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Modeling fimbriae mediated parasite–host interactions

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ABSTRACT

Type 1 fimbriae are a known virulence factor in a number of pathogenic enterobacteriaceae, including *Salmonella*, *Shigella* and *E. coli*. Yet, they are also expressed by some commensal strains, notably of *E. coli*. One hypothesis of the role of fimbriae in commensals is that they evoke a small but tolerable host immune response in order to have the host release sialic acid, which is a valuable nutrient. Genetic evidence suggests that sialic acid down-regulates fimbriation. This has been believed to enable the cells to reduce virulence when the host response is increasing, thus avoiding a full activation of host defenses. In this article we assess the plausibility of this hypothesis using mathematical models. Our models lead us to two main conclusions: A slight activation of host defenses is only possible with a carefully tuned set of parameters, whereas under a wide range of parameters and assumptions, the model predicts the host defenses to be activated to at least half their potential in response to fimbriation. Secondly, the fact that fimbriation is suppressed by sialic acid seems irrelevant for the global qualitative properties.

1. Introduction

Type 1 fimbriae are a type of bacterial adhesin that is widely distributed across pathogenic strains of bacteria, specifically in a number of enterobacteriaceae, including *Salmonella* (Collinson et al., 1996; Mller et al., 1991), *Shigella* (Snellings et al., 1997) and *E. coli*. Fimbriae allow the bacterial parasite to attach itself to host cells. They also have a pro-inflammatory effect, are an important virulence factor (Ashkar et al., 2008; Eto et al., 2007) implicated in urinary tract infections, and are thought to be a factor in a variety of chronic inflammatory diseases (Janben et al., 2001; Gupta et al., 1997).

This article will focus on type 1 fimbriae in *E. coli*. Within this species there is a large variety of strains that differ markedly in their virulence. Some strains cause serious symptoms when ingested, while others are commensals[1] of the human gut and are completely asymptomatic. Interestingly, the human gut commensal is a uro-pathogenic strain (UPEC), meaning that it is virulent when transferred to the urinary tract. The genetic regulation of type 1 fimbriation in commensal strains of *E. coli* has been studied in detail (Lahooti et al., 2005; Sohanpal et al., 2005; El-Labany et al., 2003; Sohanpal et al., 2004). While clearly associated with virulence in pathogenic strains of *E. coli*, it has been found that commensal strains also express fimbriae, although only at a low level, in the sense that only a small proportion of the total population is fimbriate. Hence, the population of bacteria is phenotypically heterogeneous with respect to type 1 fimbriae. Such differential expression of a trait in an isogenic population is called *phase variation* (Bayliss, 2009).

Mechanistically, phase variation is implemented as a molecular random bit generator. Each individual cell can randomly switch between the fimbriate and the afimbriate state. The probability of a particular cell being *fimbriate* or not depends on environmental signals (as described in Chu and Blomfield, 2006), most notably the concentration of sialic acid (SA). SA comprises a family of sugars (one of which is *N*-acetylneuraminic acid) and is released by mammalian hosts as a part of inflammatory host responses (Sohanpal et al., 2005; Severi et al., 2007). It is also a source of nutrient for many bacteria colonizing the host, including *E. coli* that can metabolize SA via the *nanATEK* operon (Chu et al., 2008). SA down-regulates the expression of fimbriae, in the sense that it reduces the rate of individual cells switching to the fimbriate state (Sohanpal et al., 2001; Chu and Blomfield, 2006; Holden et al., 2007).

While the basics of the regulation of fimbriae in commensals are well understood, the biological role of the control mechanism remains unclear. There are a number of hypotheses concerning this. Phase variation is often seen as a bet-hedging strategy (Kussell and Leibler, 2005; Thattai and van Oudenaarden, 2004) to allow optimal growth in changing environments. Another hypothesis is that it is an immune evasion mechanism; two

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[1] We use the word "commensal" here to mean non-pathogenic, and do not commit to any particular assumption about the benefit or detriment the commensal entails for the host.

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excellent reviews are van der Woude and Bäumler (2004); van der Woude (2006). Specifically in the context of type 1 fimbriation, another explanation of the biological function has recently been suggested (Chu et al., 2008; Severi et al., 2007; Chu and Blomfield, 2006; El-Labany et al., 2003): A low level expression of fimbriae could cause a "slight" activation of the host defenses—one that is tolerable and accompanied by a release of SA, so the hypothesis. The regulation of the switch between fimbriate and afimbriate could then be interpreted as the parasites balancing SA release against the risk of a full activation of host defenses.

There are a number of features of the system that suggest this interpretation: SA is a valuable nutrient for *E. coli*, but it is also an indicator of activated host defenses. Its metabolic pathways are co-regulated with fimbriation, suggesting a link between nutrient and virulence. More specifically, the switching rate of the individual cell into the fimbriate state is down-regulated by SA. This could be interpreted as a safety mechanism against too high activation of host defenses. A picture emerges where the commensal parasite usurps the presumed biological function of the host defenses for its own purposes. This is only possible if the host releases SA while the host immune reaction is weak enough to be tolerable. We will henceforth refer to this hypothesis as the *naive host model*. This model appears to unify many of the features of the system into a parsimonious explanation involving the environmental context of the parasites.

A potential problem with the naive host model, however, is that the interaction between host and parasites has a closed feedback loop, which could lead to unintuitive behaviors. The amount of SA released by the host depends on the absolute number of fimbriate cells. In turn, the absolute number of fimbriate cells will depend on the population size and the probability of each cell being fimbriate. These complications cannot be glossed over. Intuitively, it is not clear under which circumstances, or even if, this model supports a stable steady state or whether it leads to unsustainable run-away growth. The behavior of this system has never been analyzed theoretically.

