E-Article

Multiscale Immune Selection and the Transmission-Diversity Feedback in Antigenically Diverse Pathogen Systems

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abstract: Antigenic diversity is commonly used by pathogens to enhance their transmission success. Within-host clonal antigenic variation helps to maintain long infectious periods, whereas high levels of allelic diversity at the population level significantly expand the pool of susceptible individuals. Diversity, however, is not necessarily a static property of a pathogen population but in many cases is generated by the very act of infection and transmission, and it is therefore expected to respond dynamically to changes in transmission and immune selection. We hypothesized that this coupling creates a positive feedback whereby infection and disease transmission promote the generation of diversity, which itself facilitates immune evasion and further infections. To investigate this link in more detail, we considered the human malaria parasite *Plasmodium falciparum*, one of the most important antigenically diverse pathogens. We developed an individual-based model in which antigenic diversity emerges as a dynamic property from the underlying transmission processes. Our results show that the balance between stochastic extinction and the generation of new antigenic variants is intrinsically linked to within-host and between-host immune selection. This in turn determines the level of diversity that can be maintained in a given population. Furthermore, the transmission-diversity feedback can lead to temporal lags in the response to natural or intervention-induced perturbations in transmission rates. Our results therefore have important implications for monitoring and assessing the effectiveness of disease control efforts.

Keywords: antigenic diversity, immune selection, recombination, positive feedback, *Plasmodium falciparum*, individual-based model.

Introduction

Many pathogens have evolved to optimize their transmission potential by evading host immune responses. One of the most common immune evasion strategies is the exploitation of allelic polymorphisms at key antigenic sites that renders acquired responses from previous encounters ineffective. Antigenic diversity can play a key role both at the within-host level, where it helps to prolong infectious periods, and at the population level, where it facilitates reinfection of hosts with preexisting immunity.

Within-host antigenic diversity, whereby the expression of prominent immune targets are altered over the course of a single infection, results in repeated immune escape and enhances the pathogen's chance of transmission to another host. The molecular mechanism underlying the generation of within-host diversity range from error-prone replication, as in the case of human immunodeficiency virus (McMichael and Phillips 1997), to more sophisticated strategies involving programmed and reversible switches in antigen expression. The latter is often referred to as clonal antigenic variation and is found in many pathogens transmitted by insect vectors or through sexual contact, where onward transmission is more uncertain and can be interrupted for long periods of time (reviewed in Barbour and Restrepo 2000; Deitsch et al. 2009). These pathogens include the sleeping sickness–causing African trypanosomes (Borst and Rudenko 1994), the sexually transmitted bacterium *Neisseria gonorrhoeae* (Hill and Davies 2009), and the causative agent of Lyme disease, *Borrelia burgdorferi* (Barbour 1991).

Antigenic variability at the population level has an equally beneficial effect on disease transmission by allowing the parasite to infect hosts with previous exposure to the same pathogen but in an antigenically distinct form. In contrast to many childhood diseases, such as measles, where recovered individuals remain protected against reinfection for life, antigenically diverse pathogens can infect the same host multiple times, often with little or no fitness effect on subsequently infecting parasites. Pathogens may differ greatly with regard to the extent of antigenic diversity at any one point in time and space, ranging from the cocirculation of multiple serotypes, as in the cases of rotavirus (Santos and Hoshino 2005) and *Neisseria meningitidis* (Tondella et al. 2000), to the seasonal replacement of dominant strains, as observed for influenza A virus (Russell et al. 2008). However, the consequences

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in terms of expanding the pool of susceptible hosts are equivalent, and it is this ability to circumvent herd immunity that poses a considerable challenge for the development of effective vaccines.

The mechanisms underlying antigenic diversification at the population level, including mutation, recombination, and phase variation, are similar to the ones underlying within-host variability. In fact, it is often the within-host processes that generate and maintain the diversity found at the population level, and many pathogens can be found to actively exploit diversity at both scales. One of the best-studied organisms where multiscale antigenic diversity is a key factor underlying its global success is the human malaria parasite *Plasmodium falciparum*, which relies on clonal antigenic variation to prolong infectious periods and thus overcome the uncertainties associated with being vector transmitted. Transcriptional switches between members of the *var* gene family during infection changes the expression of variant surface proteins PfEMP1 (*P. falciparum* erythrocyte membrane protein 1), which are targets of adaptive immune responses and important virulence factors (Borst et al. 1995; Craig and Scherf 2001; Peters et al. 2002; Scherf et al. 2008; Kirkman and Deitsch 2012). Each parasite carries a repertoire of around 60 *var* genes, but there is little concordance between the repertoires of individual parasites (Barry et al. 2007; Kraemer et al. 2007; Rask et al. 2010; Tessema et al. 2015). Consequently, numerous infections and, hence, exposure to a large number of antigenic variants is required for individuals to acquire protection from symptomatic and life-threatening disease (Bull et al. 1998; Reyburn et al. 2005; Langhorne et al. 2008; Chan et al. 2012).

The diversity of *var* genes and *var* gene repertoires is mainly generated by frequent intra- and intergenic recombination events, respectively (Conway et al. 1999; Freitas-Junior et al. 2000; Taylor et al. 2000; Bopp et al. 2013; Claessens et al. 2014). Mitotic recombination between individual *var* genes during asexual replication in the blood has the potential to generate new *var* gene variants. These might not necessarily contribute directly to within-host immune evasion, as seen in other antigenically variable parasites, such as trypanosomes or *Babesia* (Barbour and Restrepo 2000; Deitsch et al. 2009), but may be passed on as part of the genomic *var* gene repertoire during transmission. Meiotic recombination, on the other hand, occurs during sexual replication inside the mosquito vector and operates at the genome level, where it is responsible for the creation of new *var* gene repertoires when mosquitoes are infected by more than one parasite genotype. Importantly, the probability of this happening is itself related to population-level prevalence and diversity. This is because hosts are more likely to carry multiclonal infections (Vafa et al. 2008; Gatei et al. 2015) and be infected by parasites with different antigenic repertoires (Chen et al. 2011) when prevalence and diversity are high.

The link between within-host and between-host diversity and their role in immune evasion has important but often overlooked consequences for the epidemiology of antigenically diverse pathogens. Specifically, for pathogens where infection and transmission events generate novel antigenic variants, such as *P. falciparum*, we would expect that diversity and disease prevalence are coupled via a positive feedback mechanism, with an increase in one leading to a subsequent increase in the other. This has not yet been explored in detail, however.

