Within-Host Disease Ecology in the Sea Fan *Gorgonia ventalina*: Modeling the Spatial Immunodynamics of a Coral-Pathogen Interaction

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Abstract: We develop a spatially explicit model for the within-host interactions between a fungal pathogen and the immune response by its coral host. The model is parameterized for the recent epizootic of *Aspergillus sydowii* in the sea fan *Gorgonia ventalina*, but its structure is adaptable to many other diseases attacking corals worldwide, fungal infections in other invertebrates and plants, and opportunistic fungal infections in vertebrates. Model processes include pathogen growth and spread through consumption of host tissue, chemotactic attraction of undifferentiated host amoebocytes to infections, and amoebocyte differentiation into various cell types that attack the pathogen. Sensitivity analysis shows that the spread rate of a single localized infection is determined primarily by the pathogen’s potential rate of host tissue consumption and by the host’s ability to replenish the pool of undifferentiated amoebocytes and sustain a long-term response. The spatial localization of immune responses creates potentially strong indirect interactions between distant lesions, allowing new infections to grow rapidly while host resources are concentrated at older, larger infections. These findings provide possible mechanistic explanations for effects of environmental stressors (e.g., ocean warming, nutrient enrichment) on aspergillosis prevalence and severity and for the observed high spatial and between-host variability in disease impacts.

Keywords: spatial ecology, pathogen, immune system, aspergillosis, corals, sea fan.

The progress and outcome of fungal infections in plants, invertebrates, and vertebrates are determined by the dynamics of pathogen growth and the corresponding host immune response. Most fungal infections of vertebrates are stopped at skin and surface mucosal barriers, and infections that do penetrate to deep tissues are often difficult to eradicate. Fungal pathogens are primarily opportunistic in nature, establishing when host immune defenses are weakened or breached. For example, opportunistic fungal infections in vertebrates most often occur when the host has impaired innate and/or adaptive cellular immunity (Crameri and Blaser 2002).

From 1994 to 2006, populations of the sea fan coral *Gorgonia ventalina* suffered high mortality in a Caribbean-wide epizootic of the fungal pathogen *Aspergillus sydowii* (Nagelkerken et al. 1997; Kim and Harvell 2004; Mullen et al. 2006; Harvell et al. 2007). This epizootic proceeded from an epidemic phase, with prevalence in excess of 70% of sea fans, to its current endemic phase, with low disease prevalence (Kim and Harvell 2004; K. Kim and C. D. Harvell, unpublished data). Infections are characterized by spreading lesions that strip coenenchyme (living coral tissue) from the inner gorgonin skeleton of the coral and that, in some cases, consume the skeleton. A slow or impaired initial immune response by the host may allow a fungal infection to establish in deep tissues, in particular the protein-rich skeleton of the coral and the spread of the pathogen past the initial point of infection then occurs in the skeleton. Lesions are surrounded by a zone of inducible purpling, which suggests a significant inflammatory response to infection (fig. 1). Immune responses of *G. ventalina* to pathogen infection that have been detected include production of antifungal lipid metabolites (Kim et al. 2000a, 2000b), chitinase production and release (Douglas et al. 2007), melanin deposition as a physical barrier to prevent fungal expansion (Petes et al. 2003; Alker et al. 2004; Mullen et al. 2004), and production of oxidative enzymes such as peroxidase (Mydlarz and Harvell 2007).
The sea fan immune response thus includes both chemical (diffusive enzymes) and cellular (phagocytic, barrier forming) components. The primary line of defense against spreading infections in the skeleton appears to be a cellular response facilitated by wandering totipotent cells called amoebocytes. It is believed that circulating “naive” amoebocytes differentiate into granular amoebocytes and axial epithelial cells, in addition to other lineages (Mullen et al. 2004; Mydlarz et al. 2006).

