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# Epidemiology, hypermutation, within-host evolution and the virulence of *Neisseria meningitidis*

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Many so-called *pathogenic* bacteria such as *Neisseria meningitidis*, *Haemophilus influenzae*, *Staphylococcus aureus* and *Streptococcus pneumoniae* are far more likely to colonize and maintain populations in healthy individuals asymptomatically than to cause disease. Disease is a dead-end for these bacteria: virulence shortens the window of time during which transmission to new hosts can occur and the subpopulations of bacteria actually responsible for disease, like those in the blood or cerebral spinal fluid, are rarely transmitted to new hosts. Hence, the *virulence factors* underlying their occasional pathogenicity must evolve in response to selection for something other than making their hosts sick. What are those selective pressures? We address this general question of the evolution of virulence in the context of phase shifting in *N. meningitidis*, a mutational process that turns specific genes on and off, and, in particular, *contingency loci* that code for virulence determinants such as pili, lipopolysaccharides, capsular polysaccharides and outer membrane proteins. We use mathematical models of the epidemiology and the within-host infection dynamics of *N. meningitidis* to make the case that rapid phase shifting evolves as an adaptation for colonization of diverse hosts and that the virulence of this bacterium is an inadvertent consequence of short-sighted within-host evolution, which is exasperated by the increased mutation rates associated with phase shifting. We present evidence for and suggest experimental and retrospective tests of these hypotheses.

**Keywords:** *Neisseria meningitidis*; phase shifting; contingency loci; evolution; epidemiology; virulence

## 1. INTRODUCTION

Although *Neisseria meningitidis* is best known for its role in sporadic and epidemic meningitis, it is primarily a commensal member of the human nasal pharyngeal passages, which causes disease in less than 0.01% of colonized hosts. In the US, strains of *N. meningitidis* can be isolated from the nasopharynx and sub-epithelium of the nasal passages of 5–10% of healthy humans (Odugbemi *et al.* 1992; Anker & Schaaf 2000). Even higher frequencies of asymptomatic carriage occur during epidemics, like those in the Meningitis Belt of Central Africa and in closed communities such as military bases (Di Martino *et al.* 1990; Gagneux *et al.* 2002). Although the asymptomatic colonization and shedding of these bacteria can last up to 2 years, infections that progress to disease are eliminated by treatment, immunity or host death in a matter of days or weeks (Apicella 2000), thereby reducing the time these bacteria have to be transmitted to new hosts. Moreover, the specific bacteria that are responsible for disease, such as those that invade the blood or cerebral spinal fluid, are unlikely to be transmitted to new hosts. It therefore seems reasonable to conclude that the genes responsible for the pathogenicity of *N. meningitidis*, those coding for 'virulence determinants', must evolve in response to the demands (selection pressures) associated with the commensal existence of *N. meningitidis*, rather any benefits that might arise from causing meningitis or other invasive diseases.

What are those demands? *N. meningitidis*, like other commensal bacteria, must surmount a variety of environmental hurdles to successfully colonize a genetically and immunologically diverse host population, evade the constitutive and inducible defences of these hosts and be transmitted to new hosts that are genetically and immunologically different from those from whence they came. Presumably to meet these challenges, these bacteria have highly mutable 'contingency' genes (Moxon *et al.* 1994; Bayliss & Moxon 2002), which code for lipopolysaccharides, outer membrane proteins, capsules, pili and other molecules needed for colonization and persistence in human hosts and transmission to new hosts (Hammerschmidt *et al.* 1996; de Vries *et al.* 1996). When mutations generate variation in these genes, populations of *N. meningitidis* will include cells capable of colonizing hosts that are genetically or immunologically different for their current host, and thereby facilitate their survival of the host population at large.

As appealing as this idea may be, from an evolutionary perspective, it is not sufficient to explain the existence of phase-shifting strains. Certainly, we can expect natural selection to favour individual bacterium expressing genes that directly enhance colonization, persistence and infectious transmission. It is less clear, however, how to account for the evolution of mechanisms that augment the *rate* at which these contingency genes mutate to different

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states, including states that may be detrimental to the bacterium.

In the case of *N. meningitidis*, contingency genes are turned on and off by a mutational mechanism called *phase shifting*. Depending on the strain, the *N. meningitidis* genome contains between 20 and 65 of these contingency genes, each of which contains microsatellites—extended stretches of repeated nucleotide motifs—which are hotspots for replication errors (Saunders *et al.* 2000). High rates of nucleotide additions and deletions occur in these regions, which may offset the codon reading frame and thereby transform the downstream sequence into nonsense or back into sense. The rates of phase shifting vary among genes and among strains of *N. meningitidis*. For example, switching rates in receptors responsible for iron uptake have been measured to range from $2 \times 10^{-2}$ to $7 \times 10^{-6}$ per c.f.u. (colony forming unit) (Richardson & Stojiljkovic 1999), which differ from the rates of phase variation found in other genes (Murphy *et al.* 1989; Jonnson *et al.* 1991; Hammerschmidt *et al.* 1996). Higher rates of phase variation have been associated with both the number of repeated motifs in these microsatellites (Garza *et al.* 1995; Schug *et al.* 1998; Harr & Schlötterer 2000; Kruglyak *et al.* 2000) and impaired mismatch repair systems (Bucci *et al.* 1999; Richardson & Stojiljkovic 2001).

In this investigation, we use mathematical models to identify and explore the conditions that favour the evolution and maintenance of phase shifting of contingency loci and the effects of high-rate phase shifting on the virulence of these usually commensal bacteria. We postulate that phase shifting evolved and is maintained by selection operating at an epidemiological level, as a mechanism to facilitate colonization and infectious transmission to new immunologically diverse hosts. We further postulate that, from the perspective of individual hosts, rapid phase shifting has an inadvertent downside. It increases the likelihood that colonizing populations of *N. meningitidis* will cause invasive disease. We discuss the predictions, implications and limitations of these theoretical results and possible approaches to empirically testing the hypotheses generated from this theoretical consideration.

## 2. OVERVIEW

Before immersing the reader in the relative formality of mathematical models and computer simulations, we offer an intuitive overview of our hypotheses for the evolution and consequences of phase shifting and discuss the empirical observations upon which our models are based.

### (a) *How did phase shifting evolve in* N. meningitidis*?*

Consider an inoculum of *N. meningitidis* that has entered the nasopharynx of a host that is genetically and/or immunologically different from the host whence it came. Furthermore, assume that successful colonization of this novel host requires one or several mutations to generate the necessary phenotypes. Whether or not those mutants exist in the potentially colonizing population depends on the total number of bacteria in the inoculum, the fraction of the inoculum that enters the nasopharynx, and the rates at which the required genetic change(s) is (are) generated by mutation. All else being equal, an inoculum that includes a substantial frequency of bacteria with high rates of mutation in the contingency loci associated with colonization—fast phase shifters—is more likely to include cells that can successfully colonize the new host than inocula made up primarily of cells with lower rates of phase shifting. Moreover, the specific mutant cells that facilitate colonization of the new host are more likely to be from fast phase-shifting lineages than from lineages less likely to produce appropriate mutations. As a result, the frequency of fast phase-shifting bacteria would increase within the host. If a host population includes sufficient immunological diversity, then, as a consequence of this colonization advantage in novel hosts, natural selection will favour fast phase shifters. Such host diversity may be genetically determined or may be a product of humoral responses to meningococcal antigens from, for example, *N. meningitidis* currently colonizing the nose or throat, or to other microparasites and commensals that stimulate cross-reacting antibodies.

Preliminary analysis of meningococcal strains from two worldwide epidemics revealed frequent elevations in rates of phase shifting. This is consistent with the view that the demands of between-host colonization may favour high rates of phase shifting of genes associated with colonization. Approximately half of 100 serogroup A isolates sampled from the epidemics were found to be fast shifters (rates greater than $5 \times 10^{-5}$ per c.f.u.), and estimates of this ratio continue to increase as more strains are analysed. Furthermore, the prevalence of fast shifters in this set of strains increases as the epidemic progresses in time (Richardson *et al.* 2002). One possible explanation is that, as the epidemic progresses, an increasing fraction of the population has acquired one or more antigenic variants of *N. meningitidis*. Subsequent infections of these individuals require changes in the antigenic state of the bacterium, such as those that could be generated by phase shifting. We will use two mathematical models to illustrate and quantify these epidemiological selection hypotheses for the evolution of fast phase shifting in *N. meningitidis*.