To investigate this proposed feedback between diversity and infection prevalence, we developed an individual-based model in which diversity is explicitly generated through processes underlying infection and is allowed to respond dynamically to changes in disease transmission. Using *P. falciparum* as a model system, we demonstrate how this transmission-diversity feedback can introduce temporal lags in the system's response to environmental or control-induced external perturbations, with potential implications for the assessment of intervention measures.

Methods

We developed a stochastic individual-based transmission model of *Plasmodium falciparum*, explicitly accounting for host and mosquito demographics, parasite diversity, and infection and transmission events.

Mosquito Demographics

Mosquitoes are modeled individually and can be uninfected, exposed, or infectious. Mosquitoes are assumed to die only of natural causes and are immediately replaced with new uninfected individuals to maintain a constant population. The age-related probability of death is modeled using a logistic function:

$$p_{\text{death}}(T_{\text{m}}) = \frac{0.25}{1 + e^{-0.5(T_{\text{m}} - \mu_{\text{m}})}}, \tag{1}$$

where $\mu_{\text{m}} = 32$ days is the average lifespan and T_{m} is the mosquito's age in days.

Host Demographics

Human hosts are modeled individually, and host demographic processes are modeled by assuming a daily probability of death, given as

$$p_{\text{death}}(T_{\text{h}}) = e^{-\lambda T_{\text{h}}} - 1, \tag{2}$$

where $\lambda = -1.5e^{-6}$, corresponding to a mean life expectancy of 55 years, and T_{h} is the host age in years (i.e., we assume a constant probability of death over the course of a year). We did not account for maternal protection and assumed that

on death individuals are immediately replaced by an immunologically naive newborn to maintain a constant population.

Antigen Representation and Parasite Strains

Each parasite carries a repertoire of 60 distinct antigen variants. The antigens themselves are encoded by a 32-bit binary number, which we refer to as the antigen's sequence type (*st*), where the leading (leftmost) $32 - k$ bits of this sequence determine the (immunologically defined) antigen type (a). This distinction between sequence type and antigen type allows us to consider cases where two different sequence types encode the same antigen type, in line with the fact that not every nucleotide change results in an antigenic change. The antigen type is calculated as

$$a = \left\lfloor \frac{st}{2^k} \right\rfloor \bmod A, \tag{3}$$

where $A = 2^{32-k}$ defines the size of the total antigenic space.

Within this framework, we define a parasite strain **s** by its antigen repertoire, where two parasites are considered different strains if their repertoires differ by at least one antigen, defined by its antigen type. Mathematically we can represent **s** as an A-dimensional vector containing 0s and 1s, where each element s_a indicates the presence ($s_a = 1$) or absence ($s_a = 0$) of antigen a.

An example 16-bit antigen representation with $k = 6$ is visualized in figure 1, in which the antigen-encoding bits are shaded in blue.

Transmission and Infection

Mosquitoes are assumed to bite humans at a constant rate, b. When an infectious mosquito bites a host, it transmits the infection with probability p_{trans} unless the host already has immunity to all of the infecting strain's (**s**) antigenic variants or is at its maximum capacity for concurrent infections (see below). On infection the parasite will try to express its antigenic

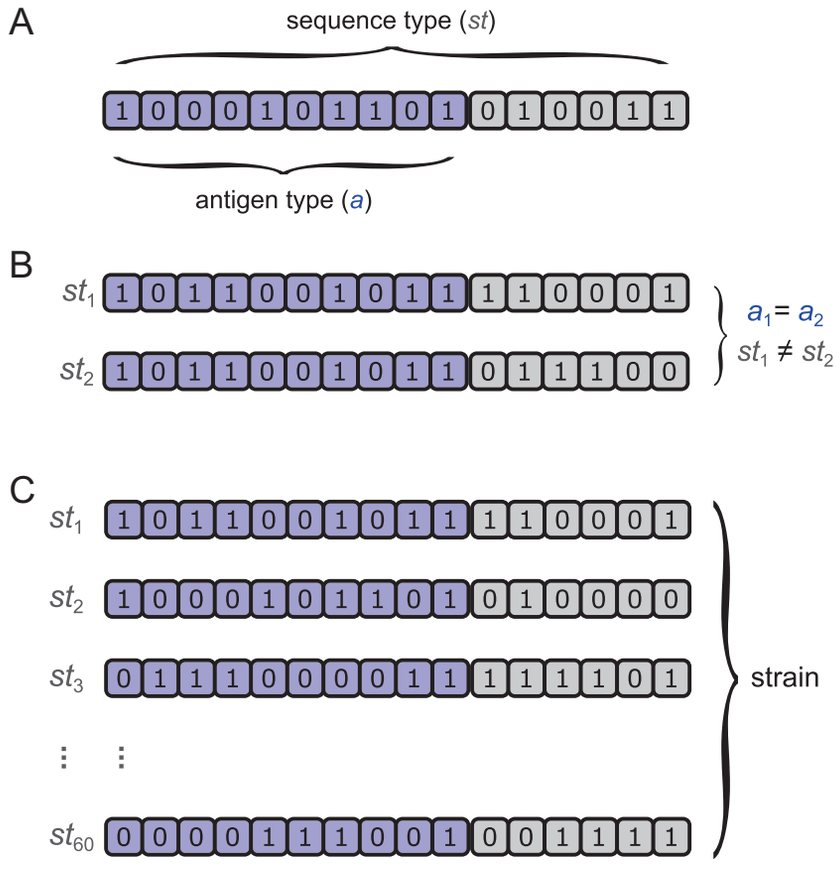

Figure 1: Example antigen representation as a 16-bit sequence. *A*, The antigen type (a) is determined by the leading 10 bits (shaded in blue), whereas the sequence type (*st*) is determined by the whole 16-bit sequence. The number of bits encoding only sequence type ($k = 6$, shaded in gray) determines the number of possible antigen representations for each antigen type. *B*, Separating antigen type and sequence type permits two different genes (st_1 and st_2) to encode the same antigen type. *C*, A parasite strain is defined by its repertoire of 60 distinct antigen types.

repertoire over the course of the infection by means of clonal antigenic variation. The within-host infection dynamics are not explicitly modeled here; instead, we calculate the duration of the infection as a function of the number of novel antigenic variants the parasite presents to the host (Holding and Recker 2015), given as

$$\omega(\mathbf{h}, \mathbf{s}) = \alpha < 1 - \mathbf{h}, \mathbf{s} >, \quad (4)$$

where α is the maximal contribution to the infection length by a novel antigen (i.e., an antigen to which the host has no immunity to). The term $\mathbf{h} = \{h_1, h_2, \ldots, h_A\}$ is the host's immune status vector (with respect to all possible variants $a \in A$), where h_a represents the degree of protection against antigen type a, with $h_a = 1$ corresponding to complete immunity to antigen a and $h_a = 0$ corresponding to complete susceptibility. Therefore, if the host has no immunity to any of the infecting parasite's antigen repertoire, the infection length will be 60α, whereas this would be reduced to $\alpha \Sigma_a (1 - h_a)$ if the host has already been exposed to a subset of the antigens.

