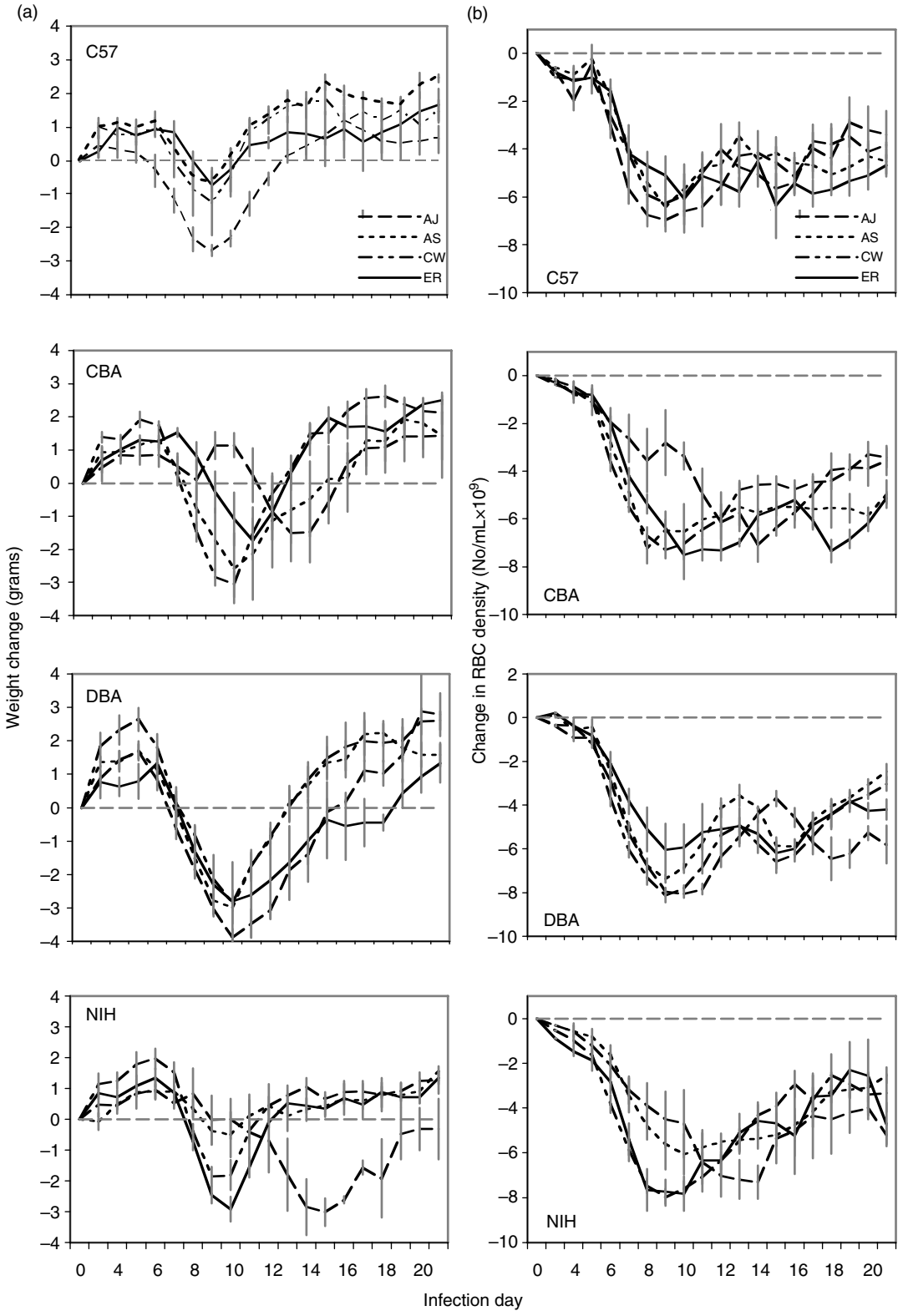

Fig. 3 Daily weight change (a) and red blood cell (RBC) density change (b), from preinoculation values (horizontal lines). Each line represents the mean for each parasite clone in each host strain as Fig. 2. Each data point is from up to six mice.