Granular amoebocytes (fig. 2A, 2B) of most invertebrates and likely sea fans contain acidophilic granules that hold lytic and antimicrobial proteins, reactive oxygen, and peroxidases that may limit or inhibit growth of the fungus. The exact process by which granular amoebocytes halt fungal growth is unknown, but several roles are hypothesized. Fungal hyphae are too large for direct phagocytosis by granular amoebocytes, but these cells may surround fungal hyphae and release toxic compounds. Minute channels (canaliculi) have been observed in host skeletal gorgonianin (E. Peters, personal communication), and these would allow toxic compounds produced by amoebocytes in the host tissue to penetrate into the skeleton. These deductions are verified by experimental studies that have demonstrated aggregation of amoebocytes in fungus-exposed coral (L. D. Mydlarz, unpublished data; fig. 2), as well as the inferred involvement of peroxidases exhibiting antifungal activity in initial stages of infection (Mydlarz and Harvell 2007).

Axis epithelial cells (fig. 2C) are the putative cell type responsible for producing melanin (E. Peters, personal communication), forming a physical barrier to contain an infection within the skeleton. These cells are typically found at the coenenchyme-skeleton border.

In this article, we develop a model for the within-host growth of a fungal pathogen in gorgonian corals, specifically infection of *G. ventailina* by *A. sydowii*, and for the innate immune response of the host. Some aspects of the model are closely tailored to this particular system, but the structure of the model may be broadly relevant to opportunistic fungal infections in plants and animals. Our
model assumes that a localized infection has just reached the gorgonin skeleton, is starting to spread there, and has already elicited the host’s chemical responses in extraskelatal tissue. In some cases, pathogen spread is successfully arrested, with the host then recovering and surviving, even though amoebocytes cannot penetrate into the gorgonin skeleton. We therefore assume that fungus in the skeleton is vulnerable to diffusible enzymes produced by amoebocytes having the potential to control the infection.

The broad goals of our study of the *A. sydowii* epizootic center on understanding the factors responsible for variability in disease impacts: (1) the large variation across sites in the prevalence and severity of infection (Dube et al. 2002; Kim and Harvell 2002, 2004; Bruno et al. 2003; Mullen et al. 2006), (2) variation among individuals at a site in their ability to resist infection and to contain an infection that has started to grow (Jolles et al. 2002; Alker et al. 2004; Kim et al. 2006), and (3) the overall waxing and waning of the epizootic. Of particular interest are the potential roles of environmental drivers (such as climate warming and human impacts on water quality) in the epizootic (Harvell et al. 2002, 2007; Bruno et al. 2003) and the potential for host evolution (loss of more susceptible genotypes) to explain or to contribute to its decline (Dube et al. 2002); we return to these issues in “Discussion.”

Our study of the model therefore aims to identify the processes and parameters that have the strongest effects on the outcome of an infection. These are the most likely candidates for mechanistic links between climate drivers and disease progression and are also under the strongest selection by pathogen-induced mortality. Specifically, we would like to know the answers to the following questions: (1) How do the timescales of different components of the fungal attack and host response affect the ultimate consequences of an infection? (2) What are the potential consequences of an increase in pathogen virulence? *Aspergillus* virulence is very sensitive to changes in temperature, increasing rapidly at temperatures stressful to the host (Alker et al. 2004), and to water quality (Bruno et al. 2003).

Fungal and microbial pathogens are currently causing significant damage to coral reefs worldwide (Harvell et al. 2004; Ward and Lafferty 2004; Kim et al. 2005; Weil et al. 2006), contributing to the global decline in coral abundance and diversity (Gardner et al. 2003). Our model’s basic structure will be adaptable to many of these coral-
pathogen interactions. More generally, our model and results demonstrate that the spatial ecology of disease within the host can be crucial to understanding and predicting the progress of a host-pathogen interaction.

Model

Our model is intended to include, in the simplest possible form, the key processes in the host coral’s cellular responses to a spreading infection. We assume that pathogen recognition and any resulting cytotoxic chemical responses occurred “instantly” when the pathogen first successfully entered the host.