In this contribution, we close this modeling gap in order to assess the plausibility of the naive host model. We present both a differential equation model and a stochastic Markov chain model of the fimbriae mediated host–parasite interaction. The models show that, under a wide range of conditions, the fimbriae–host interaction leads to a stable steady state parasite population. This steady state population is possibly of vanishing size. An implication of the model is that, except for a narrow range of parameters, any stable steady state supported by the system either leaves host defenses in-activated, or activates them significantly—this is true independent of the parameters and, indeed, independent of the whether fimbriation is repressed or enhanced by SA. We will conclude from this that the naive host model is not a satisfactory interpretation of the host–parasite interactions. Instead, we will suggest that phase variation of type 1 fimbriation could be a mechanism to enable parasites to be virulent in one environment and commensal in another.

2. Results

2.1. Base model

To derive a model we assume that the host consists of N_H cells. At any point in time, t, there are $q(t)$ parasite cells of which $n(t)$ are fimbriate. We assume that every fimbriate cell attaches to a host cell, such that there are $\lambda = n/N_H$ parasites attached to each host cell on average. The probability of any particular host cell being in contact with exactly k fimbriate parasites is described by a Poisson distribution. We will use this as an *ansatz* to derive the shape of the host activation curve, i.e., the activation of the host defenses as a function of the number of fimbriate bacteria. To simplify the problem, we assume that each of the host cells has its defenses activated if θ or more fimbriate parasites attach to it, in which case it releases sialic acid. If activated then each cell releases SA at a fixed rate. If it is in contact with fewer than θ cells then it releases nothing. We assume here that each of the host cells acts independently. This is likely to be a simplification. Since we are only interested in the qualitative shape of the response (rather than a quantitative model) and since we will not model spatial effects, it would seem excessive to take into account unknown host–host interactions.

We can now calculate the probability that an individual host cell releases sialic acid, which is also the proportion of host cells that are activated.

$$P(\text{release}) = f_\theta(\lambda) = \sum_{k=\theta}^{\infty} \frac{\lambda^k \exp -\lambda}{k!} \tag{1}$$

This sum can be expressed in terms of Γ functions for positive integer values of θ. However, for our purposes, more instructive are specific values of the solutions for particular values of θ.

The function $f_\theta(\lambda)$ has a very natural interpretation in that it formulates the probability that a particular host cell is in contact with at least θ fimbriate parasites, where λ is the average number of fimbriate parasites per host cell. It can also be taken as a measure of the global activation level of the host, with possible values ranging from zero (no activation) to one (full activation). The function $f_0(\lambda) \equiv 1$ represents a trivial case such that the host is always fully activated even in the absence of fimbriate parasites. The function $f_1(\lambda) = 1 - \exp(-\lambda)$, on the other hand, is a globally concave function for $\lambda > 0$, i.e., $f_1' > 0$ and $f_1'' < 0$ for all $\lambda > 0$. For $\theta > 1$, the functions $f_\theta(\lambda)$ are no longer globally concave. Instead, they are sigmoid functions of the average number of fimbriate cells per host cell.

Unfortunately, the functions f_θ are somewhat cumbersome to use. To simplify the analysis and for convenience, we use a Hill function (Real, 1977; Holling, 1977; Hofmeyr and Cornish-Bowden, 1997) to represent the rate at which the host releases nutrient in response to fimbriation (Fig. 1). It will turn out, below, that the qualitative properties of the system are unaffected by the specifics of the response function, but mainly depend on its overall shape, whether concave or sigmoid. In the spirit of notational economy, we use the absolute number of fimbriate parasites, n, as our free variable, rather than the average number

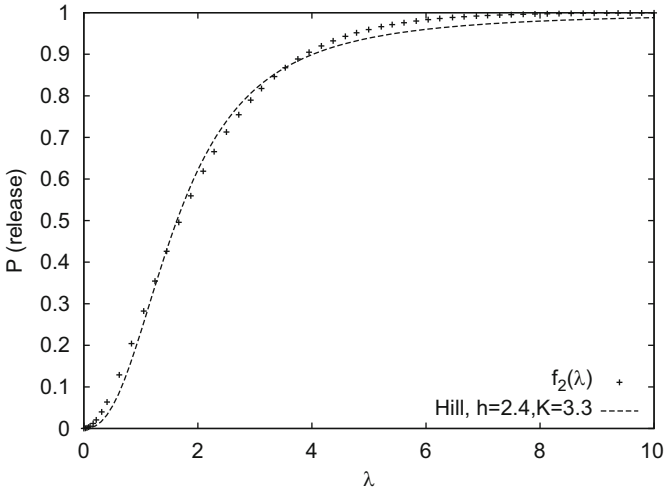

Fig. 1. The functions $f_\theta(\lambda)$ are sigmoid functions that can be fitted to Hill functions. This graph shows $f_2(\lambda)$ and a Hill function with $h = 2.43$ and $K = 3.29$.

of parasites per host cell λ. Since λ and n only differ by a constant factor N_H^{-1}, this amounts to scaling the value of the Hill constant K by N_{Hh} (a fixed number). Again, we are only interested in qualitative properties, and, hence, do not need to worry about this rescaling of the Hill function. Altogether, the rate of nutrient release is given by

$$r = \frac{n^h}{K^h + n^h}. \tag{2}$$

This rate is concave for $h \leq 1$ and a sigmoid function of n for $h > 1$.