### (b) *Why would high rates of phase shifting increase the virulence of* N. meningitidis*?*

This hypothesis—that fast phase shifting is correlated with invasive disease—rests on the idea that the virulence of *N. meningitidis* is a rare consequence of within-host evolution for proliferation into new tissues and other sites of replication. We postulate that the virulence of *N. meningitidis* does *not* confer any long-term advantage to the bacteria in its ability to spread through a community of hosts (and may even be detrimental), but rather is an inadvertent consequence of mutation and selection within individual hosts. Levin and Bull have called this phenomenon the 'short-sighted' evolution of virulence (Levin & Bull 1994; Taha *et al.* 2002).

Based on what we know about the phenotypic changes these bacteria undergo during an invasive infection, *N. meningitidis* is a particularly appealing candidate for the within-host evolution of virulence. As illustrated in table 1, different bacterial phenotypes are favoured in different tissues and sites of replication. Capsulated *N. meningitidis* with assembled pili and non-sialylated L8 lipopolysaccharides (LPS) are favoured in the nasopharynx. Uncapsulated *N. meningitidis* with unassembled pili and sialylated

Table 1. Examples of specific site- and tissue-adapted phenotypes of *N. meningitidis.*
(Capsulated strains with assembled, high molecular weight, pili and sialylated LPS are expressed in non-invasive isolates. By contrast, uncapsulated strains with minimal or S pili and non-sialylated LPS are selected for in the strains invading the epithelial cells (de Vries *et al.* 1996). Presumably, assembled pili facilitate adhesion to somatic cells, but make the bacterium a more vulnerable target of the immune system. Whereas capsules prevent desiccation during open-air transmission and make the bacteria less vulnerable to phagocytosis in the blood, they also reduce the likelihood of *N. meningitidis* invading the cells of the epithelia (Hammerschmidt *et al.* 1996; de Vries *et al.* 1996). Sialylated LPS play a dual role in evading host immunity in the blood and facilitating entry into epithelial cells (de Vries *et al.* 1996; Jennings *et al.* 1999).)

| | pili | capsule | lipopolysaccharide (LPS) |
|---|---|---|---|
| adhesion to nasopharynx | assembled | yes | L8 (non-sialylated) |
| invasion of epithelial and sub-epithelial cells | S | no | L8 (sialylated) |
| establishment and persistence in blood | assembled | yes | L3, 7, 9 |

L8 LPS are favoured in the epithelia and sub-epithelia. In addition, in the blood, capsulated *N. meningitidis* with assembled pili and L3, L9 and L7 LPS have an advantage. If each of these characters are coded by at least one contingency locus (in fact, multiple contingency loci are involved in the control of LPS biotypes and sialylation), then at least three mutations are required for a population of *N. meningitidis* that is adapted for proliferation and maintenance in the nasopharynx to become adapted for proliferation in the epithelia and sub-epithelia, and similarly, at least three more mutations are required for epithelia-adapted bacteria to become adapted for persistence and proliferation in the blood. As a result, *N. meningitidis* strains that produce these contingency loci mutations at higher rates could be more likely to be virulent than those with lower rates of phase shifting.

## 3. EPIDEMIOLOGICAL COLONIZATION SELECTION FOR PHASE SHIFTING

Here, we introduce two novel *SIR* compartmental models of the epidemiology of *N. meningitidis*, in which hosts are classified as susceptible, infected or recovered (Anderson & May 1991). With these models we illustrate and explore mathematically the conditions under which the transmission of bacterial populations through human communities would favour the evolution of phase shifting.

### (a) *Model 1: intrinsic host diversity*

The first model considers the epidemiology of two strains of *N. meningitidis*—a wild-type strain where phase shifting is limited or absent and a fast phase-shifting strain—in a host population consisting of two distinct immunological classes, A and B. We assume that human hosts are born and remain in one of these two classes and that each host is in one of the following four states with respect to *N. meningitidis*: susceptible to colonization $S_A$ or $S_B$; colonized with the wild-type strain, $I_A$ or $I_B$; colonized with phase shifters, $I_{\pi_A}$ or $I_{\pi_B}$; or cleared of the bacteria, $R_A$ or $R_B$. Note that $S_A$, $S_B$, $I_A$, $I_B$, $I_{\pi_A}$, $I_{\pi_B}$, $R_A$ and $R_B$ also represent the population densities of these host classes.

As can be seen in figure 1, transmission of the pathogen from a colonized host to a susceptible host of the *same* immunological class (A or B) occurs at rates proportional to the densities of these populations and transmission rate constants $\beta$ and $\beta_\pi$ for the wild-type and fast phase-shifting strains, respectively. Transmission from a colonized host of one class to a susceptible host of the other class requires genetic changes in the bacterial populations, that is, the transmitting cells must mutate to a state appropriate for colonization of a different host environment. Hence, transmission between hosts in different classes occurs at rates proportional to the densities of these populations, the transmission rate parameters $\beta$ and $\beta_\pi$, and the mutation rates $\mu$ and $\mu_\pi$ of the pathogens. The rate constants $\mu$ and $\mu_\pi$ represent the probabilities that an inoculum of wild-type or phase-shifting bacteria, respectively, from a host of type A (or B) will include or produce sufficient mutations to colonize a host of type B (or A). Hosts of all epidemiological classes die (or are otherwise removed from the community) at a *per capita* rate $\delta$ per day, and all colonized hosts lose the bacteria at a *per capita* rate $\rho$ per day (that is, they remain colonized for, on average, $1/\rho$ days). For simplicity, we assume that the total

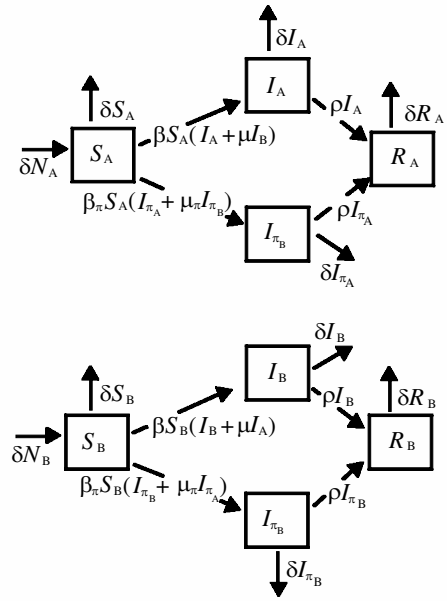


Figure 1. Epidemiological model 1: a host population with two immunological classes. Hosts are either of immunological class A or B, and are either susceptible ($S_A$ or $S_B$), infected with either wild-type ($I_A$ or $I_B$) or mutator strains ($I_{\pi_A}$ or $I_{\pi_B}$) of *N. meningitidis*, or recovered from such an infection ($R_A$ or $R_B$). Transmission to susceptible hosts occurs at rates $\beta$ and $\beta_\pi$, mutations that allow successful colonization of immunologically different hosts occur at rates $\mu$ and $\mu_\pi$, hosts recover at a rate $\rho$ per day and die at a rate $\delta$ per day, and births exactly make up for deaths.