As we are not explicitly modeling the within-host dynamics, infected humans are assumed to be equally infectious over the course of the infection. The intrinsic incubation period in the human host is not expected to have any influence on the system's dynamics (as it is very small compared with the average human life expectancy), and we therefore assumed that humans become infectious at the onset of an infection. If a susceptible mosquito bites an infectious host, it will become infected with probability p_{trans} and infectious after an extrinsic incubation period of $L = 10$ days. If the host is infected by more than one strain, the mosquito will become infected with a recombinant strain (see below).

For computational simplicity, we limited the number of concurrent infections to two. Although the multiplicity of infection can easily exceed this limit, especially in regions of high transmission intensities, this is the simplest setup that facilitates meiotic recombination in the mosquito following a bloodmeal on a coinfected host. Mosquitoes are limited to one infection, and once infected they are assumed to stay infected with that strain for life.

Immunity

By default we assumed that immunity is strictly variant specific and is the result of the parasite expressing its antigenic repertoire—or rather the subset that the host has not yet seen—over the course of an infection. We make the simplifying assumption that on infection the host's immune status changes immediately to reflect exposure to all antigens of the infecting strain's repertoire. Hence, the success of subsequent infections is subject to these changes even if a host has not yet cleared the ongoing infection.

In addition to variant-specific immunity, we also considered that exposure to variant a can induce cross protection against other, antigenically similar variants, where the degree of protection decays with distance in antigen space from a. We implemented cross-immunity by an additive transformation of the immune status vector, $\mathbf{h}$, using a Gaussian distribution with the mean corresponding to the antigen type and the strength of cross-immunity determined by the variance. The change in the immune status with exposure to antigen a can then be calculated by

$$\Delta h_{i,a} = \exp\left(-\frac{(i-a)^2}{2\gamma}\right) \quad \text{for } i \in \{1, 2, \ldots, A\}, \quad (5)$$

where γ controls the strength of cross-immunity. For computational simplicity, we truncated the tails of the Gaussian distribution to zero where $\Delta h_{i,a} < 0.01$.

Recombination

We considered diversity generation through recombination at both the gene level and the genome level. For sexual recombination, we assumed that two strains picked up by the mosquito from a multiclonal infection give rise to a single recombinant strain that infects the mosquito. Meiotic recombination thus takes place in our model at the moment of feeding, and this avoids modeling the dynamics of multiple strains within the mosquito. This also allows us to simplify mosquito infections to a single strain while still modeling the generation of new antigen repertoires and the flow of genes between strains. An important consequence of this is that it assumes that meiotic recombination can occur only between strains taken up from the same host rather than from multiple feeds from different hosts. This may thus underestimate diversity generation in our model, especially in combination with our restriction to two concurrent infections.

During meiotic recombination, the antigen repertoire of the recombinant strain is generated from the parental repertoires in a genewise manner by probability p_s. That is, we assumed that a gene can be taken from either parent strain in an independent manner. Although this might not be the most biologically realistic assumption and ignores any intragenomic structuring (e.g., by means of chromosomal location and upstream promoters), it maximizes the generation of new repertoires.

Mitotic recombination between individual genes is assumed to occur during asexual reproduction in the host (Bopp et al. 2013; Claessens et al. 2014). This involves recombination between two genes within the same antigen repertoire and in the model leads to the replacement of one of these genes with a gene encoding the recombinant antigen, leaving all other genes unmodified. It is expected that parasites carrying a novel gene resulting from mitotic recombination make

up only a small fraction of parasite population in the host and that the magnitude of (antigenic) change is too small to have any bearing on an ongoing infection. Additionally, we expect the probability of mosquitoes picking up these parasites to be much lower than the more numerous original clone. For computational reasons, we do not compute this at the within-host level. Instead, we assumed that a recombinant gene is copied to the transmitted strain with a small per-gene probability, p_c, which incorporates the probability that a recombinant parasite is taken up during the blood meal as well as the rate of intragenic (mitotic) recombination.

Mitotic recombination is implemented by adding a number, r, to the original antigen representation, where r is proportional to the difference between the original and donor antigens, given as

$$r = \kappa\theta(a_i - a_j), \tag{6}$$

where κ is a random number drawn from a uniform distribution $\mathcal{U}(-1, 1)$, θ scales the magnitude of change, and a_i and a_j are the original and donor antigen types, respectively. The new recombinant gene is then represented as

$$g_i^* = g_i + r, \tag{7}$$

where g_i is the full sequence type representation (not antigen type) of the parent antigen.

This scheme avoids having to simulate low-level biological mechanisms such as insertion and deletion of domains or nucleotide sequences between genes, leading to a computationally efficient model that emulates the main features of recombination: (i) recombination between antigenically similar donor genes is likely to produce recombinant genes that are similar to the donor genes, (ii) recombination between antigenically dissimilar genes more likely results in a recombinant antigen that differs from the original genes, and (iii) recombination events alter the sequence type but not necessarily the antigenic type, although these silent changes can accumulate over time and can eventually lead to changes in the antigen type.

Initialization

The model was initialized with both human and mosquito populations at a demographic equilibrium. A small number of mosquitoes were initialized as infected, while all humans were initialized as naive (i.e., with no prior exposure). As a result, a burn-in period was required to allow the system to reach a dynamic equilibrium. We generated an initial pool of antigens of size A_{init} from a uniform distribution over the entire sequence type space. Next we generated S_{init} strains by randomly sampling from the antigen pool without replacement (or with replacement in cases where $A_{\text{init}} < S_{\text{init}}$), which were then used to infect mosquitoes, such that an approximately equal number of mosquitoes were infected by each initial strain.