In *E. coli*, a single recombination event requires the simultaneous binding of four FimB proteins to the respective binding sites. This is commonly modeled using the Hill function, where the Hill coefficient depends on specific aspects of the system (specifically, the number of binding sites and the cooperativity between the binding sites Chu et al., 2009). The amount of FimB itself is regulated by the sialic acid concentration in the cell. We will exclude the molecular aspects in the individual cells here and assume that the rate of fimbriation depends on the total concentration of nutrient, s, in the environment of the cell:

$$r_{s+} = \frac{K_s^{h_s}}{K_s^{h_s} + s^{h_s}} \tag{3}$$

Switching from the fimbriate to the afimbriate state can be thought of as independent of the sialic acid concentration in the environment (Sohanpal et al., 2001; Holden et al., 2007), and we assume it to happen at a constant rate b. Finally, we assume that the bacteria grow while consuming the nutrient with a rate constant of 1 and die with a rate constant of m. Altogether, if we assume that there are q bacteria in the population of which n are fimbriate, then we obtain our model system defined by three differential equations.

$$\dot{s} = -qs + \frac{\nu n^h}{K^h + n^h} \tag{4}$$

$$\dot{n} = sn - mn + \frac{\nu_s K_s^{h_s}(q-n)}{K_s^{h_s} + s^{h_s}} - bn \tag{5}$$

$$\dot{q} = qs - qm \tag{6}$$

The parameters ν and ν_s determine the maximum rate of SA release and the maximum rate of switching fimbrial expression on. It is easy to see that $q(t) \geq n(t) \geq 0$ if $q(0) \geq n(0) \geq 0$, as it should be. We assume that sialic acid and both fimbriate and afimbriate cells are well mixed, thus ignoring spatial effects. In practice, perfect mixing only requires that the time scale of growth of bacteria is small compared to the diffusion of sialic acid through the system, and that both fimbriate and afimbriate cells have equal access to nutrients. Nutrient stays in the environment unless it is consumed by the cells. This is a simplifying assumption that is not necessarily met. However, one can always assume that any loss due to other sources is incorporated into the release rate of the nutrient.

2.2. Analysis

We analyze the steady state behavior of the system Eqs. (4)–(6). From Eq. (6) we get $s = m$ at steady state. Given this, we can then obtain the nullclines of the reduced system from Eqs. (4) and (5).

$$q = \frac{\nu n^h}{(K^h + n^h)m} \tag{7}$$

$$q = \kappa n = \frac{n(\nu_s K_s^{h_s} + bK_s^{h_s} + bm^{h_s})}{\nu_s K_s^{h_s}} \tag{8}$$

Note that Eq. (7) is, in essence, the activation function of the host. Hence, even though sialic acid concentration has been eliminated from the two nullclines, they still allow us to draw conclusions about the relative activation levels of the host. We will use this below.

For $h=1$ we obtain the steady state values of $n_1^*=0$ and $n_2^* = -(m\kappa K - \nu)/m\kappa$. The non-trivial solution only exists for $\nu \geq m\kappa K$. In the case of $h=2$ the set of solutions is given by

$$n_1^* = 0 \quad \text{and} \quad n_{2,3}^* = \frac{1}{2}\frac{\nu \pm \sqrt{\nu^2 - 4m^2\kappa^2K^2}}{m\kappa}$$

There exist either 1 or 3 positive real solutions. The latter is the case when $\nu > 2m\kappa K$. Finally, we can also generate the solutions for $h=3$. The analytic expression for these is rather complicated and we will suppress it here. Yet, we note that three positive real solutions exist in this case if $\nu > (1/4)^{1/3}3m\kappa K$. Apart from these three special cases, it is not possible to obtain meaningful closed form expressions for the steady states. It is possible, however, to make general statements about the existence and qualitative properties of the steady states based on the shapes of the nullclines. The first nullcline is a Hill function with Hill coefficient h, and the second nullcline is a straight line going through the origin (Fig. 2). Steady state solutions correspond to the points where these curves intersect. This means that the trivial steady state, given by $n^*=q^*=0$, always exists. For $h > 1$, there exist two positive real steady states in the special case when:

$$\frac{d}{dn}\frac{\nu n^h}{(K^h + n^h)m}\bigg|_{n=n^*} = \kappa$$

Finally, for some parameters, there exist three steady states, $n_0^* < n_1^* < n_2^*$. In this case, the middle steady state n_1^* is always unstable, whereas n_0^* and n_2^* are stable (Cherry and Adler, 2000). The system is then bistable. We conclude that the system allows either a unique, but trivial steady state, or is bistable.

The nullclines not only provide information about the size of the bacterial population, but also the extent to which the host defenses are activated. One of the two nullclines is, in essence, the activation function of the host (even though the axes of the graph correspond to the sizes of the total and fimbriate bacteria populations). Consequently, the position of the steady state on the sigmoidal nullcline indicates whether the host's immune response is on or not. The higher up the intersection on the sigmoidal curve, the higher the activation state of the host at steady state.

In the case of a bistable system and $h \geq 2$, the stable steady state is always larger than the saturation constant in the Hill function, i.e., $n^* \geq K$. In the case of $h=2$ and $h=3$, this can be explicitly shown by calculating the value of κ for which there are exactly two positive real solutions. Setting this value of κ into the original equation and solving the intersection problem recovers the result.

More generally, the same can be easily seen geometrically. For $h > 1$ and positive arguments, there is exactly one point at which a line through the origin can be a tangent to a Hill function $h(n)$. For $h=2$, this point is exactly given by $n=$K, but for higher h, it will be somewhat larger than K, namely $n_T = (h-1)^{1/h}K$. The slope of this tangent is given by $\kappa_T = h'(n_T)$. As far as our system is concerned, for any $\kappa > \kappa_T$, there will be only the trivial steady state. For any $\kappa < \kappa_T$, there are two stable steady states, and the non-trivial steady state will be greater than $n_2^* > n_T \geq K$ as long as $h \geq K$.