We therefore lump all cellular lines of defense into two broad categories: (i) granular amoebocytes (GA cells) that produce chemicals toxic to the pathogen and (ii) axis epithelial (AE) cells that attempt to encapsulate the pathogen with gorgonin and melanin in order to block hyphae from escaping from the skeleton into coenenchyme. These cells produce a basic inflammatory response without any of the immune memory mechanisms that are present in vertebrate immune systems. Because we are modeling an infection that has reached the skeleton, we focus exclusively on the interaction between the pathogen and host cellular responses and in particular neglect other components of the coral holobiont, despite their potential importance for blocking pathogen penetration to the skeleton.

As discussed in the introduction to this article, we conceptualize the sea fan host as a “sandwich” of three disk-shaped layers, an inner layer of skeleton surrounded by two outer layers of coenenchyme (fig. 3 shows this sandwich as seen from the side). Because the model considers the within-host spread of a pathogen that has penetrated to the skeleton, we omit the outer mucus layer surrounding the host. For simplicity, we derive the model initially in one spatial dimension \( -L \leq x \leq L \) (the horizontal direction in fig. 3), but for all model simulations, we use a model in which figure 3 represents a stack of three disks, seen from the side. We ignore within-layer vertical variability and assume that the two coenenchyme layers are the same in all respects. So, all state variables in the model are then functions only of radial distance from the center of the host, even though some variables really “live” in the skeleton and others only in coenenchyme.

Once and for all, we choose units so that \( L \) and the maximum thickness of the skeleton “layer” both equal 1 (so in two dimensions, each layer would be a disk of radius 1); this simplifies initial conditions and is convenient for simulations. The model state variables are as follows. (1) Host skeleton tissue \( T(x, t) \) is the amount of pathogen-free skeleton remaining at location \( x \) at time \( t \). Initial conditions are \( T(x, 0) \equiv 1 \). (2) Pathogen \( P(x, t) \) is the density of fungal hyphae at location \( x \) at time \( t \) in the skeleton.

Initial conditions will be a localized attack, such as

\[
P(x, 0) = P_0 \max(0, 1 - wx^2), \ w \gg 1.
\]

(3) Chemical signal \( S(x, t) \) occurs in the coenenchyme, an aggregate representing various substances (e.g., chemokines) produced by the host at locations where the pathogen is present. The signal then diffuses and results in recruitment of amoebocytes to the point of attack. Initial conditions are \( S(x, 0) \equiv 0 \). (4) Undifferentiated stem cell amoebocytes \( U(x, t) \) are found in the coenenchyme. The initial condition is a spatially uniform density, \( U(x, 0) \equiv U_0 \). (5) Differentiated immune cells in the coenenchyme are of two types: \( G(x, t) \) are GA cells that release substances that increase the pathogen mortality rate, and \( A(x, t) \) are AE cells that lay down gorgonin and melanin in order to encapsulate the pathogen. In addition, chemical intermediates in the production of encapsulating materials are assumed to be toxic to the pathogen and to diffuse into the skeleton. Initial conditions are \( G(x, 0) = A(x, 0) \equiv 0 \). (6) The amount of “barrier” material \( B(x, t) \) is what AE cells have laid down at the interface between the coenenchyme and skeleton. Initial conditions are \( B(x, 0) \equiv 0 \).

Pathogen is assumed to grow through the consumption of skeleton tissue, to suffer amoebocyte-induced mortality \( \mu_p(G, A) \), and to spread by advection toward healthy coral tissue. On the timescale of interest, we assume that there is no regrowth of coral tissue:

\[
\dot{T} = -qPT; \tag{1}
\]

\[
\dot{P} = \chi_P qPT - \mu_p(G, W)P - \frac{\partial}{\partial x}\left(P\eta_T \frac{\partial T}{\partial x}\right), \tag{2}
\]