Table 2. Equilibrium population distributions for epidemiological model 1.

| equilibrium | $\hat{S}_A$ | $\hat{S}_B$ | $\hat{I}_A$ | $\hat{I}_B$ | $\hat{R}_A$ | $\hat{R}_B$ |
|---|---|---|---|---|---|---|
| (1a) | $S_A$ | $S_B$ | 0 | 0 | 0 | 0 |
| (1b) | $\frac{\rho+\delta}{\beta(\mu+1)}$ | $\frac{\rho+\delta}{\beta(\mu+1)}$ | $I$ | $I$ | $\frac{\rho I}{\delta}$ | $\frac{\rho I}{\delta}$ |

density of the host population remains constant by setting the rate at which new hosts enter the population (births) exactly equal to the total rate at which hosts are removed from the population (deaths). Further, we do not allow multiple infections of a single host. With these definitions and assumptions, the rates of change in the densities of the various host subpopulations are given by the differential equations

$$\frac{dS_A}{dt} = -\beta S_A(I_A + \mu I_B) - \beta_\pi S(I_{A_\pi} + \mu_\pi I_{B_\pi}) + \delta(I_A + I_{A_\pi} + R_A),$$

$$\frac{dS_B}{dt} = -\beta S_B(I_B + \mu I_A) - \beta_\pi S(I_{B_\pi} + \mu_\pi I_{A_\pi}) + \delta(I_B + I_{B_\pi} + R_B),$$

$$\frac{dI_A}{dt} = \beta S_A(I_A + \mu I_B) - (\rho + \delta)I_A,$$

$$\frac{dI_B}{dt} = \beta S_B(I_B + \mu I_A) - (\rho + \delta)I_B,$$

$$\frac{dI_{\pi_A}}{dt} = \beta_\pi S_A(I_{A_\pi} + \mu_\pi I_{B_\pi}) - (\rho + \delta)I_{\pi_A},$$

$$\frac{dI_{\pi_B}}{dt} = \beta_\pi S_B(I_{B_\pi} + \mu_\pi I_{A_\pi}) - (\rho + \delta)I_{\pi_B},$$

$$\frac{dR_A}{dt} = \rho(I_A + I_{A_\pi}) - \delta R_A,$$

$$\frac{dR_B}{dt} = \rho(I_B + I_{B_\pi}) - \delta R_B.$$

Like other models of its ilk the population dynamics of the bacteria are not explicitly considered, but are instead incorporated into the transmission, recovery and mortality parameters, and the state of the host with respect to colonization.

We now use mathematics to ask when a strain with a high rate of mutation due to phase shifting can invade a host community that has already experienced the spread of a more slowly mutating strain. The equilibria in table 2 provide the two relevant starting conditions: either (1a) the host population completely naive or (1b) the wild-type strain is endemic to the population. Note that this is only a subset of the possible equilibria for this model. There exist stable equilibria in which all eight host populations are present.

(i) *Case 1: invasion of a completely naive host population*

First, we calculate the basic reproductive numbers, $R_0$, (Anderson & May 1991) of the invading phase-shifting strains, which are the numbers of new infections (colonizations) caused by a single infected (colonized) host. These reproductive numbers are touchstones in the analysis of dynamical *SIR* models, as they indicate when a host population is susceptible to the invasion by a commensal or pathogenic microparasite.

Consider first a population in equilibrium (1a) consisting entirely of susceptible class A and class B hosts. Suppose a single host is colonized by a phase shifting strain, $I_{\pi_A} = 1$ or $I_{\pi_B} = 1$. Then the basic reproductive number, the epidemiological potential of that population of bacteria is, without loss of generality,

$$R_{\pi_A} = \frac{\beta_\pi(S_A + \mu_\pi S_B)}{\rho + \delta}.$$

The numerator gives the rate of new colonizations caused by transmission from the original colonized host to other hosts while the denominators give the rate at which individuals leave the colonized state through recovery or death. In other words, $1/(\rho + \delta)$ is the expected duration of infection, and $\beta_\pi(S_A + \mu_\pi S_B)$ reflects the rate of transmission while colonized.

The phase-shifting strain will spread through the host population whenever $R_{\pi_A} > 1$, and hence whenever the host population size is sufficiently large $(S_A + \mu_\pi S_B) > (\rho + \delta)/\beta_\pi$. Even if $S_A \leqslant (\rho + \delta)/\beta_\pi$, meaning that the pool of susceptible class A individuals is not large enough to harbour an epidemic on its own, rapid phase shifting may enable the bacteria to frequently colonize class B hosts and thus increase the reservoir of potential hosts sufficiently to cause an epidemic. This will happen when the mutation rate in the phase-shifting strain surpasses the threshold given by $\mu_\pi > 1/S_B((\rho + \delta)/\beta_\pi - S_A)$.

(ii) *Case 2: invasion when wild-type is endemic to the host population*

Suppose a wild-type strain has spread through both immunological classes and the host population has come to a stable equilibrium that includes susceptible, colonized and recovered hosts. The second equilibrium (1b) reflects this condition. Substituting the equilibrium population densities into the basic reproductive number for the phase-shifting strain, we find

$$R_{\pi_A} = \frac{\beta_\pi(S_A + \mu_\pi S_B)}{\rho + \delta} = \frac{\beta_\pi\left(\left(\frac{\rho+\delta}{\beta(\mu+1)}\right) + \mu_\pi\left(\frac{\rho+\delta}{\beta(\mu+1)}\right)\right)}{\rho + \delta} = \frac{\beta_\pi(1 + \mu_\pi)}{\beta(1 + \mu)}.$$

The success of the phase-shifting strain depends on a trade-off between transmissibility and rate of phase shifting. The strain will spread when $(1 + \mu_\pi)/(1 + \mu) > \beta/\beta_\pi$, that is, when the *relative* rate of phase shifting outweighs any reduced transmissibility of the fast phase-shifting strain.

Many models have addressed the trade-off between transmissibility to naive hosts and virulence (Levin & Pimental 1981; Ewald 1983; Anderson & May 1991;

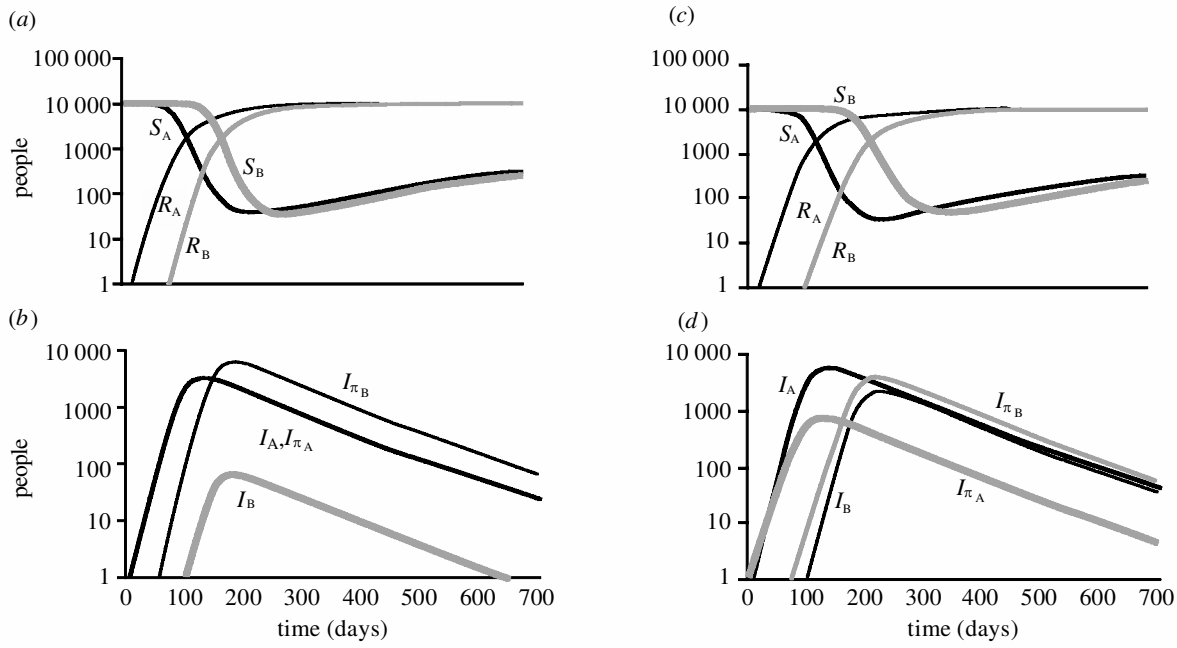


Figure 2. Simulation of epidemiological model 1: the spread of wild-type and fast phase shifters through a diverse host population. (*a*,*b*) A simulation in which both strains are equally infectious ($\beta = \beta_\pi = 1 \times 10^{-5}$). (*c*,*d*) A simulation in which the fast shifting strain has lower transmissibility than the wild-type strain ($\beta = 1 \times 10^{-5}$ and $\beta_\pi = 8 \times 10^{-6}$). Both simulations assumed $\rho = 1 \times 10^{-2}$, $\delta = 1 \times 10^{-4}$, $\mu = 1 \times 10^{-5}$, $\mu_\pi = 1 \times 10^{-3}$, and initial population sizes of $S_A = S_B = 1 \times 10^4$, $I_A = I_{\pi_A} = 1$ and $I_B = I_{\pi_B} = R_A = R_B = 0$.