Table 1 summarizes the main parameters and parameter ranges used in our model. Throughout we use the baseline values for each parameter (as indicated in table 1) unless otherwise specified.

Table 1: Main model parameters and their default values

Parameter	Description	Baseline value (range)[a]
H	Host population size	10,000 [4,000, 25,000]
μ_h	Average human life expectancy in years	55
M	Mosquito population size	10,000 [4,000, 25,000]
μ_m	Average mosquito life expectancy in days	32
L	Extrinsic incubation period	10
b	Daily mosquito bite rate	.12 [0, .5]
p_c	Mitotic recombination rate	.002 [0, .02]
p_s	Meiotic recombination rate	.01 [0, .1]
A	Size of antigen space	50,000 [60, 60,000]
S_{init}	Number of initial strains	50 [1, 1,000]
A_{init}	Number of initial antigens	3,000 [60, 60,000]
I_{init}	Number of initially infected mosquitoes	250 [50, 2,500]
R	Parasite antigen repertoire size	60
k	Number of bits encoding only sequence type	7
θ	Recombination scaling factor	1 [.005, 100]
α	Scale of per-antigen contribution to infection length	3 [.4, 45]
p_{trans}	Transmission probability	.5 [.05, 1]
γ	Strength of cross-immunity	0[b] (0, 10]

[a] Ranges indicate the values over which we have tested the robustness of the model.

[b] Cross-immunity was not considered in the default setting.

The model was implemented in C++ and is provided in a zip file, available online.[1]

Results

We investigated the effect of antigenic diversity on malaria transmission and prevalence by means of a stochastic individual-based model in which antigen variants are dynamically generated through recombination and in which the diversity of the parasite population emerges from the underlying transmission dynamics (see "Methods"). To highlight how population-level prevalence and transmission rates are related to parasite diversity, we first show the model behavior assuming static levels of antigenic diversity—that is, without accounting for recombination—before investigating the dynamical feedback between parasite antigenic diversity and malaria epidemiology.

Static Diversity

First we considered the situation in which diversity is static, that is, with mitotic and meiotic recombination turned off. Strains were initialized by randomly selecting antigens without replacement to ensure that there is no overlap between repertoires. This allowed us to assess the general effect of diversity without the added complications of immune interactions. Without the possibility of new variants entering the population, the model converged toward an equilibrium-like state dictated by the background transmission rate, here quantified as the daily biting rate, and the total level of diversity among the parasite population (fig. 2*A*). With no mechanism for the generation of new antigen variants, diversity decreased over time because parasite strains were subject to stochastic extinction. In fact, in each model run we observed that only a certain proportion of the initial set of variants was maintained over a given period of time. We therefore describe this state as a semiequilibrium because epidemiological processes are at equilibrium with respect to transmission but stochastic extinction means that diversity is slowly lost from the system over time. The size of the host population is thus a crucial factor influencing the relationship between malaria prevalence and parasite diversity. Larger populations are known to be able to maintain higher degrees of diversity, and we see the same phenomenon in our model. That is, we found a strong positive correlation between host population size and the proportion of initial antigens retained and, hence, an overall increase in prevalence (fig. 2*B*).

As expected, for a given number of antigenic variants that cocirculate in the population there is a positive but nonlinear relationship between mosquito biting rate and population-level parasite prevalence, here defined as the proportion of the population that is currently infected. After an initial steep increase in prevalence with increasing rates of transmission, the relationship plateaus, up to a point where increases in transmission do not raise parasite prevalence any further (fig. 2*C*). This scenario, which we refer to as "transmission saturated," occurs as hosts acquire immunity to the vast majority of the antigenic variants available in the population. Therefore, the attained equilibrium rate in prevalence is strongly dependent on antigenic diversity, with higher levels of diversity enabling the parasites to more readily find susceptible hosts, leading to a higher proportion of infected individuals (fig. 2*C*).

A similar relationship is also found between antigenic diversity and parasite prevalence, with increasing levels of diversity leading to an increase in prevalence, at least up to a point where further diversification does not affect prevalence any more. This scenario, here referred to as "diversity saturation," occurs when the number of malaria infections a host acquires over a lifetime is simply limited by exposure. As a result and pretty much as expected, higher levels of exposure (i.e., biting rates) will shift the equilibrium levels of parasite prevalence upward, as shown in figure 2*D*.

Dynamic Diversity

As shown in figure 2, there is a strong link between malaria prevalence and parasite antigenic diversity, that is, the degree to which the parasite can circumvent immune responses and establish infections even in preexposed and semi-immune individuals. In the examples shown above it was assumed that diversity was an initially fixed but slowly declining quantity. In reality, however, antigenic diversity in *Plasmodium falciparum* malaria is the result of dynamic processes, predominantly recombination, whose rates are determined by epidemiological parameters related to transmission and prevalence.

To demonstrate the effect of considering diversity as a dynamic property, we ran our model without recombination for a number of years until it reached a dynamic equilibrium state before turning recombination on. As illustrated in figure 3, allowing new variants and parasite strains to be generated over time leads to a significant increase in overall diversity, here defined as the proportion of all possible antigenic variants, *A* (fig. 3*A*). This increase in diversity effectively reduces population-level immunity, as hosts will not have experienced the newly generated variants before (fig. 3*B*). As a result, parasites are more likely to find susceptible hosts, leading to an increase in parasite prevalence (fig. 3*B*) and, hence, disease transmission (here measured by the entomological inoculation rate [EIR], shown in fig. 3*D*), even without changes to the transmission potential through biting rates.

1. Code that appears in *The American Naturalist* is provided as a convenience to the readers. It has not necessarily been tested as part of the peer review.