where superscript dots indicate derivatives with respect to time (e.g., \( \dot{P} = \partial P/\partial t \)). Far more complex models for fungal spread have been developed (Davidson 1998; Stacey et al. 2000; Boswell et al. 2002, 2003). These mechanistically represent the initiation and expansion of individual hyphae, nutrient uptake, and redistribution within the fungal colony, and so on, and so may have a half-dozen equations.
for the fungus alone. The model above is our attempt at a simple model that captures the phenomenology of lesion development and spread, similar in spirit to models of tumor growth that use advection and diffusion coefficients to summarize the net effect of the physical forces driving spatial expansion (e.g., Ayati et al. 2006). In particular, the last term in equation (2) should not be taken literally as saying that fungal hyphae are moving within the skeleton at velocity \( \eta_p (\partial T / \partial x) \). Rather, it represents a net redistribution of total fungal biomass due to hyphal tip expansion and nutrient translocation within the fungal colony, which, in more complex models, leads to advection and diffusion terms. Histology on our study system generally shows a sharp front of pathogen spread, so we assume that advection is dominant and omit pathogen diffusion.

Signal production, per unit of living host coenenchyme tissue, is assumed to be proportional to the amount of pathogen present (with rate constant \( \alpha \)). Signal chemicals spread by diffusion and degrade at some constant rate \( \mu_s \):

\[
\dot{S} = \alpha P T - \mu_s S + D_s \frac{\partial^2 S}{\partial x^2} \tag{3}
\]

(for description of parameters, see table 1). Signal diffusion is not affected by the pathogen because pathogen is in the skeleton while these chemicals are diffusing in the coenenchyme. The production term \( \alpha P \) is based on experimental evidence (L. D. Mydlarz, unpublished data) that (1) some pathways leading to signal production are elicited by dead fungus, that is, even in the complete absence of pathogen activity, and (2) a higher dose of pathogen leads to a higher level of response in the elicited pathways. We therefore assume that signal production by each unit volume of host tissue occurs in response to the amount of pathogen locally present rather than, for example, the local pathogen activity level. For simplicity, we assume a linear dose-response relationship rather than a saturating response.

Undifferentiated cells are more complicated because multiple processes are involved. We assume that these begin their lives wandering slowly through the coral tissue; when a signal is sensed, they head toward where it is being produced by moving up the signal concentration gradient, and they differentiate into GA and AE cells at a per-cell rate proportional to the signal concentration. The equation representing these processes is

\[
\dot{U} = K_U - \mu_u U - (\delta_u + \delta_A) SU - \frac{\partial}{\partial x} \left( U \eta_u \frac{\partial S}{\partial x} \right) + D_u \frac{\partial^2 U}{\partial x^2} \tag{4}
\]

The first three terms are production of undifferentiated cells, natural mortality at rate \( \mu_u \), and differentiation at rates assumed to be proportional to the local signal concentration. Differentiation into cell types other than GA and AE cells is assumed not to depend on signal concentration and is included in \( \mu_v \) for the purposes of this model, those cells are “lost” from the system. The next term is directed movement toward increasing signal concentration. Its biological meaning is that the velocity of directed movement is proportional to the gradient in signal concentration, with proportionality constant \( \eta_u \). The last term represents random diffusive movements of naïve amoebocytes.

GA and AE cells arise from differentiation of naive cells and are lost through natural mortality and additional mortality imposed by the pathogen. Histology (fig. 2; L. D. Mydlarz, unpublished data) shows that GA and AE cells aggregate at the margins of a growing pathogen attack, so we assume that these cells “chase” the pathogen by moving in the direction of increasing signal concentration:

\[
\dot{G} = \delta_v SU - \mu_v (P) G - \frac{\partial}{\partial x} \left( G \eta_v \frac{\partial S}{\partial x} \right) \tag{5}
\]

\[
\dot{A} = \delta_u SU - \mu_u (P) A - \frac{\partial}{\partial x} \left( A \eta_u \frac{\partial S}{\partial x} \right) \tag{5}
\]