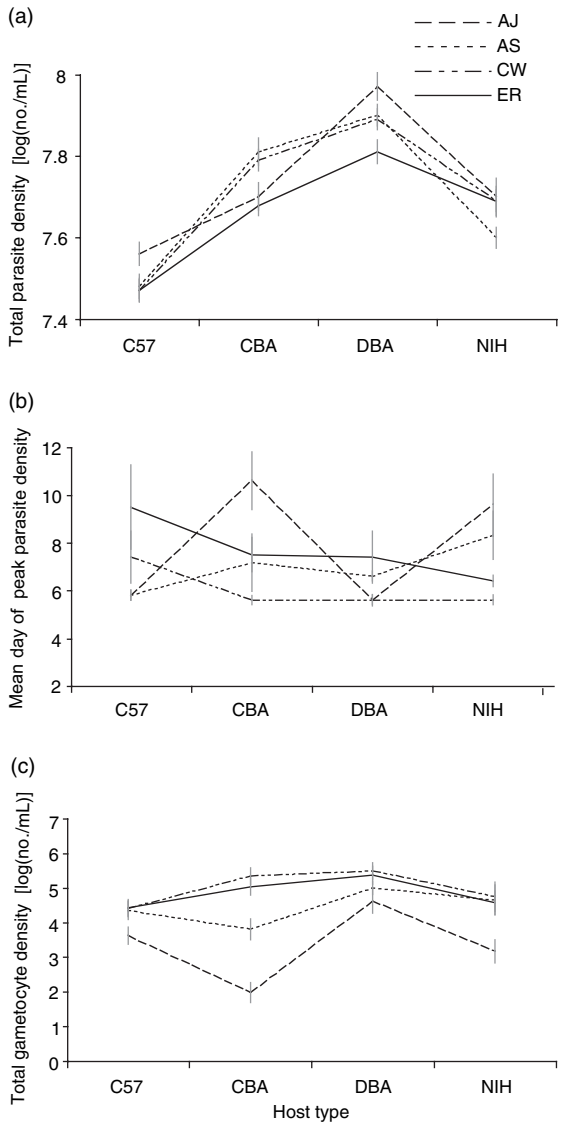

Fig. 4 Host-by-parasite interaction plots for resistance and transmission potential demonstrated by the (a) least square means of total parasite density, (b) mean day of peak parasite density, and (c) least square means of total gametocyte density, shown for each parasite clone (AJ, AS, CW or ER) in each host strain (C57, CBA, DBA and NIH) with the associated standard error. Each data point is for up to six mice.

8.4, $P = 0.001$ respectively; CBA: $F_{3,18} = 2.0$, $P = 0.14$; DBA: $F_{3,18} = 0.46$, $P = 0.7$). In both of these host strains, the parasite clones ranked from least to most virulent were: AS, CW, ER and AJ. Parasite differences in minimum RBC density could be detected in host strains C57 and DBA ($F_{3,16} = 4.4$, $P = 0.019$; $F_{3,17} = 3.2$, $P = 0.049$ respectively; CBA: $F_{3,18} = 1.7$, $P = 0.19$; NIH: $F_{3,17} = 1.3$, $P = 0.30$), with the same rank order.