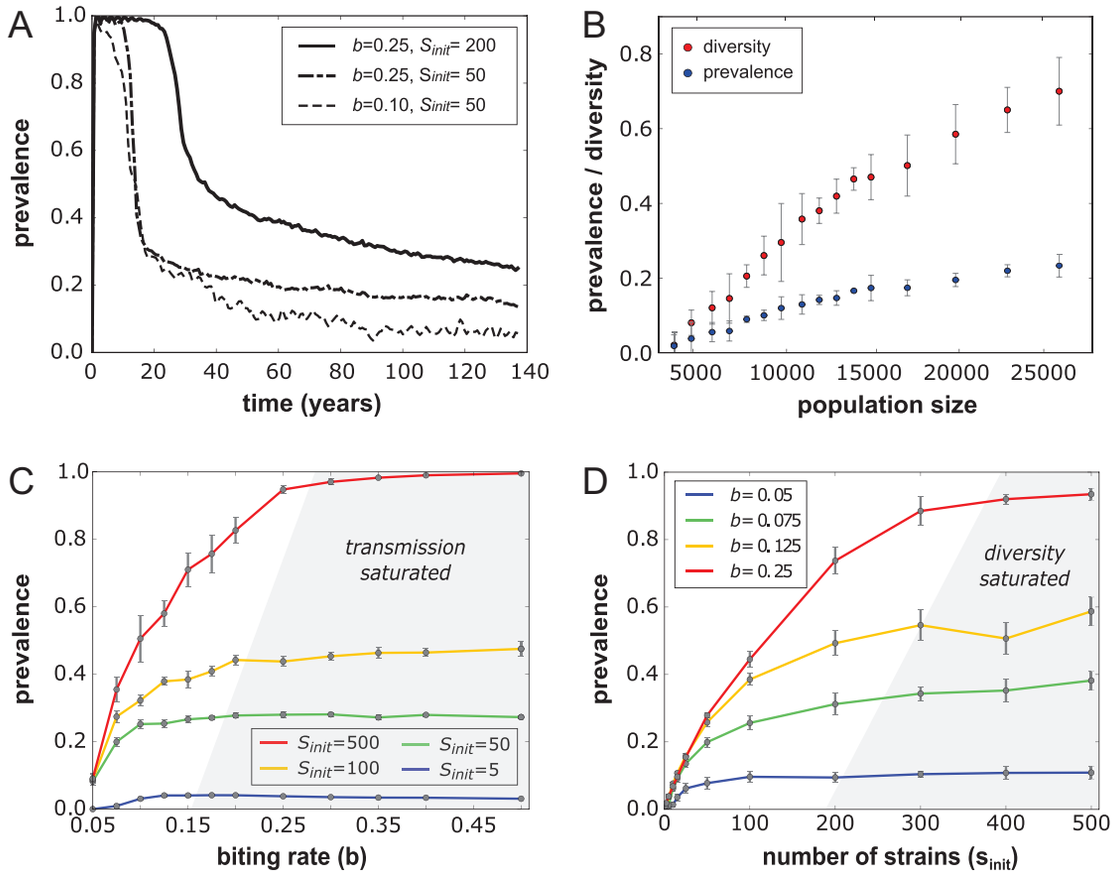

Figure 2: Relationship between transmission potential, antigenic diversity, and malaria prevalence. *A*, Simulated time series showing how malaria prevalence, defined as the proportion of the population infected by the parasite, converges toward an endemic equilibrium determined by the daily biting rate b and antigenic diversity S_{init}. *B*, Diversity, here measured as the proportion of initially circulating antigenic variants that are maintained in a population, is positively correlated with the size of the host population (assuming equal $M:H$ ratios), which also affects the equilibrium levels of malaria prevalence. *C*, Equilibrium levels of malaria prevalence as a function of the transmission potential (biting rate) under different levels of antigenic diversity. In all cases, prevalence plateaus and does not increase further with increasing biting rates; we refer to this regime as "transmission saturated." *D*, Equilibrium levels of malaria prevalence as a function of diversity under different levels of transmission, showing a plateauing behavior where prevalence does not increase any further with increasing levels of diversity; we refer to this regime as "diversity saturated." Results for *B*–*D* are based on 10 model runs, with error bars indicating the SEs around the mean. Parameter values are as in table 1 unless stated otherwise.

Not surprisingly, we found a strong correlation between the recombination rate and the system's response with regard to these epidemiological determinants.

Figure 3 demonstrates the positive feedback between parasite antigenic diversity and disease prevalence in the population. What is apparent is that this process is bounded in that the system will settle into a new equilibrium balanced between diversity generation, determined by recombination and background transmission rates, and diversity loss, due to demographic and immune selection–associated risk of extinction. It is interesting to note that diversity reaches an equilibrium after prevalence plateaus. This is because despite being linked through a positive feedback mechanism, diversity and prevalence are limited by different factors. Diversity is limited by the ability of the parasite population to retain antigenic variants, which itself depends on the parasite population size and, hence, prevalence. Prevalence, on the other hand, can be limited by either transmission (diversity saturation; fig. 2*D*) or diversity (transmission saturation; fig. 2*C*). As the parasite population diversifies, the system transitions into a diversity-saturated state, causing prevalence (and parasite population size) to plateau while diversity can still increase toward its maximum.

Immune selection in particular has a strong and expected effect on both antigenic diversity and parasite prevalence. That is, theoretical models have repeatedly shown how cross-immunity can structure antigenically variable pathogen populations into sets of strains with nonoverlapping antigenic