In biological terms, this means that the "nutrient release system" of the host is at least moderately activated; that is, releasing nutrient at a rate of at least half the maximum rate (or at least half of the host cells are in contact with θ or more fimbriate cells). Concretely, this can be seen directly from the graphs in Fig. 2. For higher Hill coefficients h, a moderate activation is only

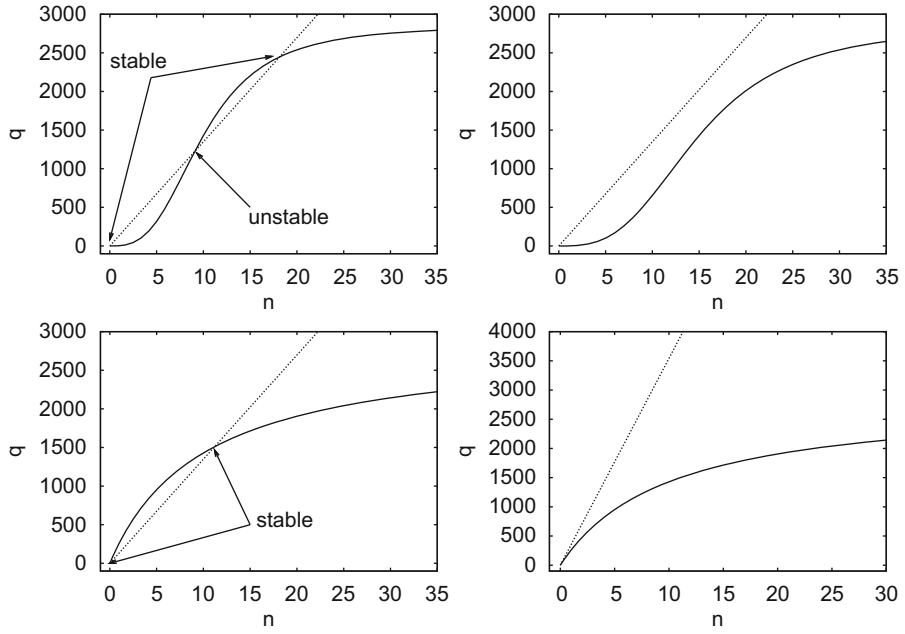

Fig. 2. The nullclines of the system. The two curves in each graph correspond to Eq. (8). Their intersection gives the steady state. Note, however, that one of the nullclines originates from the SA activation function. It is therefore possible to draw from them conclusions about the activation state of the host. (Top) the parameters used in this example are: $h=h_s=3$, $\nu_s=10$, $K_s=20$, $b=30.5$. Furthermore, $K=10$ on the left-hand side and $K=15$ on the right-hand side. (Bottom) the nullclines for $h=1$. The parameters are the same, but $K=10$ in both graphs. The graph on the right-hand side has a parameter of $b=80.5$.

possible within a relatively small range of parameters. On the other hand, for most parameters that allow bistable behavior, the non-trivial stable steady state will be close to the saturation value of nutrient release.

The situation is different, however, for a Hill coefficient $h \leq 1$. In that case, the system supports two stable states for some parameters, and there is no restriction on the position of the steady state. Specifically, stable states that do not saturate the response function of the host are possible.

2.3. Variations of the model

We consider a variation on the basic model of Eq. (6) by changing the regulation function. Let us assume that, instead of being repressed by sialic acid, fimbriation is activated by it. The resulting system has state variables $\dot{\bar{s}},\dot{\bar{q}},\dot{\bar{n}}$. The differential equations for the first two variables are the same as in Eq. (6), and we replace the differential equation for $n(t)$,

$$\dot{\bar{n}} = s\bar{n} - m\bar{n} + \nu_s \underbrace{\frac{\bar{s}_s^{h_s}}{K_s^{h_s} + \bar{s}^{h_s}}}_{①} (\bar{q} - \bar{n}) - b\bar{n}. \tag{9}$$

The key difference between Eqs. (9) and (5) is that the Hill function in the third term of Eq. (9) has changed from a Hill repressor function to a standard Hill function.

For a given set of parameters, this change certainly leads to a quantitative change of the transient and long-term behavior of the model. However, with respect to the qualitative dynamics of the system, in terms of the existence, range, and stability of steady states, the modified model has the same properties as the original one. In particular, the nullclines for the modified system have the same formal structure as Eq. (8), although the proportionality constant κ needs to be re-defined in terms of the system parameters. This suggests that, at least as far as the steady state of the system is concerned, the fact that fimbriation is repressed by sialic acid is not relevant.

In fact, it can easily be seen that the regulation function (term ① on the right-hand side of Eq. (9)) can be changed to an arbitrary function of $f(s)$ without affecting the qualitative behavior of the system, as long as f is independent of $\bar{n}$ and $\bar{q}$. In general, the slope of the nullcline would then be given by the constant $\bar{\bar{\kappa}}$

$$\bar{\bar{\kappa}} = \frac{n\nu_s f(s) + b}{\nu_s f(s)}. \tag{10}$$

Once again, this system has the same global stability properties as Eq. (6).

Another variation of the model considers an alternative source of nutrient in addition to sialic acid. We obtain this second modified system (with variables $\hat{s}, \hat{n}, \hat{q}$) by adding an extra growth term in the last line of Eq. (6). We are not concerned with the precise nature of these additional nutrient sources, but assume that they contribute to the population some constant growth L. The differential equations for $\hat{s}$ and $\hat{n}$ are the same as their counterpart in Eq. (6), but there is a new equation for $\hat{q}$.

$$\dot{\hat{q}} = \hat{q}\hat{s} - \hat{q}m + L \tag{11}$$

A closer inspection of this system shows that, for L small, Eq. (11) has the same overall qualitative behavior as Eq. (6), although the lower stable steady state will be $q^* > 0$. Intuitively, this can be easily understood by considering that the additional nutrient source will always support a limited parasite population, even in the absence of sialic acid. For very high values of L, the bistable character of the system is lost, and there exists exactly one stable steady state (Appendix B).