Relationships between traits

In parasite-centred models of virulence evolution, it is frequently assumed that parasite densities and/or transmission will be positively correlated with virulence (Frank, 1996). We found that total gametocyte density was indeed positively correlated with total parasite density (Fig. 6a; $F_{1,74} = 24.4$, $P < 0.001$). Importantly, this relationship was not influenced by a host-by-parasite interaction (host × parasite × total parasite density; $F_{9,43} = 0.43$, $P = 0.9$). Likewise, total parasite density was correlated with maximum weight loss in a linear positive manner (Fig. 6b; $F_{1,74} = 32.8$, $P < 0.001$) and, again, there was no interaction (host × parasite × total parasite density; $F_{9,43} = 0.88$, $P = 0.5$). For both of these relationships, the 2-way interactions of host × total parasite density and parasite × total parasite density were also nonsignificant ($F_{1,52} = 1.1$, $P = 0.35$; $F_{1,52} = 1.1$, $P = 0.37$ respectively). There was no relationship between gametocyte density and weight loss (Fig. 6c; $F_{1,74} = 1.0$, $P = 0.31$) nor any host-by-parasite by weight loss interaction (host × parasite × weight loss; $F_{9,43} = 1.0$, $P = 0.39$). The two-way interactions of host × weight loss and parasite × weight loss, were significant predictors of gametocyte density ($F_{9,52} = 3.4$, $P = 0.02$; $F_{9,52} = 3.1$, $P = 0.03$), so that relationships between virulence and transmission potential depend on both the host strain and the parasite clone, but not on host-by-parasite interactions.

Discussion

In this study, we used the rodent malaria model system of *P. chabaudi* in laboratory mice to determine if host-by-parasite interactions were involved in determining the virulence, resistance and transmission potential of rodent malaria infections. The availability of a range of *P. chabaudi* clones and a range of distinct hosts provided us with an opportunity to test biological assumptions implicit in both evolution of virulence and coevolutionary theoretical models in a medically relevant animal model.

We detected host genotype-by-parasite genotype interactions, but found that they were generally of small effect size, primarily arising from the effects of one parasite clone in one particular host strain (Table 1a vs. 1b). Even with the inclusion of this treatment group, the host–parasite interaction term explained only 4.2% and 6.0% of the variance in the total parasite density and gametocyte density respectively (Table 1a). For virulence, host–parasite interactions explained more of the variance: for minimum weight, the interaction explained 8.9% of the variance, while for red blood cell density, it explained 34.2% of the variance. Again though, much of this was due to one particular host–parasite combination (Table 1a vs. 1b). Overall, the interactions we did detect showed patterns akin to both types of interactions

Table 1 The proportion of variance explained in resistance, transmission potential and virulence by the main factors of parasite clone and host strain, as well as the host-by-parasite interaction in a *Plasmodium chabaudi* infection for (a) all treatment groups, and (b) excluding the parasite clone AJ in host strain CBA, which displayed highly unusual parasite kinetics (Fig. 2; see text).

	Parasite	Host	Host × Parasite
(a)			
Total parasite Density	$F_{3,59}$ = 5.4; 3.4% **	$F_{3,59}$ = 135.1; 84.1% ***	$F_{3,59}$ = 2.3; 4.2% *
Total Gametocyte Density	$F_{3,56}$ = 36.4; 36.4% ***	$F_{3,56}$ = 29.9; 30.2% ***	$F_{3,56}$ = 2.1; 6.0% *
Weight	$F_{3,71}$ = 4.8; 3.9% **	$F_{3,71}$ = 96.3; 78.4% ***	$F_{3,71}$ = 3.7; 8.9% **
Red Blood Cell Density	$F_{3,72}$ = 3.9; 22.3% *	$F_{3,72}$ = 7.0; 39.7% **	$F_{3,72}$ = 2.0; 34.2% *
	Parasite	Host	Host × Parasite
(b)			
Total parasite Density	$F_{3,64}$ = 6.6; 4.4% **	$F_{3,64}$ = 130.7; 87.5% ***	$F_{8,56}$ = 1.1; 1.8% ns
Total Gametocyte Density	$F_{3,66}$ = 31.2; 47.4% ***	$F_{3,66}$ = 32.3; 49.4% ***	$F_{8,56}$ = 0.49; 2.1% ns
Weight	$F_{3,67}$ = 2.5; 5.0% *	$F_{3,67}$ = 99.2; 80.4% ***	$F_{8,56}$ = 3.4; 7.4% **
Red Blood Cell Density	$F_{3,72}$ = 3.9; 17.2% **	$F_{3,72}$ = 11.6; 22.0% ***	$F_{8,56}$ = 0.5; 7.8% ns

Our virulence measures were the 'minimum weight', with initial weight as a covariate and the 'minimum red blood cell density', with initial red blood cell density as a covariate (see text for more details). The percentage explained by each factor does not always add to 100% as some of the variance is explained by other terms in the model.
*P < 0.05; **P < 0.005; ***P < 0.0001; NS, nonsignificant.

