Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals

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S-KaiC—the source of the crucial nonlinear feedback (Fig. 4A).

Using rate constants and a KaiA concentration dependence (table S2) derived solely from data on the non-oscillatory partial reactions (Fig. 2 and figs. S3 and S4), this simple model predicts (Fig. 4B) essential features of the circadian oscillator—period (~21 hours in the model), amplitude of total phosphorylation, sequential appearance of the phosphoforms, and the larger magnitude of the T-KaiC peak (see also fig. S7). This predictive ability suggests that the model captures the key elements of the in vitro oscillator. Modifying the model to explicitly treat the formation of KaiA-KaiC complexes (*9*) likely responsible for promoting KaiC phosphorylation makes it consistent with the observation that the oscillations are rather insensitive to the total concentration of Kai proteins (*9*) (supporting online text and fig. S8).

The following picture of the origin of stable oscillations emerges (fig. S6A). Starting from the unphosphorylated state, KaiA promotes phosphorylation that is kinetically favored at T432; subsequent phosphorylation at S431 produces ST-KaiC. ST-KaiC can decay via dephosphorylation of T432 to produce S-KaiC, but S-KaiC accumulation is slow because KaiA both inhibits that dephosphorylation and promotes rephosphorylation of S-KaiC to ST-KaiC. Thus, S-KaiC levels remain low until a substantial pool of ST-KaiC has formed. When S-KaiC levels do rise, KaiA activity is reduced, promoting dephosphorylation of ST-KaiC and thereby causing it to rapidly decay into S-KaiC. Thus, S-KaiC accelerates its own production (from ST-KaiC), which causes its concentration to overshoot the point at which KaiA is completely inactivated; this overshoot yields a reservoir of S-KaiC that permits extended inactivation of KaiA even as S-KaiC concentrations decrease through dephosphorylation. In the absence of KaiA activity, T-KaiC and ST-KaiC both dephosphorylate, and S-KaiC—which dephosphorylates more slowly—becomes the dominant remaining phosphorylated species. Eventually enough S-KaiC dephosphorylates for KaiA activity to return, and the cycle begins anew.

To focus on the essential slow dynamics and to be able to derive model parameters directly from our experimental data, our model ignores some known biochemical properties of the Kai proteins and abstracts others into the rate constants. KaiC exists as a hexamer (*4*), and we have neglected possible effects that depend on the state of the entire hexamer. Further, monomer exchange between hexamers (*9*) is not explicitly included, and we assume that inhibition of KaiA via KaiB occurs instantaneously upon formation of S-KaiC. In actuality, inhibition appears to take approximately 1 hour (fig. S9), possibly due to slow interaction between KaiB and KaiC or slow exchange of monomers between hexamers. These neglected effects have the potential to increase both the tendency of the system to oscillate and the amplitude of oscillation, but the success of our simplified model suggests that they are not part of the fundamental mechanism.

A recent report from the Kondo group (*26*) describes the differential phosphorylation of S431 and T432 during the circadian cycle and the interaction of KaiB with KaiC phosphorylated on S431. By using phosphomimetic KaiC mutants, they provide information about ordered phosphorylation complementary to and consistent with our kinetic study of wild-type KaiC.

The most striking behavior of the cyanobacterial circadian oscillator in vivo is its precision: Even with asynchronous cell division and an absence of external cues, the clock of a single cell and its offspring maintains precision to a small fraction of a day over several weeks (*27*). A reductive understanding of the various aspects of the clock—especially that of the core Kai oscillator presented here—should enable us to understand the effects of random fluctuations and variable environments. The *Synechococcus* clock provides an ideal model system for understanding how cells perform quantitative functions in highly variable intra- and extracellular environments.

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Supporting Online Material

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Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals

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Hosts can in principle employ two different strategies to defend themselves against parasites: resistance and tolerance. Animals typically exhibit considerable genetic variation for resistance (the ability to limit parasite burden). However, little is known about whether animals can evolve tolerance (the ability to limit the damage caused by a given parasite burden). Using rodent malaria in laboratory mice as a model system and the statistical framework developed by plant-pathogen biologists, we demonstrated genetic variation for tolerance, as measured by the extent to which anemia and weight loss increased with increasing parasite burden. Moreover, resistance and tolerance were negatively genetically correlated. These results mean that animals, like plants, can evolve two conceptually different types of defense, a finding that has important implications for the understanding of the epidemiology and evolution of infectious diseases.