We see that our model shows either bistable behavior or is mono-stable. If it is mono-stable, then fimbriation is essentially not activated. In the bistable regime, the SA release of the host is in a significant state of activation, unless $h < 2$ for which there are parameters that allow a low activation of sialic acid release.

2.4. Relation of model to previous experiments by other authors

The regulation of type 1 fimbriation has been studied experimentally by other authors; see for example Sohanpal

et al. (2005). Unfortunately, our prediction that the fimbriation levels are not sensitive to the sign of the regulation can neither be confirmed nor refuted from existing data in the literature. The reason is as follows: Existing experiments normally study the switching probability as a function of SA concentration in a defined medium. By assuming a fixed amount of nutrient supplied to the bacteria, these experiments do not represent the dependence of the amount of SA on the number of fimbriate bacteria. They thus break a central feedback element in model Eq. (6) that is also thought to be present under physiological conditions in the gut. In order to test our model experimentally, it would be necessary to explicitly include the feedback from the host to the colony into the experimental design.

In practice, the authors of Sohanpal et al. (2005) measured the dependence of the fimbriation rate on SA by growing a colony in a defined medium. After a certain time has passed, the proportion of fimbriate cells could be determined which, in turn, would allow the switching rate to be calculated. We can model this experiment by assuming $\dot{s}=0$ and $s(t)=s_0$. Furthermore, if we assume that the switching has equilibrated, then we obtain the fraction of fimbriate cells by solving

$$\dot{n}=\frac{v_s K_s^{h_s}(q-n)}{K_s^{h_s}+s^{h_s}}-bn=0 \tag{12}$$

for n/q, which yields

$$\frac{n}{q}=\frac{v_s K_s^{h_s}}{v_s K_s^{h_s}+bK_s^{h_s}+bs^{h_s}}. \tag{13}$$

Eq. (13) models qualitatively the behavior of fimbriae in defined media with a fixed SA concentration. Fig. 3 illustrates this for various parameters, which shows that the fimbriation level now depends on the amount of SA. Qualitatively this behavior is the same as that reported by Sohanpal et al. (2005).

If we changed the sign of the interaction in the context of Eq. (13) (i.e., assume that SA activates fimbriation rather than represses it), more sialic acid would clearly lead to higher fimbriation rates. We conclude that, in order to test the predictions of our model, the experimental design must include the feedback from the host. In this case, the qualitative features of the system will be independent of the sign of the regulation function. This prediction is yet to be addressed experimentally.

2.5. Stochastic model

We designed a stochastic equivalent of the system Eq. (6) using a continuous time stochastic Markov chain (MC) model to represent the system (Eq. (14)). Each state of the model can be described by a 3-tuple (q,n,s), where each entry has the same meaning as the corresponding state variable in the differential equation model, although $q,n,s\in\mathbb{N}$ in the MC model. We describe the model in terms of its possible state transitions and rates.

$$\begin{aligned}
&q\longrightarrow q+1 : (q-n)s\\
&q\longrightarrow q-1 : mq\\
&n\longrightarrow n+1 : ns\\
&n\longrightarrow n+1 : (q-n)v_s\frac{K_s^h}{K_s^h+K_s^h}\\
&n\longrightarrow n-1 : bn+mn\\
&s\longrightarrow s+1 : v\frac{n^h}{n^h+K^h}\\
&s\longrightarrow s-1 : qs
\end{aligned} \tag{14}$$

For parameters that lead to reasonably sized numbers, that is, $q,n,s\gg 1$, the MC-model is a stochastic version of the differential equation model. Fig. 4 shows a comparison of one path of the MC model and a numerical solution of a differential equation using the same parameters.

The MC-model displays the same bistable behavior as the differential equation model. However, depending on the parameters, stochastic fluctuations can cause a transition between the two deterministic steady states. Fig. 5 illustrates one example of a bistable system. For about 18 time units, it remains in the low stable state before (stochastically) making the transition to the high state.

The particular transition probabilities between the two stable states will depend on the parameters and will, in general, not be symmetric. The major determinant for the switching probability is the size of the basins of attraction of the stable states in the deterministic model. In particular, close to the mono-stable regime, the stochastic model spends much more time in the trivial state than in the other stable steady state. In general, when the deterministic model is bi-stable, so is the stochastic. Hence,

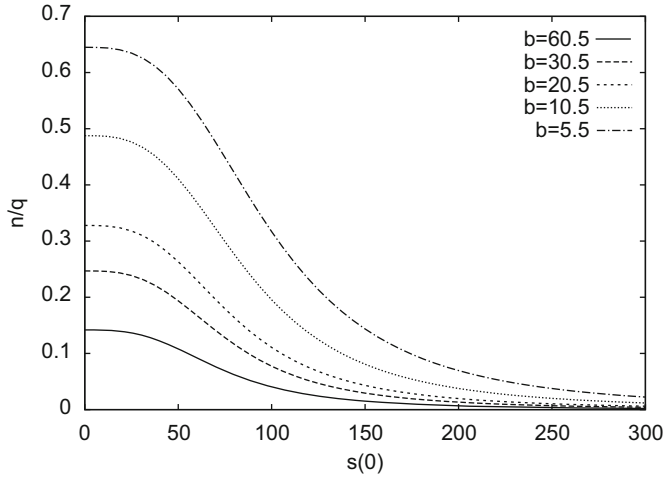

Fig. 3. The equilibrium proportion of fimbriate cells as given by Eq. (13) for various values of b. Compare this with (Sohanpal et al., 2005). The parameters used in this example are: $h_s=3$, $v_s=10$, $K_s=20$.