Barrier material is simple—it is laid down and stays there:

\[
\dot{B} = \beta A. \tag{6}
\]

Simplifying the Model

Even this “simplest” model has an overabundance of parameters. To reduce their number, we make some further simplifying assumptions, as follows. Undifferentiated amoebocytes do not travel far until they are needed: \( D_u = 0 \). Knowing little about pathogen counterattack to host immune responses, we ignore it and assume that GA and AE cells have constant and equal mortality at rate \( \mu_r \). We assume that all host immune cells have the same advection coefficient \( \eta_v = \eta_u = \eta_A = \eta_I \). We assume that pathogen mortality rate is a linear function of immune cell densities, \( \mu_r (G, A) = \mu_r + m_r G + m_r A \). AE cells are presumably less toxic to the pathogen than GA cells (\( 0 < m_r < m_c \)) because the toxicity of AE cells is a by-product of encapsulation rather than their primary function. Lacking information, we use the midpoint of the potential range and assume \( m_r = m_c / 2 \).

The parameter count can be reduced further by appro-
appropriate choice of units for the state variables ("rescaling"). The thickness of each "layer" of the host has already been scaled to 1. We can also choose units of concentration for pathogen, signal, amoebocytes, and barrier material so that \( U_0 = \alpha = \beta = \chi = 1 \) and note that \( U_0 = 1 \) implies \( K_0 = \mu_0 \), so that amoebocytes equilibrate to uniform density 1 in the absence of pathogen. We could also scale time so that \( q = 1 \), but it is useful to keep the model in real time (days) for parameterization based on empirical observations. The rescaled model is then

\[
\dot{T} = -qPT, \\
\dot{P} = qPT - (\mu_A + m_cG + 0.5m_cA)P \\
- \frac{\partial}{\partial x}\left(P\eta_T\frac{\partial T}{\partial x}\right), \\
\dot{S} = PT - \mu_A S + D_s \frac{\partial^2 S}{\partial x^2}, \\
\dot{U} = \mu_v(1 - U) - (\delta_c + \delta_A)SU \\
- \frac{\partial}{\partial x}\left(Uh\frac{\partial S}{\partial x}\right), \\
\dot{G} = \delta_cSU - \mu_G \frac{\partial}{\partial x}\left(Gh\frac{\partial S}{\partial x}\right), \\
\dot{A} = \delta_sSU - \mu_A \frac{\partial}{\partial x}\left(Ah\frac{\partial S}{\partial x}\right), \\
\dot{B} = A.
\]

A consequence of scaling the model so that \( U_0 = 1 \) is that \( m_c \) represents the product of immune cell density and the per-cell effect on the pathogen. What matters for lesion growth is the total effect of the immune response, so an increase in either of these components is equivalent in our model. Additionally, \( \mu_v \) now represents the replenishment rate of the undifferentiated amoebocyte pool when it falls below its steady state level (now scaled to equal 1).

Finally, we move up in dimension, modeling a sea fan as a cylinder of (scaled) radius 1 and (scaled) thickness 1 for each layer. We consider initial conditions that are radially symmetric (a small infection at the center of the host) and continue to ignore within-layer vertical structure in the host. Solutions remain radially symmetric at all times because the model on the unit disk is invariant under rotation about the origin, so all state variables depend only \( r \), radial distance from the center of the host. Then, when we use the radially symmetric version of the continuity equation (see, e.g., Edelstein-Keshet 1988, p. 421), the model equations are

\[
\dot{T} = -qPT, \\
\dot{P} = qPT - (\mu_A + m_cG + 0.5m_cA)P \\
- \frac{1}{r}\frac{\partial}{\partial r}\left[rP\eta_T\frac{\partial T}{\partial r}\right], \\
\dot{S} = PT - \mu_A S + D_s \frac{\partial^2 S}{\partial r^2}, \\
\dot{U} = \mu_v(1 - U) - (\delta_c + \delta_A)SU \\
- \frac{1}{r}\frac{\partial}{\partial r}\left[rUh\frac{\partial S}{\partial r}\right], \\
\dot{G} = \delta_cSU - \mu_G \frac{\partial}{\partial r}\left(Gh\frac{\partial S}{\partial r}\right), \\
\dot{A} = \delta_sSU - \mu_A \frac{\partial}{\partial r}\left(Ah\frac{\partial S}{\partial r}\right), \\
\dot{B} = A.
\]

The only change from equations (7) is that the transport terms are modified to describe radial advection and diffusion.