Defense against pathogenic microorganisms and other parasites can be divided into two conceptually different components: resistance (the ability to limit parasite burden) and tolerance (the ability to limit the disease severity induced by a given parasite burden) (*1*–*4*). It is important to distinguish between these two components because, by definition, resistance has a negative effect on

parasites, whereas tolerance does not; as a result, their relative importance will have substantial consequences for the ecology and evolution of host-parasite interactions (*2*, *5*–*7*). The distinction between resistance and tolerance has attracted considerable attention in studies of the evolution of plant defense against parasites and herbivores (*3*, *6*, *8*). In this area, the emerging pattern is that plants generally exhibit genetic variation for both resistance and tolerance (*1*, *6*, *9*, *10*).

When it comes to animals, numerous studies have demonstrated genetic variation for resistance, where resistance is typically measured as the inverse of parasite burden (*11*–*14*). However, little is known about whether animals may also show genetic variation for tolerance. Yet together, resistance and tolerance are the two components of antipathogen defense that determine disease severity. Suggestive evidence for tolerance in animals comes from a study of α^+-thalassemia, a monogenic hemoglobin disorder in humans that protects against malaria. Individuals that are hetero- or homozygous for this mutation do not have lower infection intensities of *Plasmodium falciparum* than individuals that are homozygous for the wild type, but the degree of anemia at high infection intensities is diminished, thereby reducing mortality from malaria (*15*). Thus, it seems α^+-thalassemia affects tolerance but not resistance to *P. falciparum*. So far as we are aware, no study has yet formally disentangled genetic variation in these two components of defense in any animal host-parasite system.

In the plant literature, tolerance is usually defined as the slope of host fitness against infection intensity (*1*, *16*, *17*). In other words, the tolerance of a host genotype is its reaction norm to infection intensity. A tolerant genotype is one in which disease severity is relatively unaffected by increasing pathogen burden, whereas the fitness of a less tolerant genotype declines more rapidly as pathogen burdens rise (Fig. 1). If the reaction norms of different host genotypes vary (that is, if there is a statistical interaction between host genotype and infection intensity), then there is genetic variation for tolerance. We have borrowed this approach to defining and measuring genetic variation in tolerance from the plant literature and applied it to a malaria model system (*Plasmodium chabaudi* in laboratory mice) to investigate whether animal hosts may show genetic variation for tolerance and whether resistance and tolerance are correlated traits.

P. chabaudi is widely used as a model of human malaria (*18*, *19*). Previous studies have shown that there is considerable variation among mouse strains (i.e., genetic variation) for resistance to *P. chabaudi* (*20*–*22*). To investigate whether there is also genetic variation for tolerance, we performed an experiment with five different inbred mouse strains (*23*). Mice were infected with one of three different *P. chabaudi* clones or left uninfected in a fully factorial design. The experiment was performed in three experimental blocks separated in time. As with human malaria, one of the main causes of morbidity and mortality in rodent malaria is anemia. *P. chabaudi* also causes weight loss in mice. The degree of red blood cell (RBC) loss and weight loss is correlated with infection intensity and predicts mortality (*24*). To test for variation in tolerance, we therefore used minimum RBC density and minimum weight during the infection as measures of disease severity (analogous to host fitness used in the plant literature). Specifically, we tested whether the slopes of the relations between infection intensity and minimum RBC density or minimum weight differed between mouse strains.

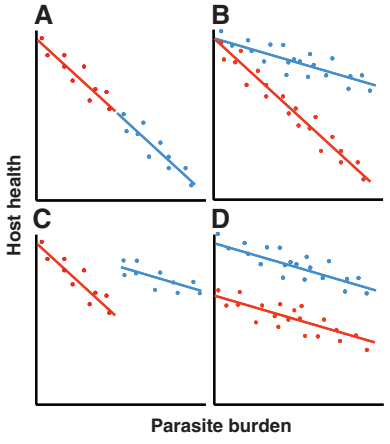

Fig. 1. Schematic figure showing reaction norms of two host genotypes (red or blue line) for disease severity across a range of infection intensities in individual hosts (dots). (**A**) Two equally tolerant genotypes differing in resistance; here, the red genotype has lower parasite burdens (is more resistant) and thereby maintains a higher health status when infected. (**B**) Two equally resistant genotypes (same average parasite burden), but here the red genotype is less tolerant (health declines faster with increasing parasite burden). (**C**) Genotypes differ in both tolerance and resistance; here, the more tolerant genotype (blue) is less resistant, so that both genotypes end up having, on average, the same health status when infected. (**D**) Host genotypes differ in neither resistance (same average parasite burden) nor tolerance (same slope). Instead, the genetic difference in health status is due to a difference in intercept, so that the difference exists even when animals are uninfected. It is thus indicative of genetic differences in "general vigor" (*8*) and has nothing to do with defense against the infectious agent in question. Because of the possible existence of variation in general vigor, tolerance has to be defined as a reaction norm, and so it can only be measured and compared across groups of animals (*17*). Thus, in contrast to resistance, it is not possible to compare the tolerance of two individual hosts. Demonstrating genetic variation for tolerance therefore requires that disease severity be assessed in animals of the same genotype across a range of infection intensities; a difference in slope between genotypes indicates genetic variation for tolerance.