Antia *et al.* 1994). We question the trade-off between transmissibility and mutability. If, instead, we consider virulence versus mutability we find very analogous results. For example, in the second case, the criterion for invasion of the phase-shifting strain becomes $(1 + \mu_\pi)/(1 + \mu) > (\rho + \delta)/(\rho + \delta_\pi)$. The phase-shifting strain can spread when the mutation rate is high enough to overcome any additional virulence associated with rapid phase shifting ($\delta_\pi > \delta$). In § 3b, we discuss the relationship between phase shifting and virulence in detail.

(iii) *Case 3: simultaneous introduction wild-type and phase-shifting strains into a naive host population*

The situation where the host population is naive to the bacterium and both strains are introduced at once is not particularly amenable to the kind of formal analysis described above. Instead, we analyse this situation through numerical simulations of the system of differential equations that define the model. We demonstrate that, under these conditions, populations can reach stable equilibria with all eight host states present. Our simulations of the model employ Euler's method with a constant step size d$t$. (All simulations described in this article were programmed and run with Berkeley Madonna software. Copies of all programs are available to interested readers.)

In figure 2 we illustrate the case where the wild-type strain mutates at a rate two orders of magnitude lower than the phase-shifting strain, $\mu_\pi = 10^2\mu$. First we assume that the two strains are identical except for their rates of phase shifting (figure 2*a*,*b*), we then assume that the phase-shifting strain has a lower rate of transmission than the wild-type strain, $\beta_\pi < \beta$ (figure 2*c*,*d*). In the former case, the wild-type and phase-shifting strains initially spread through the class A host population with equal force. The phase-shifting strain, by its frequent mutations that facilitate the rapid colonization of novel environments, enters and spreads through the class B host population ahead of the wild-type strain. In the latter case, the rapid phase-shifting strain is a weaker colonizer and thus spreads more slowly through the class A host population than the wild-type strain. Despite its lower prevalence, however, the phase-shifting strain again more rapidly produces mutants capable of colonizing class B hosts than the wild-type strain.

### (b) *Model 2: epidemic induced diversity*

Our second model of colonization selection for elevated mutation rates also considers the epidemiology of two strains—a wild-type strain and a phase-shifting strain. In this model, however, the host population does not harbour intrinsic immunological diversity, but rather, gains immunological diversity via infection and recovery. We assume that human hosts are in one of five states with respect to *N. meningitidis*: (i) completely naive, $S$; (ii) experiencing a first colonization, $I$ or $I_\pi$; (iii) cleared of the first colonizing population, $R$; (iv) colonized a second time by an antigenically different strain, $\mathcal{J}$ or $\mathcal{J}_\pi$; or (v) cleared of the second colonization, $Q$; where $S$, $I$, $I_\pi$, $R$, $\mathcal{J}$, $\mathcal{J}_\pi$ and $Q$ also represent the population densities of these host classes.

As can be seen in figure 3, transmission of the bacteria from a first-time colonized host ($I$ or $I_\pi$) to a naive host ($S$) or from a second-time colonized host ($\mathcal{J}$ or $\mathcal{J}_\pi$) to a cleared host ($R$) occurs at rates proportional to the densities of these populations and transmission rate parameters ($\beta$ and $\beta_\pi$). These two forms of transmission do not involve any mutations in the colonizing cells. By contrast, transmission from a second-time colonized host to a naive host or from a first-time colonized host to a host that has cleared the first colonizing bacteria *does* require

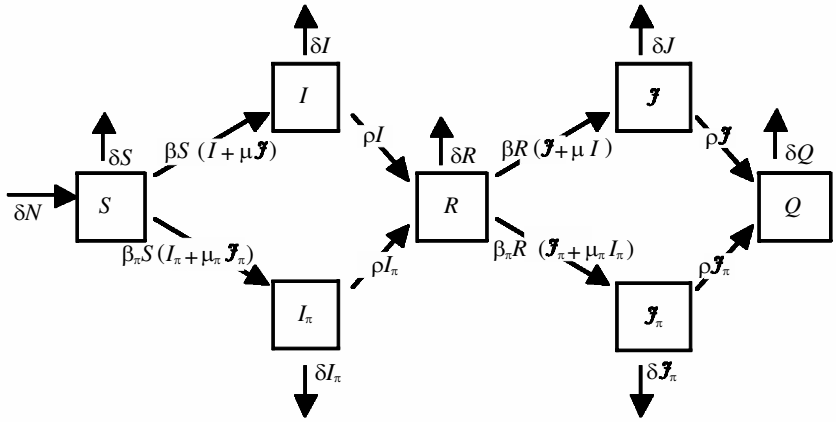


Figure 3. Epidemiological model 2: reinfection of previously colonized hosts. Hosts are either naive ($S$), experiencing a first colonization by *N. meningitidis* ($I$ or $I_\pi$), cleared of the first colonizing population ($R$), colonized a second time by an antigenically different strain ($J$ or $J_\pi$), or cleared of the second colonization ($Q$). Transmission to susceptible hosts occurs at rates $\beta$ and $\beta_\pi$, mutations that allow successful colonization of immunologically different hosts occur at rates $\mu$ and $\mu_\pi$, hosts recover at a rate $\rho$ per day and die at a rate $\delta$ per day, and births exactly make up for deaths.

mutations in the infecting cells. Hence, these forms of transmission occur at rates proportional to the densities of the populations involved, the transmission rate parameters $\beta$ and $\beta_\pi$ and the mutation rates $\mu$ and $\mu_\pi$ of the pathogens. Again, we assume that hosts die or are otherwise removed from the community at a rate of $\delta$ per day. As before, by setting the number of new, naive host entering the community through birth or migration equal to this mortality rate, we maintain a constant total density.

Using these definitions and assumptions, the rates of change in the densities of the host of these different states are given by the differential equations,

$$\frac{dS}{dt} = -\beta S(I + \mu J) - \beta_\pi S(I_\pi + \mu_\pi J_\pi) + \delta(I + I_\pi + J + J_\pi + R),$$

$$\frac{dI}{dt} = \beta S(I + \mu J) - (\rho + \delta)I,$$

$$\frac{dI_\pi}{dt} = \beta_\pi S(I_\pi + \mu J_\pi) - (\rho + \delta)I_\pi,$$

$$\frac{dR}{dt} = -\beta S(J + \mu I) - \beta_\pi S(J_\pi + \mu_\pi I_\pi) + \rho(I + I_\pi) - \delta R,$$

$$\frac{dJ}{dt} = \beta R(J + \mu I) - (\rho + \delta)J,$$

$$\frac{dJ_\pi}{dt} = \beta_\pi R(J_\pi + \mu I_\pi) - (\rho + \delta)J_\pi,$$

$$\frac{dQ}{dt} = \rho(J + J_\pi) - \delta Q.$$

As before, we derive the conditions under which a phase-shifting strain can invade a population. Table 3 gives the two relevant equilibria. Also, as before, the first in this list (2a) represents a naive population. In the second (2b), the wild-type strain is endemic to the entire population. Again, these are a subset of the possible equilibria for this model. There exist stable equilibria in which all seven host populations coexist.

(i) *Case 1: invasion of a completely naive host population*

In the case of a totally naive population, there are no hosts that have already cleared a bacterial infection and the condition for invasion by the fast phase-shifting strain reduces to the classical reproductive number $R_\pi = \beta_\pi(S + \mu_\pi R)/(\rho + \delta) = \beta_\pi S/(\rho + \delta)$. Thus, under these conditions, the epidemiological fitness of the bacteria does not depend on its rate of phase shifting.