**Boundary Conditions**

Equations (8) hold on the open interval \( 0 < r < 1 \), and we need to specify the boundary conditions at \( r = 0 \) and \( r = 1 \). The conditions at \( r = 0 \) follow from symmetry: a smooth, radially symmetric function on the unit disk must have zero radial derivative at \( r = 0 \); that is,

\[
\frac{\partial T}{\partial r}(0, t) \equiv 0,
\]

and the same is true for all other state variables.

At \( r = 1 \), the natural boundary condition is zero flux: nothing leaves or enters at the host boundary. The flux for \( T \) and \( B \) in the model is 0 everywhere. The flux for \( S \) is proportional to \( \partial S/\partial r \), so we must have \( (\partial S/\partial r)(1, t) \equiv 0 \). The fluxes for \( U \), \( G \), and \( A \) are also proportional to \( \partial S/\partial r \), so no additional conditions are needed for these variables. The flux for \( P \) is proportional to \( P\eta_T\partial T/\partial r \), so one term in this product must be 0 at the boundary. Enforcing either \( P = 0 \) or \( \partial T/\partial r = 0 \) would require some kind of “external” interference with the host-pathogen inter-
teraction. We therefore impose the zero-flux condition in a way that is biologically meaningful: we make the boundary impenetrable to the pathogen by having the advection coefficient \( \eta_p \) and its spatial derivative fall smoothly to 0 in a small zone near \( r = 1 \), for example, \( \eta_p(r) = \eta_p(0) (1 - r^{20})^5 \). The only effect of this assumption is to slow an infection that is about to finish spreading through the entire host, so that it does not affect model predictions about spread versus containment of a localized initial infection. So long as \( \eta_p(x) \) varies smoothly, the exact shape of the cutoff has very little effect on model solutions (e.g., using \( \eta_p(r) = \eta_p(0) (1 - r^{40})^5 \) rather than the expression above had no effect on disease severity or total tissue consumption after 120 days to three significant figures, at our default parameter values). As discussed in appendix B, for numerical solutions, a small amount of “artificial diffusion” is added to the pathogen equation (also with zero flux at the boundary), as a result of which the pathogen can spread up to the boundary but not through it.

**Timescales and Parameter Ranges**

Plausible values and ranges for parameters are collected in table 1, and the rationale for these estimates and ranges is discussed in appendix A. Because the estimates are all somewhat speculative, we consider a wide range of parameter values in exploring the model’s behavior. The parameter values reflect a large separation of timescales between pathogen growth and host response. Immune response begins within hours (Olano and Bigger 2000), whereas pathogen growth and spread occur on a much slower timescale (months to years), with daily relative growth rates of 1% or smaller. This large separation of timescales has important consequences for the model’s behavior and its relative sensitivity to changes in different parameters.

**Results**

We study the model numerically using methods described in appendix B. We first present model results for selected parameter values and then carry out parameter sensitivity analyses to answer the questions posed in the introduction to this article.

Figure 4 shows successful spread of a lesion. Within a few days, the host has mounted a cellular response. Signal has spread, resulting in the recruitment and differentiation of amoebocytes that are concentrated near the margins of the spreading infection. This initial response is strong enough to kill off half the initial infection by time \( t = 10 \). Within 60 days, however, the supply of amoebocytes has been depleted, and the pathogen can proliferate despite the host response. In figure 5, the host response is effective enough to limit the pathogen. After the initial strong attack, the host is able to sustain a level of amoebocytes that overcomes the pathogen. Note, however, that without any barrier to its lateral spread, the pathogen spreads broadly even while its total abundance is reduced by the host response.