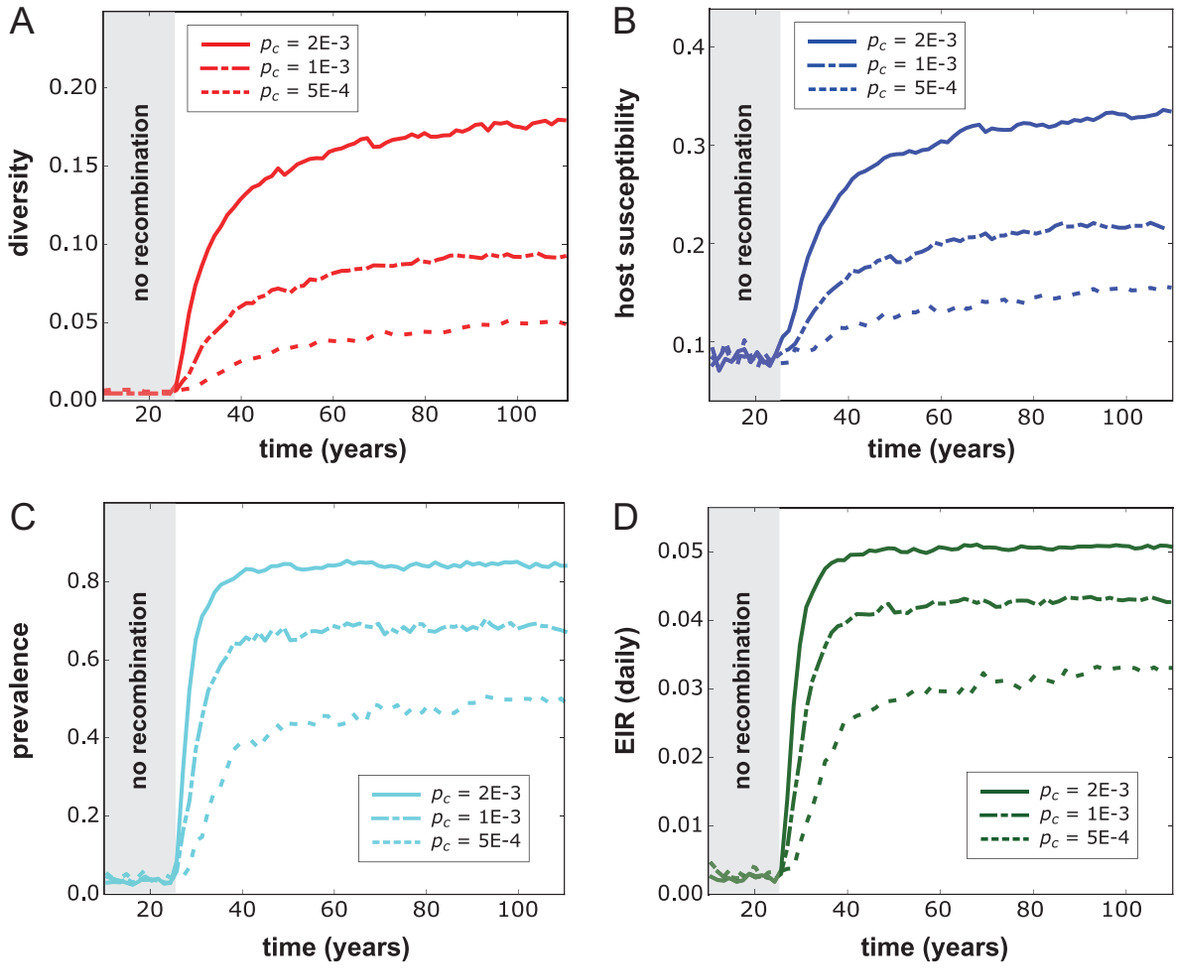

Figure 3: Diversity as an emergent property of infection and transmission. *A*, Allowing for recombination to create new antigenic variants and antigenic repertoires significantly increases the level of diversity among the parasite population, here defined as the percentage of the assumed maximum level of diversity. *B*, As diversity increases, host susceptibility increases as parasites carrying novel variants find it easier to reinfect individuals with prior immunity. *C*, Increasing diversity and host susceptibility leads to higher malaria incidence and population-level prevalence. *D*, Increasing the number of infected hosts increases the overall transmission intensity (entomological inoculation rate [EIR]) even without changes to the biting rate. Different lines denote different rates of recombination (p_c), showing how higher rates of diversity generation relate positively with parasite prevalence and disease transmission. Parameters values are as in table 1 unless stated otherwise.

repertoires (Gupta et al. 1996; Gupta and Anderson 1999), although different population structures can also emerge depending on the degree of cross-immunity and assumptions regarding transmission and recombination (Artzy-Randrup et al. 2012). In our model, increasing the degree of cross-immunity that a variant antigen elicits against antigenically similar variants enhances the selection pressure on the pathogen to find susceptible hosts, leading to an increased risk of extinction and thus a decrease in overall diversity and parasite prevalence. This is demonstrated in figure 4, where we simulated our model using the same assumption about transmission and recombination under increasing immune-selection pressure (degree of cross-immunity, γ).

Diversity and Prevalence under Changing Transmission Rates

As diversity and prevalence are coupled dynamically and are partially determined by the transmission potential in terms of mosquito biting rate, we hypothesized that there must be a lag in the system's response to temporal changes in disease transmission, which could be caused by changes in mosquito population density or bed net usage. We analyzed this by increasing or decreasing the biting rate over a period of 4 years and recorded the resulting response in antigenic diversity (fig. 5*A*, 5*C*) and parasite prevalence (fig. 5*B*, 5*D*) over time.

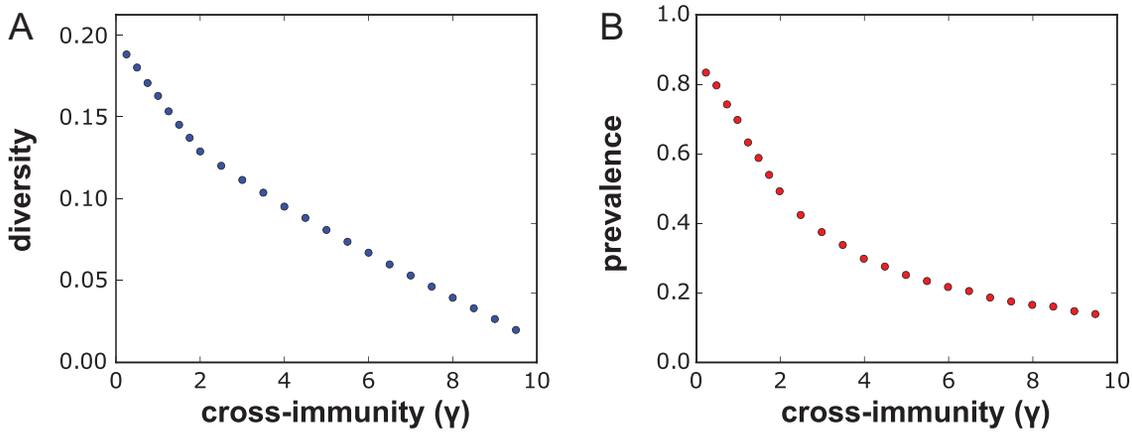

Figure 4: Antigenic diversity and malaria prevalence as a function of immune selection pressure. Cross-immunity determines the degree of inhibition that each antigenic variant elicits against antigenically similar variants, such that higher levels of cross-immunity increases the selection pressure on the parasite population, which in turn limits the number of variants that can be maintained in a population (*A*) and thus decreases the overall level of malaria prevalence (*B*). Each point is the average equilibrium level based on 10 model runs. Parameters values are as in table 1 unless stated otherwise.