(ii) *Case 2: wild-type endemic to the host population*

Next, consider a population in equilibrium (2b) consisting of susceptible hosts, hosts colonized by the initial antigenic type bacteria (wild-type), and hosts that have cleared those bacteria. Under these conditions, the basic reproductive number for the phase shifting strain is:

$$R_\pi = \frac{\beta_\pi}{\beta}\left(\frac{\delta + \rho}{\mu\rho + \delta + \rho} + \frac{\mu_\pi\rho}{\mu\delta + \mu\rho + \rho}\right).$$

Here again, there is a trade-off between the relative rate of transmission and mutation, as seen by $\beta_\pi$ and $\mu_\pi$ in the numerator and $\beta$ and $\mu$ in the denominator. As in the previous model, the higher the rate of phase shifting, the more likely the strain is to spread through a host population with a persistent wild-type strain. Unlike the previous model, however, the mortality and recovery rates influence the epidemiological potential of the phase-shifting strain.

(iii) *Case 3: simultaneous introduction of wild-type and phase-shifting strains into an uncolonized host population*

We simulate the simultaneous entry of a wild-type and phase-shifting strain into a naive host population. Figure 4 illustrates the case where the wild-type strain mutates at a rate two orders of magnitude less than the phase-shifting strain. In figure 4*a*,*b*, the two strains are identical except for their rates of phase shifting, and in figure 4*c*,*d*, the phase-shifting strain has a lower rate of transmission than the wild-type strain. As in the previous simulations, the fast phase-shifting strain has an advantage due to its increased mutability. Once a sufficient pool of recovered hosts exists, the phase-shifting strain quickly evolves to spread through that reservoir of hosts. Even when the phase-shifting strain is less transmissible than the wild-type strain, its elevated mutation rate allows it to exploit the recovered pool significantly more quickly than the wild-type strain.

Table 3. Equilibrium population distributions for epidemiological model 2.

| equilibrium | $\hat{S}$ | $\hat{I}$ | $\hat{R}$ | $\hat{\mathcal{J}}$ | $\hat{Q}$ |
|---|---|---|---|---|---|
| (2a) | $S$ | 0 | 0 | 0 | 0 |
| (2b)[a] | $\frac{(\rho+\delta)^2}{\beta(\mu\rho+\delta+\rho)}(1+\varepsilon)$ | $-\frac{\delta\mu S\mathrm{A}}{\mathrm{BC}}$ | $\frac{\mathrm{A}}{\beta\mathrm{C}}$ | $-\frac{\delta\mathrm{AD}}{\beta\mathrm{BC}}$ | $-\frac{\rho\mathrm{AD}}{\beta\mathrm{BC}}$ |

[a] $\mathrm{A}=(\rho+\delta-S\beta)(\rho+\delta)$, $\mathrm{B}=S\beta(\delta+\rho(1-\mu))+(\delta+\rho)^2$, $\mathrm{C}=\mathrm{S}\beta(\mu^2-1)+\delta\rho$, $\mathrm{D}=-S\beta+\delta+\rho$, and $0<\varepsilon<\mu(\mu\delta+\mu\rho+\rho)/(\delta+\rho)(1-\mu^2)$.

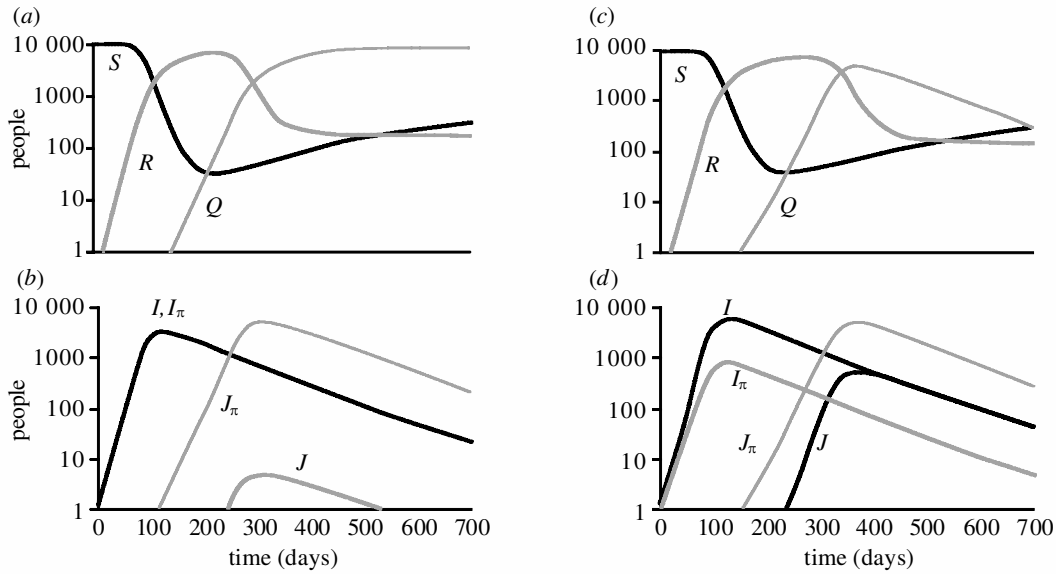


Figure 4. Simulation of epidemiological model 2: the transmission of wild-type and fast phase shifters to both susceptible and recovered hosts. (*a*,*b*) Illustrate a simulation in which both strains are equally infectious ($\beta=\beta_\pi=1\times10^{-5}$). (*c*,*d*) Illustrate a simulation in which the fast shifting strain has lower transmissibility than the wild-type strain ($\beta=1\times10^{-5}$ and $\beta_\pi=8\times10^{-6}$). Both simulations assumed $\rho=1\times10^{-2}$, $\delta=1\times10^{-4}$, $\mu=1\times10^{-5}$, $\mu_\pi=1\times10^{-3}$, and initial population sizes of $S=1\times10^4$, $I=I_\pi=1$ and $R=\mathcal{J}=\mathcal{J}_\pi=Q=0$.

## 4. PHASE SHIFTING AND THE VIRULENCE OF *NEISSERIA MENINGITIDIS*

We use a simple heuristic to illustrate the relationship between the rate of mutation in contingency loci and likelihood that colonization will progress to invasive disease. In this model, there are three within-host habitats in which *N. meningitidis* can proliferate: (H1) the nasopharynx, (H2) the blood and (H3) the cerebrospinal fluid (CSF). We assume that the successful migration from one of these within-host habitats to another requires genetic changes in the bacterial population. We also assume that mutants capable of colonizing a new within-host habitat are also capable of proliferating in the habitat whence they came, at possibly a lower rate than their ancestral clone. As depicted in figure 5, the model allows bacteria to move between habitats in single steps and in only one direction, in particular, from H1 to H2 and from H2 to H3. For example, mutants capable of proliferating in the CSF are derived from bacteria in the blood and once they enter the CSF they do not return to the blood. We assume that all bacteria have an equal probability of moving from one habitat to the next, yet only those with appropriate genotypes will survive in the next habitat. We index a strain $\mathrm{B}_{xy}$ according to its current habitat ($x$) and its genotype ($y$). There are two strains, $\mathrm{B}_{11}$ and $\mathrm{B}_{12}$, that can colonize H1 (the nasopharynx), two strains, $\mathrm{B}_{22}$ and $\mathrm{B}_{23}$, that can proliferate in H2 (the blood) and one strain that can grow in H3, $\mathrm{B}_{33}$, where the $\mathrm{B}_{xy}$s represent both the densities (bacteria per millilitre) and designations of these populations.

Within each habitat the bacteria grow in a logistic manner, so that during the growth phase, the rate of increase in the density of a clone $y$ in habitat $x$ depends on its exponential growth rate, $r_{xy}$ per hour, the total density of bacteria in that habitat, $N_x$ cells per ml, and the carrying capacity of that habitat (the maximum density that can be maintained), $k_x$ bacteria per millilitre. The time during which a population of bacteria can proliferate in a habitat is limited. At $\tau_1$ and $\tau_2$ hours after initial colonization, the bacterial population in habitats H1 and H2, respectively, begin to decline because of the host defences. Using these definitions and assumptions, the rates of change in the densities of the bacterial populations in the three different habitats are given by

$$\frac{\mathrm{d}\mathrm{B}_{xy}}{\mathrm{d}t}=r_{xy}\mathrm{B}_{xy}\left(\frac{1-N_x}{k_x}\right)-\mathrm{d}_{xy},$$

where $\mathrm{d}_{xy}=0$ when $t<\tau_x$.