**Local Sensitivity Analysis by Latin Hypercube Sampling**

To answer our key questions, we need to compare the impacts of different parameters on the outcome of an infection. We began with a local sensitivity analysis, meaning that we considered the effects of relatively small changes in parameters away from their default values. Local sensitivity analysis is typically performed by making very small changes in each parameter, one at a time, and using the results to compute the partial derivative of some

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**Table 1: Default parameter values and the ranges of values used for global sensitivity analysis of the scaled model (eqq. [8])**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Default values</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>( q )</td>
<td>Pathogen virulence (intrinsic per capita rate of increase; day (^{-1} ))</td>
<td>.02, .05</td>
<td>( \times (.5-2) )</td>
</tr>
<tr>
<td>( \mu_p )</td>
<td>Pathogen intrinsic per capita mortality (day (^{-1} ))</td>
<td>.002, .005</td>
<td>( \times (.1-5) )</td>
</tr>
<tr>
<td>( m_i )</td>
<td>Toxic effect of GA cells on pathogen</td>
<td>.002, .02</td>
<td>( \times (.5-2) )</td>
</tr>
<tr>
<td>( \rho_p )</td>
<td>Toxic effect of AE cells on pathogen, relative to that of GA cells</td>
<td>.5</td>
<td>( 1-9 )</td>
</tr>
<tr>
<td>( \eta_p )</td>
<td>Pathogen advection coefficient</td>
<td>( 5 \times 10^{-4} )</td>
<td>( \times (.1-10) )</td>
</tr>
<tr>
<td>( D_s )</td>
<td>Signal diffusion coefficient</td>
<td>.1</td>
<td>( \times (.2-5) )</td>
</tr>
<tr>
<td>( \mu_s )</td>
<td>Signal decay rate (day (^{-1} ))</td>
<td>.1</td>
<td>( \times (.1-10) )</td>
</tr>
<tr>
<td>( \rho_u )</td>
<td>Undifferentiated amoebocyte turnover rate (day (^{-1} ))</td>
<td>.1</td>
<td>( \times (.1-10) )</td>
</tr>
<tr>
<td>( \delta_{ci}, \delta_{ci} )</td>
<td>Rate coefficients for amoebocyte differentiation into GA and AE cells</td>
<td>1</td>
<td>.5-10</td>
</tr>
<tr>
<td>( \mu_i )</td>
<td>Mortality rate of GA and AE immune cells</td>
<td>.1</td>
<td>( \times (.1-10) )</td>
</tr>
<tr>
<td>( \eta_i )</td>
<td>Advection coefficient of host immune cells (naive, GA and EA)</td>
<td>1.0</td>
<td>( \times (.1-2) )</td>
</tr>
</tbody>
</table>

Note: We considered two sets of default values for the first three parameters listed, corresponding to different assumptions about the potential growth rate of the pathogen in the absence of host immune response. GA = granular amoebocytes; AE = axis epithelial cells.

Entries of the form \( \times (x-y) \) indicate that the range of parameters considered ran from \( x \) to \( y \) times the default value.
model outcome (e.g., total tissue consumed) with respect to each parameter. But because our parameter estimates are highly uncertain, we instead considered the effects of parameter variations across a multidimensional "hypercube" formed by allowing each parameter to vary by up to $\pm 50\%$ from its default value.