In cases of both increasing and decreasing transmission potential the model showed a predictable response, with higher transmission rates leading to higher levels of diversity and prevalence rates and vice versa. However, in particular in those cases where we simulated an increase in transmission (fig. 5*A*, 5*B*), we also observed a certain inertia where both diversity and prevalence kept increasing for many years despite no further changes to the biting rate. This can be explained by the positive feedback loop between diversity and prevalence, where a change in one property has a delayed downstream effect. Interestingly, though, we found that the system would generally respond quicker to decreases in transmission, although even in those cases it took many years for the system to attain a new state of equilibrium. An explanation for this is that because of high selection pressure it is easier for antigenic variants to become extinct than for new variants to be generated and become established in the population.

The system's intrinsic inertia also leads to the phenomenon of hysteresis, where different rates of transmission can have very different outcomes in terms of diversity and prevalence, depending on whether there has been an increase or a decrease in the biting rate. This is shown in figure 5*E* and figure 5*F*, which depict the levels of diversity and parasite prevalence during the transition from low to high (blue lines) followed by high to low (red lines) mosquito biting rates for 100 repeat simulations. What is clear from these graphs is that the relationship between malaria prevalence and other external factors that could influence its transmission potential is highly nonlinear and time lagged to the point where observed changes in malaria incidence, for example, could be due to changes in mosquito abundance that had happened a considerable period of time in the past.

Discussion

Here we analyzed the diversity-transmission feedback and its implication for the epidemiological dynamics of antigenically diverse pathogens. Using an evolutionary framework in which diversity is an emergent property of the dynamic processes underlying infection and transmission events, we have demonstrated how population-level parasite prevalence and incidence are intrinsically linked via diversity, and how this can create temporal lags in how the system reacts to perturbations in disease transmission rates. Although we concentrated our analysis on the human malaria parasite *Plasmodium falciparum*, our results should be broadly applicable to other multistrain disease systems where novel antigenic variants can be readily generated through processes related to infection and transmission and where variants might equally be lost from the population through stochastic extinction. For those systems, we would then expect to find a nonlinear and time-varying relationship among incidence, prevalence, and the pathogen's transmission potential, implying that diversity needs to be considered explicitly in theoretical approaches trying to elucidate this relationship from empirical data.

For *P. falciparum* malaria, epidemiological studies have revealed a strong nonlinear relationship between transmission intensity, determined by means of the EIR, and parasite prevalence, which increases steeply under low to medium

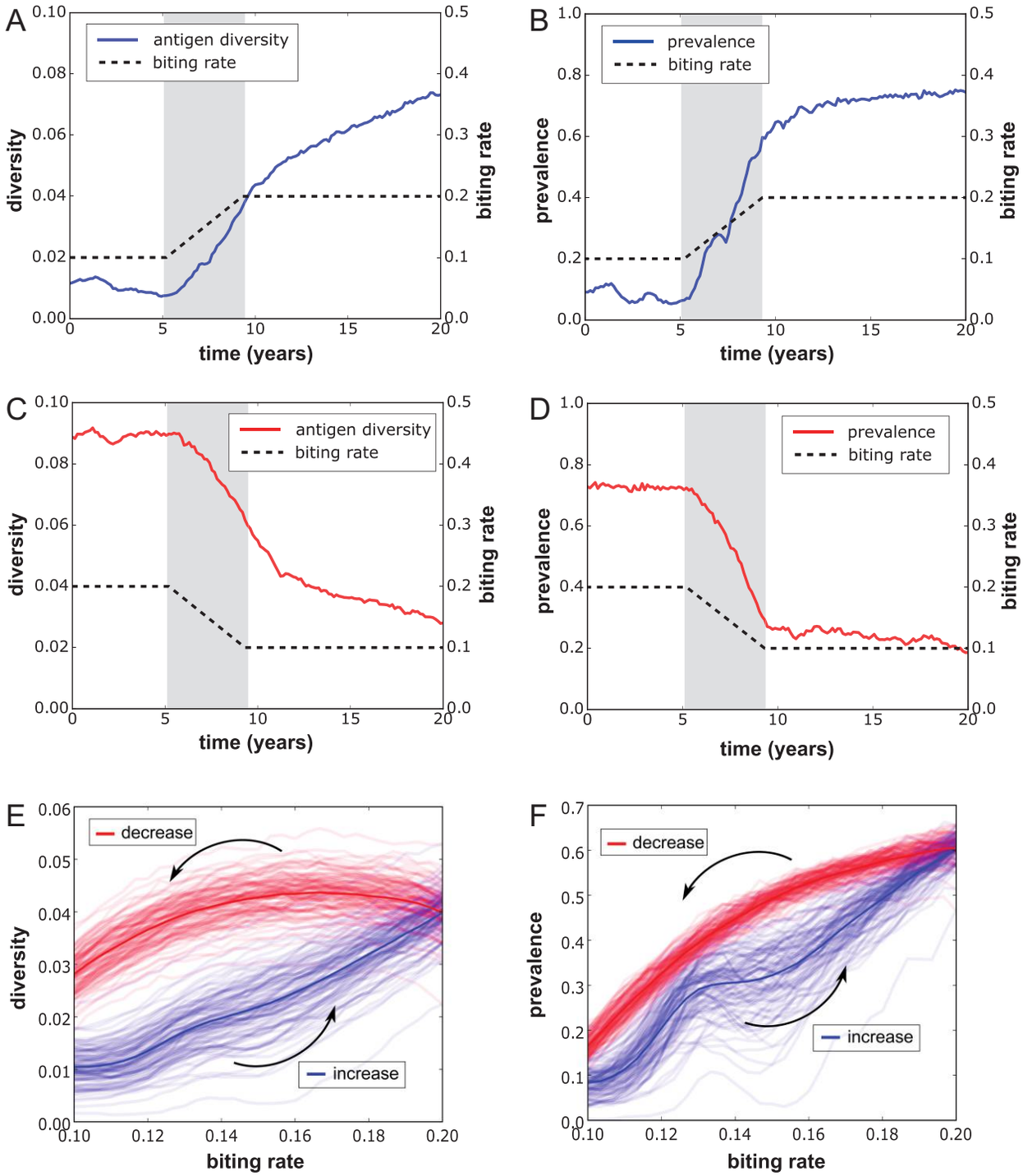

Figure 5: Antigenic diversity and malaria prevalence in response to changes in transmission. As antigenic diversity and parasite prevalence are linked via a dynamic feedback loop, our model predicts a temporal lag in the response to both increases (*A*, *B*) and decreases (*C*, *D*) in transmission rates (mosquito biting rate, black dashed lines). The periods over which the biting rate was changed is highlighted in gray. The system also exhibits a degree of inertia, with changes in diversity and prevalence taking place many years after the biting rate has settled onto a new value. The different rates at which the system responds to changes in the transmission rate can result in different levels of diversity (*E*) and prevalence (*F*), depending on whether there has been a reduction (red lines) or an increase (blue lines) in transmission. *A*–*D* show the results of a single model run, and *E* and *F* show the results of 100 model runs, with the bold lines representing the averages. Parameter values are $M = 8{,}000$, $H = 8{,}000$, $p_c = 0.001$, $A_{init} = 2{,}400$, and $S_{init} = 40$; other parameter values are as in table 1.