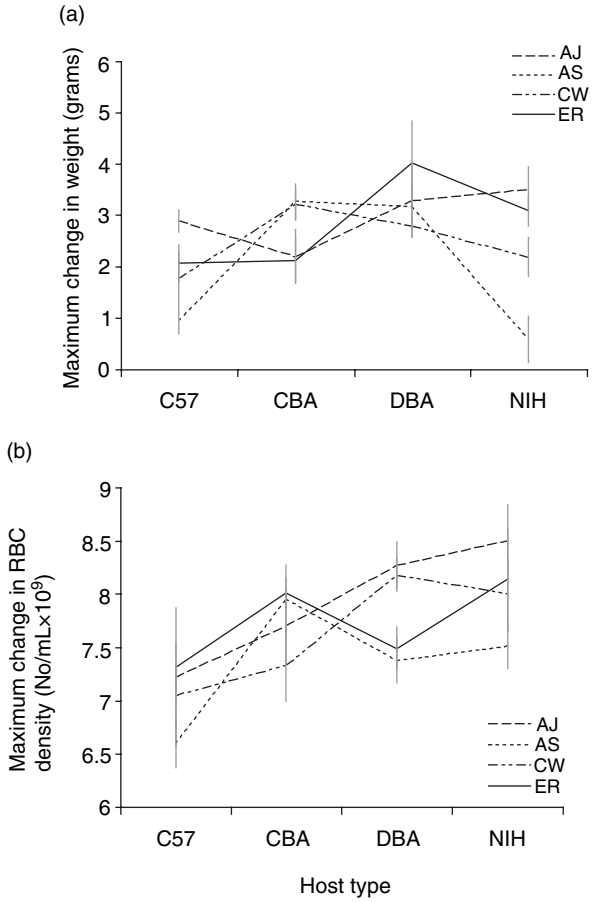

Fig. 5 Host-by-parasite interactions in virulence demonstrated by (a) the mean maximum change in weight and the (b) mean maximum change in RBC density from preinoculation values, with the associated standard error, averaged across two experiments. Each data point is up to six mice.

(Fig. 1c,d). Parasite clone effects were at times more pronounced in certain host strains than in others, while crossing of reaction norms were also observed (Figs 4 and 5). The limited size of our interaction effects, however, clearly inhibits our ability to make precise comparisons to the simplified scenarios of Fig. 1.

Experimental tests of virulence optimality theory and estimates of genotype-by-genotype interactions are very rare in vertebrate disease systems. In animal models of malaria, most work has been concentrated on different parasites in a single host genotype (e.g. Jarra & Brown, 1989; Mackinnon & Read, 1999; Paul *et al.*, 2004; de Roode *et al.*, 2005) or a single parasite line in different host genotypes (e.g. Stevenson *et al.*, 1982; Fortin *et al.*, 2001). We are aware of only two other malaria studies that simultaneously examined both host and parasite genotypes; neither found host–parasite interactions and both found, as we did, that host genotype was a relatively more important determinant of both resistance and virulence than was parasite genotype. In the first study, three different mouse strains were infected with either a low virulence clone of *P. chabaudi* or a more virulent line derived from it by serial passage. For these two highly related lines, there was no evidence of host–parasite interactions for any of the variables examined (Mackinnon *et al.*, 2002). The other study involved two unrelated clones in two strains of mice and again found no host–parasite interactions for virulence (de Roode et al. 2004). The four treatment combinations in that experiment were also present in the experiments we report here and included the AJ–CBA combination we found to be a marked outlier responsible for most of the host–parasite interactions (Table 1, Fig. 2). In that earlier study, the dynamics of clone AJ in CBA mice was much more in line with what we found for all other host–parasite combinations. We have no explanation for the difference