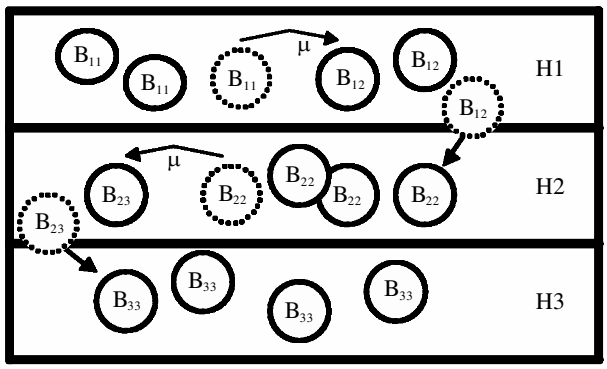


Figure 5. Movement and mutation of bacteria within a host. For bacterial type $B_{xy}$, $x$ indicates the location of a bacterium within the host and $y$ indicates the genetic state of the bacterium. For example, bacteria of type $B_{1y}$ ($B_{11}$ and $B_{12}$) can proliferate in host habitat H1. Bacteria of type $B_{12}$ can migrate into habitat H2, and in doing so, become $B_{22}$.

We use a Monte Carlo procedure to model the mutation and the habitat migration processes, and the Euler method with a constant step size d$t$ for solving the differential equations. At any give time there is a constant probability $\mu$ (the mutation rate) that a cell will mutate into the next stage. Hence, at any given time interval, the probability that a $B_{xy}$ cell will mutate is $p_{xy} = \mu B_{xy} V \mathrm{d}t$, where $V$ is the total volume in its habitat (the existence of mutants depends on the total number of cells, not their densities). In our simulation of this process we use values of d$t$ such that the probability of more than one mutant being generated at any given time is negligible. To determine when a mutant occurs, we generate a uniformly distributed random number $x$ ($0 < x < 1$). If $x < p_{xy}$, a mutant enters the $B_{x\,y+1}$ bacterial population and density of bacteria in the $B_{xy}$ population is reduced by $1/V$.

We use a similar procedure to model the movement of a mutant from the habitat in which it was generated to the next habitat (to which it is better adapted). For this, we assume a constant probably, $h$, for the movement of a single $B_{12}$ bacterium from H1 to H2, which thus becomes $B_{22}$, and for the movement of a single $B_{23}$ bacterium from H2 to H3, which thus becomes $B_{33}$. At any given time, the probability of movement of a bacterium of genotype $y$ moving from habitat $x$ to $x + 1$ is $q_{xy} = hBq_{xy}V\mathrm{d}t$.

### (a) *Simulation results*

In figure 6, we present three qualitatively distinct outcomes resulting from simulation with the parameter values provided in the legend to figure 6: (*a*) the colonizing population is cleared before the bacteria enter the blood; (*b*) the bacteria establish a population in the blood, but are cleared before any enter the CSF; and (*c*) the bacteria establish a population in the CSF. The frequency at which each of these outcomes obtains depends, of course, on the values of the parameters.

By varying the mutation rate while holding all other parameters constant, we illustrate the contribution of the mutation rate (rate of phase shifting) to the likelihood of an invasive infection—a bacteraemia or meningitis (proliferation in the CSF). As shown in table 4, we performed 100 simulations at four different mutation rates ($10^{-8}$, $5 \times 10^{-8}$, $10^{-7}$ and $5 \times 10^{-7}$). As the mutation rate increased, so did the number of infections that progressed to bacteraemia (successful colonization of H2) and meningitis (successful colonization of H3). In these simulations, populations in H1 and H2 begin to decline 40 and 80 h after initial colonization, respectively. Therefore, if invasive disease does not occur with the first 100 h, it will not occur at all.

## 5. DISCUSSION

*Neisseria meningitidis* persists through the colonization of multicellular hosts, proliferation in those hosts and transmission to new hosts at a rate at least equal to the rate at which they are cleared from colonized hosts. There is no evidence that causing disease in colonized hosts provides *N. meningitidis* with an advantage. If anything, the bacteria responsible for invasive disease, those in the blood and CSF, are not transmitted. Why then does *N. meningitidis* cause disease in some hosts? A broad answer to this evolutionary question is that disease is coincidental and that the evolution and maintenance of the virulence determinants of this bacteria is a side-effect of natural selection for the persistence of the organism in a community of hosts. In this investigation, we have studied the evolution and maintenance of a particular kind of virulence determinant—the regions responsible for the phase shifting of genes that Moxon and colleagues call contingency loci (Moxon *et al.* 1994).

Our epidemiological models provide a simple explanation for the evolution of fast phase shifting. Assuming that: (i) the contingency loci include genes responsible for colonizing new hosts and evading host defences; and (ii) under some conditions, the successful transmission from one host to another requires mutations in some of these contingency loci, then the genes that augment the mutation rate at these loci, like those responsible for phase shifting, may be favoured as the bacteria spread through a community of hosts. Whether or not phase-shifting regions will be selected in this way depends on the genetic and immunological constraints that limit transmission and the costs associated with fast phase shifting, which may include more rapid clearance from hosts, higher rates of host mortality or lower rates of transmission.

We also postulate that the virulence of these normally commensal bacteria, their capacity to cause bacteraemia and meningitis, is an inadvertent by-product of mutation and selection of these bacteria within colonized hosts, and high rates of phase shifting of contingency loci simply increase the likelihood of this detrimental outcome. In our model of the within-host population dynamics of colonizing populations of *N. meningitidis*, genes that increase the mutation rate of loci responsible for colonization will also increase the likelihood of disease. This model is based on the empirically supported assumption that the invasion and/or proliferation of these bacteria into new within-host habitats, such as the blood and meninges, requires genetic changes in their population. This evolutionary by-product of rapid phase shifting falls decidedly into the category of *short-sighted evolution of virulence* (Levin & Bull 1994). That is, the invasion of different within-host habitats provides an immediate growth advantage to the bacteria, but also entails long-term disadvantages in that the bacteria may enter sites such as the meninges that preclude future

Table 4. Within-host model simulation results.

| $\mu$ | number of failed infections | number of infections causing bacteraemia | number of infections causing meningitis |
|---|---|---|---|
| $10^{-8}$ | 92 | 8 | 0 |
| $5 \times 10^{-8}$ | 53 | 34 | 13 |
| $10^{-7}$ | 43 | 30 | 27 |
| $5 \times 10^{-7}$ | 1 | 5 | 94 |

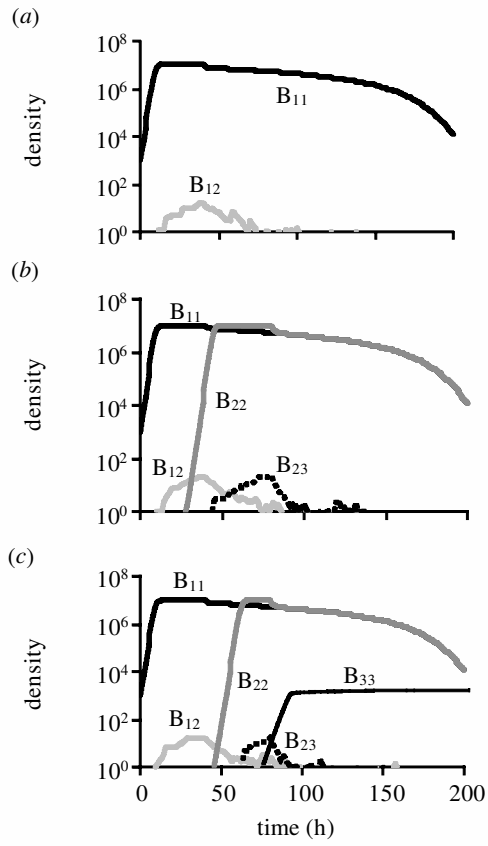


Figure 6. Simulation of the within-host population dynamics of a meningococcal colonization and invasive infection. Parameter values: growth rates $r_{11} = r_{22} = r_{33} = 1.0$ and $r_{12} = r_{23} = 0.95$ per hour; habitat carrying capacities $k_1 = k_2 = 10^7$ and $k_3 = 10^4$ cells; habitat migration rate $h = 10^{-3}$ per cell per hour, habitat volume $V = 1.0$ ml, timing of host defences $\tau_1 = 40$ h and $\tau_2 = 80$ h; and mutation rate $\mu = 5 \times 10^{-8}$ per cell per hour. (*a*) Simulation run terminating with the loss of the colonizing population and no invasive disease. (*b*) Simulation run terminating with a bacteraemia but no CSF infection. (*c*) Simulation terminating with meningitis.

transmission to new hosts, or the proliferation of the bacteria may cause the host to die before the bacteria are transmitted to other hosts. (For a general consideration of the evolution of virulence and the role of trade-offs, like this trade-off between the colonization of novel hosts and virulence, see Day (2003).)