As usual in this host-parasite system (*25*, *26*), there was a distinct peak in parasite density around day 8 post-inoculation. Minimum RBC density occurred

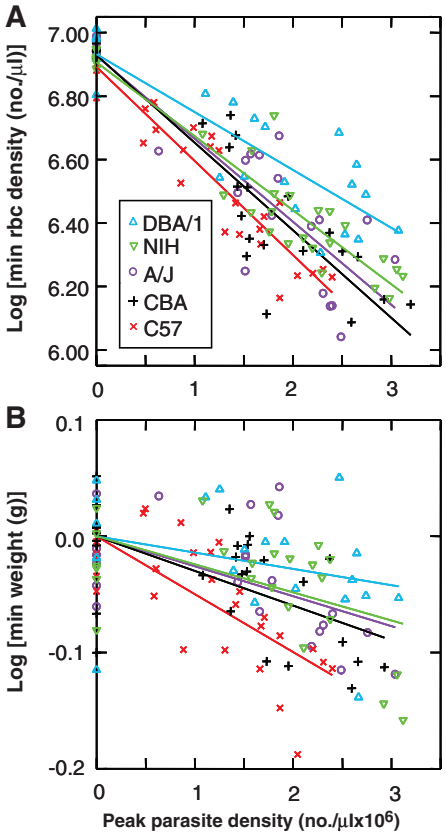

Fig. 2. Variation for tolerance among mouse strains. (**A**) Minimum RBC density (log-transformed) versus peak parasite density. Mouse strain × parasite density: $F_{4,117} = 6.08$, $P = 0.0002$; parasite density: $F_{1,117} = 173.3$, $P < 0.0001$; mouse strain: $F_{4,117} = 0.20$, $P = 0.94$; and experimental block: $\chi^2 = 22.1$, $P < 0.0001$. Initial RBC density [$F_{1,116} = 0.80$, $P = 0.37$], the quadratic terms [parasite density^2: $F_{1,117} = 0.76$, $P = 0.38$; strain × parasite density^2: $F_{4,111} = 0.33$, $P = 0.86$], and the interaction between block and strain ($P > 0.25$) were not significant and were therefore excluded from the model. (**B**) Minimum weight (log-transformed) versus peak parasite density. Strain × parasite density: $F_{4,110} = 6.06$, $P = 0.0002$; parasite density: $F_{1,111} = 8.09$, $P = 0.0053$; parasite density^2: $F_{1,111} = 34.4$, $P < 0.0001$; mouse strain: $F_{4,110} = 2.76$, $P = 0.031$; initial weight: $F_{1,111} = 140$, $P < 0.0001$; and experimental block: $\chi^2 = 18.1$, $P < 0.0001$. Strain × parasite density^2 [$F_{4,105} = 1.20$, $P = 0.31$] and the interaction between block and strain ($P > 0.25$) were not significant, and these terms were therefore excluded. To facilitate the comparison of slopes and because the initial weight (the intercept) differed between strains but for the present purposes is an irrelevant main effect when testing for resistance and tolerance, the reaction norms for weight have been scaled so that all genotypes have an intercept of zero. DBA/1, NIH, A/J, CBA, and C57 are the different mouse strains.

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around day 11, on average 2.49 ± 0.88 (mean ± SD) days after the peak parasite density. Minimum weight occurred around days 10 to 11, on average 1.60 ± 2.89 days after the peak parasite density (fig. S1).

To test for variation in resistance among mouse strains, we performed an analysis of peak parasite density against mouse strain and parasite clone. Peak parasite density differed between mouse strains [$F_{4,102}$ = 15.54, P < 0.0001] and parasite clones [$F_{2,103}$ = 64.81, P < 0.0001], but there was no strain-by-clone interaction [$F_{8,102}$ = 0.66, P = 0.73]. There was also a significant effect of experimental block (χ^2 = 47.4, P < 0.0001), but no interactions between block and strain and/or clone (P > 0.25). Thus, as in previous studies (*20*–*22*, *25*), mouse strains differed in resistance, and parasite clones differed in the infection intensity that they induced.