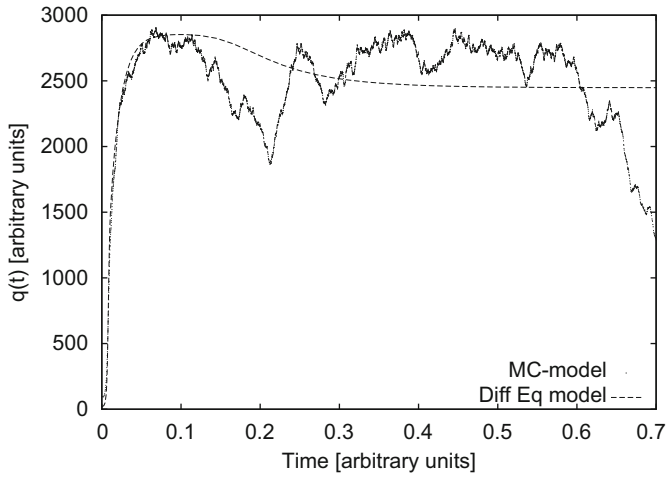

Fig. 4. Comparing the deterministic function with the Markov Chain model. The parameters used in this example are: $h_s=3$, $v=20\,000$, $v_s=10$, $K=10$, $K_s=20$, $m=70$, $b=30.5064$.

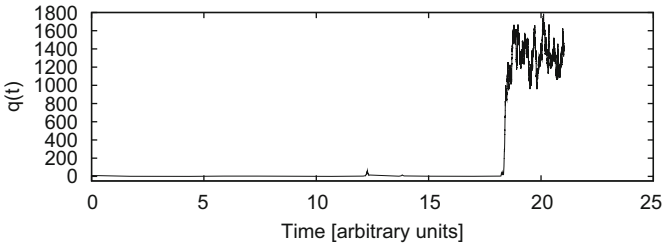

Fig. 5. Noise-induced transition between the stable steady states. The parameters used in this example are: $h_s=3$, $v=20\,000$, $v_s=10$, $K=10$, $K_s=20$, $m=70$, $b=30.5064$. We chose the initial conditions corresponding to $q(0)=10$, $n(0)=0$, $s(0)=1$. The system remains in the trivial steady state initially until it spontaneously makes the transition to the high state at around $t=18$. This is a manifestation of the transition between the two stable states caused by random fluctuations in stochastic systems, as discussed in the main text. Note that the precise time of the transition is stochastic and will differ strongly between runs.

we expect real systems in the bi-stable regime to occasionally switch between the possible steady states, i.e., between virulence and non-virulence.

3. Discussion

Prima facie, the naive host model is an attractive interpretation of the biological function of phase variation of type 1 fimbriation. In order to understand whether or not this interpretation is plausible as well, it is necessary to revisit the two fundamental assumptions of the naive host model. Firstly, underlying the model is the idea that the parasites express fimbriae just enough for the host to release nutrient, yet not enough for the host to activate intolerable defense mechanisms. In this sense, the release of nutrients by the host would be a "vulnerability" in the host defenses that the parasite exploits. Assuming such a vulnerability is only plausible if it is a systemic weakness that is hard to avoid by the host. If, on the other hand, this vulnerability only exists under very specific circumstances (i.e., within a narrow range of parameters), then it should be interpreted as an adaptation of the host, rather than a vulnerability.

Secondly, a fundamental assumption of the naive host model is that fimbrial expression is regulated such that it is compatible with commensalism (simply because the naive host model attempts to explain why commensals express fimbriae). This means that the naive host model can only be considered plausible if there is a way for the host defenses to be only mildly activated by phase-varying fimbriae.

Based on our understanding of the biology of fimbriation, we assume that the release of sialic acid in response to fimbriation is linked to the activation of host defenses. While it is known that the fimbriation probability of an individual cell is reduced if the amount of SA increases (Sohanpal et al., 2005), the quantitative link between nutrient release and host defenses is unknown. In any case, it is likely to vary between cell-types and species. In order to be able to interpret our mathematical model biologically, we need to consider various possibilities. We will find that none of these possibilities is compatible with the naive host model.

Our steady state analysis showed that, if the activation function is sigmoid with $h \geq 2$, then there are only two possible activation states of the host defenses. If fimbriae are expressed then host defenses are activated at half the maximum, at least. Alternatively, it could be that the host defenses are completely de-activated, which corresponds to the trivial steady state with no fimbrial expression (Fig. 2 (top)). This general conclusion holds for all parameters as long as the activation function is sigmoid. It is also robust with respect to many structural changes of the model equations.

This scenario is not compatible with the parasites being commensals, and is therefore a problem for the naive host model. For one, half the host-cells being activated is a significant activation of the host defenses and is difficult to reconcile with commensalism (and *a fortiori* with the naive host model). Furthermore, half-activation of the host-defenses represents a lower bound. For most parameters supporting bi-stability, the steady state will be significantly higher than half-activation.