transmission levels but then plateaus in more intense transmission settings (Beier et al. 1999; Smith et al. 2005; Okello et al. 2006). Mathematical models trying to elucidate this relationship have often focused on the slow acquisition of immunity without explicitly taking diversity into consideration. In those frameworks, it is simply the notion that higher prevalence leads to higher infection rates that can generate the observed relationship under the assumption that hosts require a sufficiently high number of infections to become immune (see, e.g., Dietz et al. 1974; Molineaux 1985; Killeen et al. 2000; Gu et al. 2003; Smith et al. 2006, 2008; Mandal et al. 2011). An obvious limitation of these approaches is their inability to consider the effect of diversity on immune acquisition, effectively treating all epidemiological settings similarly.

Here we considered an alternative formulation of immunity that explicitly depends on the host's exposure to the parasite's variant antigens. Importantly, this formulation relaxes previous assumptions about the number of infections required for a host to acquire immunity, which in our model arises naturally through the interplay of antigenic diversity and transmission intensity. In fact, the central aspect of our model was to consider antigenic diversity not as a static quantity but rather as a dynamic property of the system that is regulated through multiscale processes related to infection, transmission, and immunity. That is, we considered these processes linked by a positive feedback loop. Under this assumption, novel antigenic variants can be generated through mitotic recombination during infections. These new variants, if transmitted, are disseminated throughout the parasite population by means of meiotic recombination, which we assumed acts only at the repertoire level. This in turn will help the parasite to circumvent preexisting immunity and thus facilitate the generation of further diversity.

Importantly, the continuous generation of new antigenic variants does not result in ever-increasing levels of diversity but is counterbalanced by the loss of diversity due to stochastic extinction, where small host populations and strong immune selection pressure significantly increase the risk of parasite strains becoming extinct. Assuming that recombination rates and immune interference between antigenic variants (e.g., cross-immunity) are intrinsic properties of the parasite and the host, our results suggest that each host-pathogen ecosystem will attain its own state of equilibrium with regard to parasite diversity and population-level prevalence.

There are various caveats to our model, mostly related to our simplifying assumptions about how hosts acquire immunity and how antigenic variants and immunity relate to infection length and thus a strain's probability of onward transmission. For the majority of multistrain disease systems very little is known about how acquired immunity affects the transmission success of subsequent infections, although a general decrease with repeated exposure would be a reasonable assumption. In this respect, shortening the infectious period and keeping the per-bite or per-contact transmission probability constant is akin to lowering the transmission probability for the same length of infection. Furthermore, adding cross-protective immunity, where the risk or length of infection decreases as a function of accumulated exposure or similarity to previously infecting strains, does not lead to significantly different outcomes. This means that our results are fairly robust to changes in the underlying assumptions about immunity and should therefore be applicable to other disease systems.

The feedback between infection and diversity described in this work not only leads to temporal lags in the responsiveness of the system to changes in the transmission potential but also introduces a certain degree of inertia. Delays in a dynamical system's response to external perturbations are expected; however, in our case we observed transient behaviors in parasite prevalence and diversity many years after the assumed changes in mosquito biting rates. In epidemiological terms, this implies that trying to infer the causative factors of observed changes in disease incidence might be more complicated than previously appreciated and would have to take into account potential changes that took place many years in the past. Together with the possibility that in a given endemic setting the system could be in a state of transmission saturation, evaluating the effectiveness of control measures could show significant discrepancies, especially when the evaluation period is too short for the system to have reached a new endemic equilibrium.

In summary, we have shown that diversity plays a crucial role in the epidemiology of antigenically diverse pathogens by linking multiscale immune selection with population-level parasite prevalence and incidence. Our results thus argue for a renewed effort to understand how acquired immunity to an antigenically diverse pathogen is shaped by its diversity and how diversity itself is determined by immunity and immune selection.

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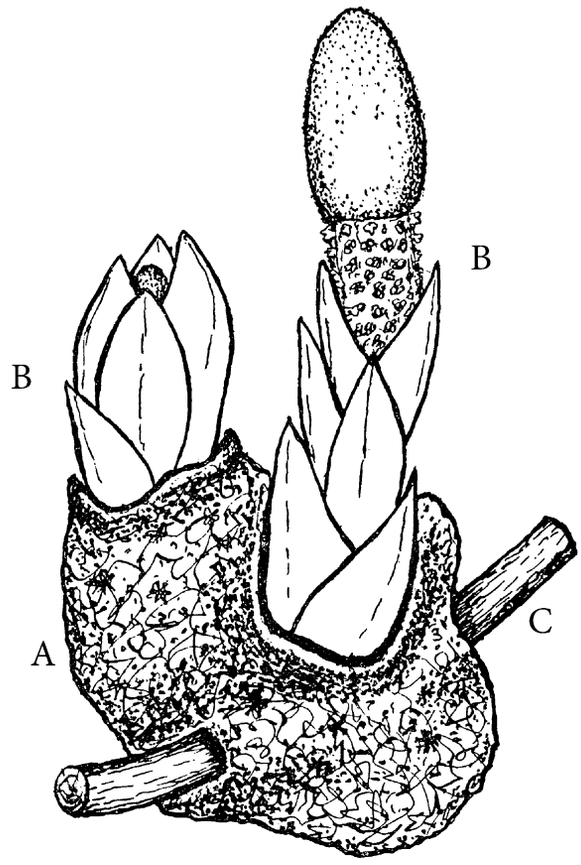

"Pen-sketch of *Bananophora hildenbrandtii*, a native of the Comoro Islands, off the east coast of Africa. After Kerner. . . . The leaves of this species, although large, are scaly and not functional. *A*. Amorphous, fleshy mass. *B*, *B*. Flower stems springing from the mass. *C*. The root-host." From "The Phenogamous Parasites" by Charles A. White (*The American Naturalist*, 1908, 42:12–33).