The models presented here are obviously simplifications of the epidemiology of *N. meningitidis* and the biology of their colonization and disease. They are useful because they allow us to explore these processes unambiguously and quantitatively, and to generate testable hypotheses. The assumptions underlying these models and the predictions generated from the analysis of their properties provide hypotheses that can be tested with both prospective (experimental) and retrospective studies. Already, some of these hypotheses are supported by prior empirical observations. There are five such hypotheses.

(i) High rates of phase shifting enable *N. meningitidis* to better transmit among immunologically diverse hosts, and, in particular, to hosts that have had previous exposure to *N. meningitidis*.
(ii) Strains with high rates of phase shifting will increase in prevalence towards the end of outbreaks.
(iii) Colonization of each host habitat (nasopharynx, epithelial cells, blood, CSF) requires the expression of different combinations of contingency loci.
(iv) High rates of phase shifting of loci associated with colonization increase the likelihood that *N. meningitidis* will cause invasive disease.
(v) Invasive disease shortens the transmission window, hence virulent infections lead to fewer secondary colonizations than colonizations that do not lead to invasive disease.

*In vivo* tests of these hypotheses are currently limited by the lack of a good animal model for *N. meningitidis*. With such an animal model, hypothesis (i) can be tested following the methods employed by Marc Lipsitch and colleagues in their studies of *Pneumococcus* colonization in laboratory mice (Lipsitch *et al.* 2000). If hypothesis (i) is valid, then strains with phase-shifting genes associated with capsular or other surface antigen-encoding genes, or even global mutators will be more likely to colonize previously immunized mice (or mice with genetically determined immunological differences) than those with lower rates of phase shifting (or without fast shifting genes).

Hypothesis (ii) is illustrated in figures 2 and 4, where the fast phase-shifting strain reaps the benefits of frequent mutation only after the epidemic has spread extensively through the initial immunological class. In figure 2, a surge in colonization by fast phase shifters follows the first successful transmission of the fast phase-shifting strain from a host of the original immunological class to a host of the yet uninfected immunological class, an event that will occur only after the strain has reached sufficient prevalence in the original immunological class. In figure 4, the fast phase-shifting strain realizes its advantage only after a sufficiently large reservoir of recovered hosts exists. These trends are consistent with an analysis of strains from two worldwide epidemics of *N. meningitidis* (Richardson *et al.* 2002), and can be further tested with similar retrospective studies of epidemic strains collected by WHO, the CDC and other *Neisseria* epidemiologists

(Achtman *et al.* 2001; Zhu *et al.* 2001). One can empirically measure rates of phase variation (Richardson *et al.* 2002) and analyse the frequency of fast shifting strains as a function of both the time-course of the epidemic and the vaccination and health history of the affected individuals.

Hypothesis (iii) is at least partly supported by the empirical work in Hammerschmidt *et al.* (1996) and de Vries *et al.* (1996), which demonstrates that successful colonization of each habitat within a host (nasopharynx, epithelium and blood) requires the expression of a unique combination of contingency loci. Although these observations are consistent with the assumption that genetic changes are required for *N. meningitidis* to move from one within-host site to another, it still requires further validation. If (iii) is correct, then bacteria that successfully colonize the mucosa of the nasopharynx, for example, will be at a disadvantage in the sub-epithelium when competing with closely related strains that have already adapted to the sub-epithelial environment. With an animal model (ideally an inbred animal model), this hypothesis could readily be tested by reconstruction experiments in which mixtures of genetically marked strains isolated from the two within-host habitats, for example, the blood and sub-epithelium, are introduced into each of the two habitats and the changes in their relative frequencies monitored. In accord with this hypothesis, in this competition the strain from the habitat should perform better than that from the other habitats.

With an animal model, hypothesis (iv) can also be addressed through reconstruction competition experiments. In this case, the concern is whether a strain with a high rate of phase shifting at specific contingency loci is more likely to colonize a new host or a new within-host habitat than one with low rates of phase shifting. The experiment would follow those described in Moxon & Murphy (1978) and Pluschke *et al.* (1983). In this case, the colonizing mixture contains one strain with high rates of phase shifting for an array of candidate contingency loci and another strain that does not. In accord with this hypothesis, when introduced into a within-host habitat, such as the nasopharynx, the strain with the higher rate of phase shifting of these contingency genes would be more likely to subsequently invade a new within-host habitat, like the blood, than that with a lower rate of phase shifting.

Without an animal model for *N. meningitidis*, hypotheses (iii) and (iv) could, in a general way, be tested using other bacteria for which there are animal models and that have both phase-shifting loci and only occasional virulence. A good candidate is the infant rat model for *Haemophilus influenzae* (Moxon & Murphy 1978). These two hypotheses as well as hypotheses (i), (ii) and (v) can also be tested retrospectively in human populations using strains collected by the WHO, the CDC and other *Neisseria* epidemiologists (Achtman *et al.* 2001; Zhu *et al.* 2001). To achieve this, one could measure rates of phase variation (Richardson *et al.* 2002) and analyse the frequency of fast shifting strains as a function of both the time-course of the epidemic and the vaccination and health history of the affected individuals. It is important to extend such analyses to include strains taken from asymptomatic carriers (Vogel *et al.* 1998). One can also determine the relationship between the frequency of contingency genes associated with phase-shifting mechanisms, rates of phase shifting and virulence. In accord with the short-sighted evolution hypothesis for the virulence of these bacteria, the incidence of invasive disease should be proportional to the frequency of contingency genes with phase shifting and the rate of phase shifting of those genes.

The phase shifting of contingency loci can be viewed as a 'virulence determinant' in *N. meningitidis*—a mechanism that occasionally leads to invasive infection by these typically commensal bacteria. There are several such bacteria, each with a different spectrum of virulence determinants, including adhesins, outer membrane proteins, capsules and even toxins, which have evolved and are maintained by natural selection. To understand the selective forces responsible for virulence factors in bacteria that are not invariably pathogenic, one has to first consider the relevance of these factors to their non-pathogenic existence and their contribution to the epidemiology of these bacteria. By shifting the perspective from single molecules and factors (which dominate the study of virulence) to a more holistic view of bacteria evolving in a dynamic environment, we will gain new insight into the virulence of bacteria that are usually commensal and, ideally, design more effective treatment, prophylaxis and vaccination strategies for controlling disease caused by these bacteria.

Finally, we offer a caveat not considered above. Within human populations, there is substantial genetic variation that contributes to both the likelihood of a host being colonized by a potentially pathogenic bacteria and the effects of that infection on morbidity and mortality (Sorensen *et al.* 1988). With the exception of the first epidemic model (which considers host genetic variation in susceptibility to specific strains), we neglect to consider the genetic variation underlying susceptibility to *N. meningitidis* in general, and the genetic variation underlying the likelihood of colonization progressing to invasive disease. We recognize that such variation is likely to influence both the virulence and spread of *N. meningitidis*, and believe that the models presented here may serve as a theoretical foundation for future research into the epidemiological consequences of genetic variation.