One way to recover commensalism is to assume that the host activation curve is globally concave, i.e., $0 < h \leq 1$. As can be seen from Fig. 2 (bottom), in addition to the trivial state, this scenario is compatible with a weak activation of host defenses. In order to realize this possibility the intersection of the straight line with the concave curve must be within a narrow range of parameters where there is a stable steady state compatible with a low activation of host defenses. The concave curve is steepest, and the slope is greatest, nearest to the origin of the curve. Consequently, a small change of the angle of the straight line in Fig. 2, corresponding to a small change of parameters, leads to a disproportionally large change of the steady state. This is due to the geometry of the nullclines. Realizing a low host activation therefore requires careful co-evolutionary fine-tuning of the parameters (both of the host and the parasite) and is difficult to reconcile with the naive host model. If $1 < h < 2$ then the system would allow stable steady states with low activation levels; yet, again, there is a high sensitivity to parameters.

There is another possible way in which our model would allow commensalism. Under a wide range of parameters, the host–fimbriae interaction allows two stable steady states, namely the activated one and a trivial one with little or no activation of the host-defenses. This tells us that commensalism could correspond to the population residing in the trivial steady state where host-defenses are not activated. In this case, we would need to assume $L > 0$ (see Eq. (11)). This scenario begs the question as to why the cell maintains the complicated regulatory network of virulence factors when, in fact, it does not use them. An intriguing potential interpretation for this is that phase variation of fimbriae supports different virulence strategies for different environments or, equivalently, in some environments the population lives at the trivial steady state, while in others at the activated one.

In the context of this scenario the commensal environment could have parameters that allow only one steady state, which would be the trivial steady state. This corresponds to the scenarios on the right-hand side of Fig. 2 and is unproblematic. Alternatively, the trivial steady state can also be realized in a bi-stable system, corresponding to the scenarios on the left-hand side of Fig. 2. This latter case is somewhat more complicated and requires some clarification. A bi-stable deterministic system will not leave a stable steady state once occupied: once commensal, always commensal. The snag is that real populations do not behave like deterministic systems, because real systems consist of a *finite* number of cells and are thus subject to stochastic fluctuations. The resulting behavior can be quite different from that of their deterministic equivalents; see Fig. 4 for an illustration. In particular, fluctuations can lead to spontaneous transition between stable states; Fig. 5 illustrates a simulated example. The time at which a particular transition takes place cannot be predicted, although the relative time the system spends in one or the other state will in the long run depend on the parameters (i.e., the environment) in a predictable, deterministic way. Hence, while occasional switching between the steady states would be inevitable in a bi-stable system, proper (co-evolutionary) fine-tuning of parameters could ensure that the system spends most of its time in a particular steady state (i.e., commensalism or virulence).

Hence, our steady state analysis shows that phase variation can naturally lead to different virulence properties in different environments. This could be adaptive if there is a preferred environment, with ideal conditions allowing steady growth rates without virulence. If, on the other hand, the bacteria are transferred to a less suitable environment, it would be more beneficial to switch to a high growth and high transmission rate strategy, i.e., virulence. This possibility is supported by the observation that the gut commensal *E. coli* is a uro-pathogenic strain.

It is enlightening to compare our interpretation with other accounts of phase variation as an adaptation to different environments (Kussell and Leibler, 2005; Thattai and van Oudenaarden, 2004). The basic idea of these is that different phenotypes vary in their growth characteristics across likely environmental conditions. A mix of phenotypes in a clonal population can then provide overall faster growth when environmental conditions are fluctuating. This strategy is often referred to as *bet-hedging*. Our model led us to another possible mechanism of phase variation as a means of adjusting to different environments that is subtly different from standard bet-hedging scenarios. In our account, we assumed that there is feedback, not only from the environment to the heterogeneity of the population (i.e., the fimbriation levels), but also the other way round (i.e., environmental conditions depend on fimbriation levels). The parasites are thus not passively tolerating the environmental conditions but, to some extent, manipulating them. In the case of phase variation of fimbriation, the condition individual cells find themselves in depend on the state of the population as a whole.

A consequence of this is that the switching rate assumes an entirely different role. In bet-hedging scenarios, it should reflect the frequency of environmental changes in order for the mean fitness of the population to be optimised (King and Masel, 2007). This can be illustrated using the example of sporulation (Maughan et al., 2007, 2009) in bacteria, which is a state of low growth but high resistance to adverse environmental states. Cells switch more or less randomly into this state of sporulation. Clearly, the rate of switching into sporulation needs to be finely tuned to optimise fitness; too high a rate and the cell loses out on expected growth, too low a rate and the cell does not sufficiently guard against adverse effects. In the scenario that is proposed here for fimbriation, the switching rate regulates the virulence of the group as a whole in that it determines the virulence characteristics of the parasites. The relationship between fitness and switching rate is therefore entirely different from that in the case of bet-hedging (see Chu, 2008 for details).

There is one theoretical possibility to save the naive host model. We could assume that host defenses and sialic acid release are activated by the same stimuli (i.e., attachment of fimbriae) but not necessarily to the same extent. This is illustrated schematically in Fig. 6, where we assume that both activation curves are of Hill type with the same h but different Hill constants, namely K_s and K_f, respectively. Depending on exactly how K_s and K_f are related, this case could allow a high rate of nutrient release with a low rate of host defense activation. Note that, in this scenario, the particular shape (i.e., whether globally concave or sigmoid) does not matter as much as the relationship between the nutrient and the defense response. For any shape, there are parameters that could allow a low activation of the immune response combined with a high activation of the nutrient response. This scenario is compatible with both significant release of SA and low activation of host defenses. However, it is difficult to see how this situation could be evolutionarily stable. Hosts with mutations that reduced the release of SA would leak less energy and be better off, hence the naive host model does not represent an evolutionarily stable state.