To test for genetic variation for tolerance, we performed analyses of minimum RBC density and minimum weight against peak parasite density (both linear and quadratic terms), mouse strain, their interactions, and pre-inoculation values of RBC density or weight. In the case of both RBC and weight loss, there were highly significant interactions between strain and parasite density (Fig. 2). Thus, there was variation among mouse strains in tolerance measured in terms of either anemia or weight loss. This conclusion is robust to the inclusion of parasite clones in the statistical models, the exclusion of uninfected animals from the analyses, or the use of different infection intensity measures (see supporting online material text).

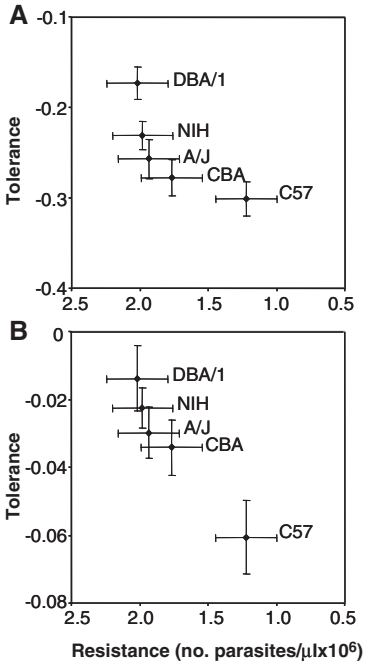

Fig. 3. Trade-off between tolerance and resistance. (**A**) Correlation between resistance (inverse of peak parasite density) and tolerance in the form of minimum RBC density during infection (that is, tolerance measured as the slope of a regression of minimum RBC density against peak parasite density). (**B**) Correlation between resistance and tolerance in the form of minimum weight during infection (that is, tolerance measured as the slope of a regression of minimum weight against peak parasite density). Plots show mean ± SEM for each mouse strain.

To test whether these two estimates of tolerance were correlated, we calculated the slopes of minimum weight and RBC density against the peak parasite density for each mouse strain. There was a significant correlation between the two measures of tolerance (Spearman's rank correlation, r_s = 1.0, n = 5 mouse strains, P < 0.05).There were also significant negative correlations between resistance and both measures of tolerance (r_s = –1.0, n = 5, P < 0.05 in the case of both RBC loss and weight loss) (Fig. 3). DBA mice, for example, were more tolerant and less resistant than C57s, which were the opposite. Thus, reduced tolerance is a cost of resistance and vice versa.

Our studies demonstrate that the conceptual and analytical framework developed by plant evolutionary biologists can also be used to reveal genetic variation for tolerance to infectious diseases in animals. The existence of genetic variation for both resistance and tolerance means that host defense can take a variety of evolutionary trajectories in response to pathogen pressure. The mechanistic basis of the genetic variation in tolerance we report remains to be determined. Variation in tolerance measured as RBC loss could occur because either the rate of regeneration of RBCs or the rate of destruction of RBCs by parasites and/or host immune responses varies among strains. The correlation between tolerance measured as RBC and weight loss suggests that there is a common underlying factor between these two forms of tolerance. In plants, where tolerance has long been studied, genes conferring disease tolerance have yet to be identified at the molecular level (*6*). In our disease model, resistance and tolerance were traded off against each other (Fig. 3). A similar trade-off has previously been demonstrated in the context of plant defense against herbivory (*2*). In the case of infectious diseases, a trade-off could arise if the price of more aggressive immune control of infection is increasing collateral damage (immunopathology).

Our findings, if they prove to be general, have important implications for our understanding of the ecology and evolution of animal host-parasite interactions. First, whereas the evolution of resistance has a negative effect on the prevalence of the infectious agent in the host population, tolerance should have a neutral or positive effect. Thus, resistance and tolerance have contrasting effects on the epidemiology of infectious diseases (*5*, *7*). Second, hosts and parasites are commonly thought to be engaged in antagonistic coevolution, where evolution of host resistance selects for counteradaptations in the parasite, which selects for improved resistance in the host and so on, leading to open-ended nonequilibrium evolutionary dynamics (*27*). However, tolerance does not have a negative effect on the fitness of the parasite, and so it cannot fuel antagonistic coevolution in the same way as is expected of resistance. Genetic variation for tolerance can therefore be expected to allow the sort of host evolution that will substantially dampen antagonistic coevolution (*6*).

Beyond evolutionary ecology, there is a clear need to recognize and separate the two components of disease defense in the context of animal breeding. For instance, attempts to enhance yield in agricultural animals by artificial selection on disease resistance traits or on total yield in the face of infection [often referred to as "resilience" (*28*)] could generate a variety of more or less desirable outcomes, depending on how resistance or yield varies with tolerance. The experimental and analytic approach used here is readily transferable to domestic animals where it could be used to work out optimal selection strategies.

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Supporting Online Material

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References

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