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## REFERENCES

Achtman, M. (and 10 others) 2001 Molecular epidemiology of serogroup a meningitis in Moscow, 1969 to 1997. *Emerg. Infect. Dis.* **7**, 420–427.

Anderson, R. M. & May, R. M. 1991 *Infectious diseases of humans: dynamics and control.* Oxford University Press.

Anker, M. & Schaaf, D. 2000 WHO report on global surveillance of epidemic-prone infectious diseases. World Health Organization.

Antia, R., Levin, B. R. & May, R. M. 1994 Within-host population dynamics and the evolution and maintenance of microparasite virulence. *Am. Nat.* **144**, 457–472.

Apicella, M. A. 2000 *Neisseria meningitidis*. In *Principles and practices of infectious diseases* (ed. G. L. Mandell, J. E. Bennett & R. Dolin), pp. 1896–1909. New York: Churchill Livingstone Inc.

Bayliss, C. D. & Moxon, E. R. 2002 Hypermutation and bacterial adaptation. *Am. Soc. Microbiol. News* **68**, 549–555.

Bucci, C., Lavitola, A., Salvatore, P., Giudice, L. D., Massardo, D. R., Bruni, C. B. & Alifano, P. 1999 Hypermutation in pathogenic bacteria: frequent phase variation in meningococci is a phenotypic trait of a specialized mutator biotype. *Mol. Cell* **3**, 435–445.

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

de Vries, F. P., van der Ende, A., van Putten, J. P. M. & Dankert, J. 1996 Invasion of primary nasopharyngeal epithelial cells by *Neisseria meningitidis* is controlled by phase variation of multiple surface antigens. *Infect. Immun.* **64**, 2998–3006.

Di Martino, M., Calì, G., Astorre, P., Usai, G., Ferrari, R. & Stroffolini, T. 1990 Meningococcal carriage and vaccination in army recruits in Italy. *Boll. Ist. sieroterap. milan.* **69**, 357–359.

Ewald, P. W. 1983 Host–parasite relations, vectors, and the evolution of disease severity. *A. Rev. Ecol. Syst.* **14**, 465–485.

Gagneux, S., Hodgson, A., Smith, T., Wirth, T., Ehrhard, I., Morelli, G., Genton, B., Binka, F., Achtman, M. & Pluschke, G. 2002 Prospective study of a serogroup X *Neisseria meningitidis* outbreak in northern Ghana. *J. Infect. Dis.* **185**, 618–626.

Garza, J. C., Slatkin, M. & Freimer, N. B. 1995 Microsatellite allele frequencies in humans and chimpanzees with implications for constraints on allele size. *Mol. Biol. Evol.* **12**, 594–603.

Hammerschmidt, S., Müller, A., Sillmann, H., Mühlenhoff, M., Borrow, R., Fox, A., van Putten, J., Zollinger, W. D., Gerardy-Schahn, R. & Frosch, M. 1996 Capsule phase variation in *Neisseria meningitidis* serogroup B by slipped-strand mispairing in the polysialyltransferase gene (siaD): correlation with bacterial invasion and the outbreak of meningococcal disease. *Mol. Microbiol.* **20**, 1211–1220.

Harr, B. & Schlötterer, C. 2000 Long microsatellite alleles in *Drosophila melanogaster* have a downward mutation bias and short persistence times, which cause their genome-wide underrepresentation. *Genetics* **155**, 1213–1220.

Jennings, M. P., Srikhanta, Y. N., Moxon, E. R., Kramer, M., Poolman, J. T., Kuipers, B. & van der Ley, P. 1999 The genetic basis of the phase variation repertoire of lipopolysaccharide immunotypes in *Neisseria meningitidis*. *Microbiology* **145**, 3013–3021.

Jonnson, A. B., Nyberg, G. & Normark, S. 1991 Phase variation of gonococcal pili by frameshift mutation in pilC, a novel gene for pilus assembly. *EMBO J.* **10**, 477–488.

Kruglyak, S., Durrett, R., Schug, M. D. & Aquadro, C. F. 2000 Distribution and abundance of microsatellites in the yeast genome can be explained by a balance between slippage events and point mutations. *Mol. Biol. Evol.* **17**, 1210–1219.

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

Levin, S. & Pimental, E. 1981 Selection of intermediate rate of virulence in parasite–host systems. *Am. Nat.* **117**, 308–315.

Lipsitch, M., Dykes, J. K., Johnson, S. E., Ades, E. W., King, J., Briles, D. E. & Carlone, G. M. 2000 Competition among *Streptococcus pneumoniae* for intranasal colonization in a mouse model. *Vaccine* **18**, 2895–2901.

Moxon, E. R. & Murphy, P. A. 1978 *Haemophilus influenzae* bacteremia and meningitis resulting from the survival of a single organism. *Proc. Natl Acad. Sci. USA* **75**, 1534–1536.

Moxon, E. R., Rainey, P. B., Nowak, M. A. & Lenski, R. E. 1994 Adaptive evolution of highly mutable loci in pathogenic bacteria. *Curr. Biol.* **4**, 24–33.

Murphy, G. L., Connell, T. D., Barritt, D. S., Koomey, M. & Cannon, J. G. 1989 Phase variation of gonococcal protein II: regulation of gene expression by slipped-strand mispairing of a repetitive DNA sequence. *Cell* **56**, 539–547.

Odugbemi, T., Ademidun, O., Agbabiaka, A. & Banjo, T. 1992 Nasopharyngeal carriage of *Neisseria meningitidis* among school children at Ijede, Lagos State, Nigeria. *Ethiop. Med. J.* **30**, 33–36.

Pluschke, G., Mercer, A., Kusecek, B., Pohl, A. & Achtman, M. 1983 Induction of bacteremia in newborn rats by *Escherichia coli* K1 is correlated with only certain O (lipopolysaccharide) antigen types. *Infect. Immun.* **39**, 599–608.

Richardson, A. R. & Stojiljkovic, I. 1999 HmbR, a hemoglobin-binding outer membrane protein of *Neisseria meningitidis*, undergoes phase variation. *J. Bacteriol.* **181**, 2067–2074.

Richardson, A. R. & Stojiljkovic, I. 2001 Mismatch repair and the regulation of phase variation in *Neisseria meningitidis*. *Mol. Microbiol.* **40**, 645–655.

Richardson, A. R., Yu, Z., Popovic, T. & Stojiljkovic, I. 2002 Mutator clones of *Neisseria meningitidis* in epidemic serogroup A disease. *Proc. Natl Acad. Sci. USA* **99**, 6103–6107.

Saunders, N. J., Jeffries, A. C., Peden, J. F., Hood, D. W., Tettelin, H., Rappuoli, R. & Moxon, R. E. 2000 Repeat-associated phase variable genes in the complete genome sequence of *Neisseria meningitidis* strain MC58. *Mol. Microbiol.* **37**, 207–215.

Schug, M. D., Hutter, C. M., Wetterstrand, K. A., Gaudette, M. S., Mackay, T. F. & Aquadro, C. F. 1998 The mutation rates of di-, tri- and tetranucleotide repeats in *Drosophila melanogaster*. *Mol. Biol. Evol.* **15**, 1751–1760.

Sorensen, T. I., Nielson, G., Anderson, P. & Teasdale, T. 1988 Genetic and environmental influences on premature death in adult adoptees. *New Eng. J. Med.* **318**, 727–732.

Taha, M., Deghmane, A., Antignac, A., Zarantonelli, M. L., Larribe, M. & Alonso, J. 2002 The duality of virulence and transmissibility in *Neisseria meningitidis*. *Trends Microbiol.* **10**, 376–382.

Vogel, U., Morelli, G., Zurth, K., Claus, H., Kriener, E., Achtman, M. & Frosch, M. 1998 Necessity of molecular techniques to distinguish between *Neisseria meningitidis* strains isolated from patients with meningococcal disease and from their healthy contacts. *J. Clin. Microbiol.* **36**, 2465–2470.

Zhu, P. (and 15 others) 2001 Fit genotypes and escape variants of subgroup III *Neisseria meningitidis* during three pandemics of epidemic meningitis. *Proc. Natl Acad. Sci. USA* **98**, 5234–5239.

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