There are also questions as to the evolutionary origin and stability of fimbriae themselves. Addressing these in full is beyond the scope of this contribution, yet a few comments are in place. Expression of fimbriae comes to the cell at a metabolic cost. Under laboratory conditions, this translates to overall lower growth rates of fimbriate cells compared to afimbriates. Thus, there is a potential shared resource problem lurking here, because non-fimbriate cheaters could still benefit from any nutrient released by the host in response to fimbriate cells. There are a number of potential solutions to this in the form of group selection mechanisms or inclusive fitness considerations (Traulsen and Nowak, 2006; Buckling et al., 2007; Griffin et al., 2004; Kreft and Bonhoeffer, 2005; Wild et al., 2009). These shared-resource problems are common in biology; yet it is unclear to what extent they actually apply to the specific case of type 1 fimbriation. Unlike laboratory strains, *in vivo* fimbriate cells may not be at a net disadvantage, despite the metabolic investment necessary to produce fimbriae. For one, fimbriate cells could have preferential access to nutrients released by the host to which they are attached. Moreover, fimbriate cells may be less prone to be transferred to different (worse) environments. In the absence of a better understanding of the details of the host–parasite interactions, it is difficult to develop a proper understanding of the inter-parasite evolutionary dynamics.

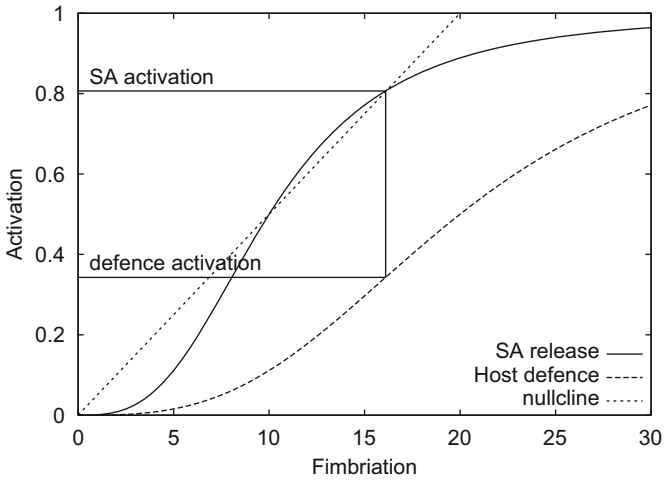

Fig. 6. A schematic illustration of the case where defense and host activation have different Hill constants. The lower sigmoid curve represents the host activation, the upper one the nutrient release function. The straight line is the nullcline of the system. The levels of nutrient release and host defense activations are indicated.

Altogether, we conclude that the naive host model is not compatible with our model. Instead, we propose that phase-variation of type 1 fimbriae in *E. coli* is a mechanism for enabling an environment-specific virulence response.

In this contribution, we have relied primarily on qualitative considerations. These can give important and general insights into the possible dynamics of the system. To create a better understanding of the actual behavior of the system, it is essential to make quantitative predictions. For this to be possible, it is necessary to build more detailed models based on a quantitative understanding of the system.

Appendix A. Material and methods

To integrate the differential equations we used the standard integrator of the Maple 13 computer algebra system. The Markov-chain model was simulated using the simulation function of the Prism (v. 3.3) model checker (Kwiatkowska et al., 2001).

The fit in Fig. 1 was obtained by using the standard fitting method of the gnuplot software package. The asymptotic standard error of the fit was 2.285% and 4.521% for h and K, respectively.

Appendix B. Steady states for the system Eq. (11)

We discuss the number of steady states for the system Eq. (11) when $L>0$. Unfortunately, the additional term complicates the analysis somewhat, such that it is no longer possible to obtain the steady state value of $\hat{s}^*$ directly by solving Eq. (11). Hence, the problem of finding the steady state points requires finding the intersections of three surfaces.

$$\hat{q}=\frac{\hat{n}(-\hat{s}K_s^{h_s}-\hat{s}^{h_s+1}+b\hat{s}^{h_s}+mK_s^{h_s}+m\hat{s}^{h_s}+v_sK_s^{h_s}+bK_s^{h_s})}{v_sK_s^{h_s}}$$

$$\hat{q}=\frac{v\hat{n}^h}{(K^h+\hat{n}^h)\hat{s}}$$

$$\hat{q}=\frac{L}{-\hat{s}+m} \tag{B.1}$$

Inspection of the first two lines in this system shows that these are a family of Hill-functions and straight lines parameterized by $\hat{s}$. As far as their intersection is concerned, our corresponding argument for Eq. (6) applies for each pair of curves corresponding to the same value of s. To find the overall steady states of the equivalent hatted system, we have to identify values of $\hat{s}$ that yield the same values of $\hat{q}$ as points of intersections between the straight line and the Hill function.

If we assume a set of parameters such that the original system given by Eq. (6) has three steady state solutions and $L\ll 1$, then the hatted system will have three steady states as well. To see this, consider that in the case of $L=0$, the steady states are given by the intersection of the first two equations in (B.1) with the surface $s\equiv m$. For $L\to 0$, this surface is continuously approached.

On the other hand, assuming the un-hatted system has three steady states, then there is a choice for L such that the system of Eq. (11) has at most one steady state. To see this, observe that the third line in Eq. (B.1) has a minimal value of L/m for $\hat{s}=0$. Choose L such that $2L/m>v$, then by the geometric arguments given above in the context of Eq. (6), it is not possible for the two lower steady states to be intersected by the third surface of Eq. (B.1).

Finally, Eq. (11) has at least one steady state. From the third line in Eq. (B.1) we see that the size of the population is positive and finite if $\hat{s}\le m$. Re-inserting the third line into the second line, we obtain a solution for $\hat{s}$,

$$\hat{s}=\frac{vg(\hat{n})}{L+vg(\hat{n})}m\le m. \tag{B.2}$$

Here, $g(\hat{n})$ is a shorthand for the Hill function in the second line of Eq. (B.1).

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