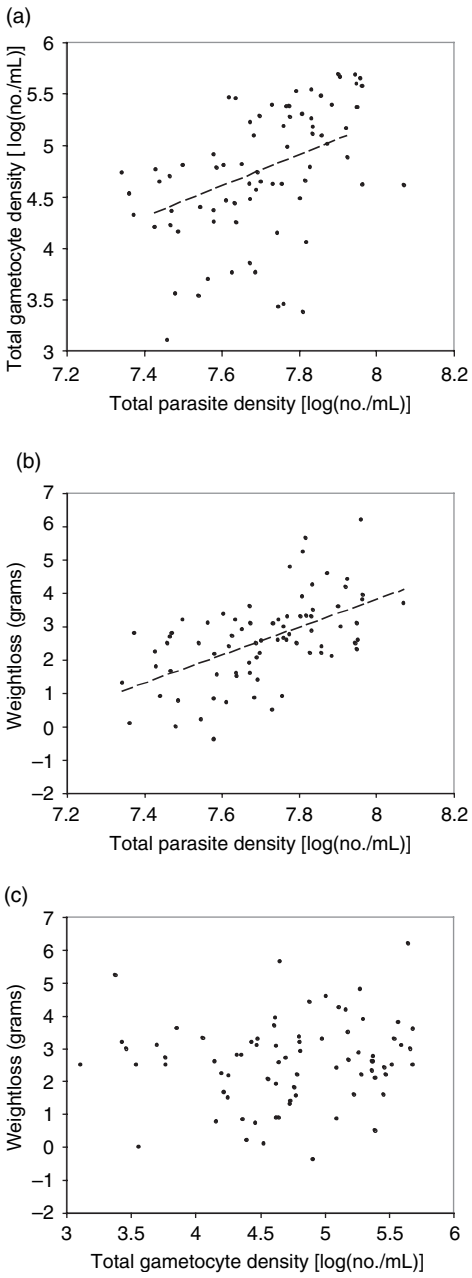

Fig. 6 The relationships between (a) total parasite density and transmission potential (total gametocyte density) (b) virulence (weight loss) and total parasite density and (c) virulence and transmission potential (total gametocyte density), during *Plasmodium chabaudi* infection. Each data point is a single mouse.

between the studies (same laboratory, mouse supplier, clonal lineages, diets, gender and approximate mouse weights), but we do note that the aberrant AJ–CBA dynamics we report here occurred in both our experimental blocks.

Our experiment thus emphasizes how the response to selection on parasite virulence could vary depending on host genotype: virulence variation was detectable in C57 mice but not, for example, in CBAs. As a consequence, it could be expected that the parasite response to selection on virulence would be more rapid in C57 mice than in CBAs. Genetic and/or phenotypic host heterogeneity may therefore affect virulence evolution (see also (Gandon & Michalakis, 2000; Gandon *et al*., 2002; Ganusov *et al*., 2002). Selection experiments involving serial passage of parasites have demonstrated that both naïve and semi-immune hosts can select for increased virulence, but passage through semi-immune hosts causes a more rapid virulence increase (Mackinnon & Read, 2004b). These different rates of evolution could be explained if the expression of virulence variation differed between the naïve and semi immune hosts, analogous to the differences we saw between host strains. Together these experiments show that predicting the response of selection on parasites will require understanding of how host genotype and/or phenotype affects the expression of virulence.

Determining whether semi-immunity affects the expression of virulence variation is not a relatively simple matter of repeating the experiments here using semi-immune animals. Immunity to malaria has a strain-specific component (e.g. Martinelli *et al*., 2005), so that choice of immunizing strain becomes critical. Crucially, though, such experiments would allow us to determine if host immunity, including immunity generated by vaccination, will enhance the importance of parasite genotype relative to the effects of host genotype as the determinant of virulence and resistance.

The experimental results we report here showed that highly specific host-by-parasite interactions neither dominated nor were wholly absent. It may be that our choice of host strains or of parasite clones is unrepresentative of genetic diversity of *Plasmodium*–host interactions in the field. If a generality does exist, control strategies may be greatly aided by knowing whether parasite-centred models or coevolutionary models best capture malaria evolution. Of course, as with our experiments, the real world may involve aspects of both.

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