For each default parameter set, parameter sets in the corresponding hypercube were generated by Latin hypercube sampling (McKay 1992; Blower and Dowlatabadi 1994), a generalization of Latin square experimental designs that is an efficient way of sampling high-dimensional parameter spaces. We used a grid of 200 evenly spaced
values for each parameter, which produced 200 parameter sets for the model. For each parameter set, we initialized the model with a local infection at the center of the fan and ran the model to compute at $t = 120$ the total pathogen abundance, the total amount of remaining healthy host skeleton tissue, and the total amount of barrier material laid down. A 120-day run was long enough for the pathogen spread rate to stabilize, thus giving a good indication of the longer-term outcome, but allowed more runs than full-year simulations.
The relative importance of each parameter was assessed by multiple regression of each model response variable (total pathogen, skeleton, or barrier) on parameter values. We log transformed both response variables and parameters because this gave fitted regressions that accounted for 97% or more of the variance in each response variable without the need for any interaction terms. Regression coefficients on this scale correspond to the “elasticity analysis” typically used to study matrix population models. The regression coefficient for each parameter can be interpreted as the percent change in the response variable resulting from a 1% change in each parameter, averaged over the parameter hypercube that was sampled.

Figure 6 summarizes the results, which are very similar for the two default parameter sets. Not surprisingly, total pathogen \( P \) and host tissue consumption \( T \) respond very similarly to parameters. For these measures of pathogen success, the most important parameter characterizing the pathogen is \( q \), the pathogen’s intrinsic growth rate, followed by \( \eta_P \), which controls its ability to spread spatially. Pathogen mortality \( \mu_P \) is less important. For the host, the parameters that determine its speed of initial response (\( \eta_I, \delta_G, \) and \( \delta_A \)) are considerably less important than the parameters \( \mu_I, \mu_U, \) and \( \mu_G \), which determine the strength of the host’s long-term immune response (the number of GA and AE cells and their per-cell impact). An increase in the signal spread rate (governed by \( D_S \)) is actually detrimental to the host: the faster initial response is outweighed by the fact that broader signal diffusion makes it harder for GA and AE cells to locate the pathogen precisely and concentrate where they are needed most. The results for total barrier \( B \) are also easy to understand: more barrier is formed when the amoebocyte pool has fast replenishment (high \( \mu_U \)), low mortality (low \( \mu_I \)), and a greater proportion of AE than GA cells (high \( \delta_A \), low \( \delta_G \)).

**Global Sensitivity Analysis by Latin Hypercube Sampling**

For a global sensitivity analysis, we allowed parameters to vary over the full ranges listed in table 1, and we again used Latin hypercube sampling with 200 parameter sets. However, to have balanced coverage of parameter variations above and below the default values, we used grids that were even on a log scale (i.e., the log-transformed grid points for each parameter were evenly spaced between the log-transformed minimum and maximum of the parameter’s range). Responses over this wider range were not fitted well by log-scale multiple linear regression. Much better fits were obtained with rank transformation of parameters and responses, which “straightens out” monotonic nonlinear relationships. These accounted for more than 80% of the variance in each response variable, without any interaction terms. Because most of the variance was explained by simple linear models, we did not pursue more general or flexible sensitivity measures (e.g., adding interaction terms or using a nonparametric method such as Sobol indexes).

The conclusions from global sensitivity analysis (fig. 7) reinforce those from the local sensitivity analysis. The parameters that determine pathogen spread versus containment are the pathogen’s intrinsic virulence (determined by \( q \)) and ability to spread within the host and the host’s ability to mount a sustained defense.

![Figure 6: Results of local sensitivity analysis using Latin hypercube sampling and linear regression of log (model response) on log (parameters). The Y-axis is the regression coefficient for each parameter, which is the average elasticity for that parameter over the sampling region. Results are shown for three response variables: total pathogen (red diamonds), amount of host skeleton tissue consumed by the pathogen (green circles), and total amount of barrier material (blue squares), all 120 days after the initial infection. Note the difference in scaling between the top (more virulent fungus, default \( q = 0.05 \)) and the bottom (less virulent fungus, default \( q = 0.02 \)) panels.](image)
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Figure 7: Results of global sensitivity analysis using Latin hypercube sampling and multiple linear regression of rank-transformed response on rank-transformed parameters. The Y-axis is the regression coefficient for each parameter. Response variables are the same as in figure 6.

Multiple Points of Infection

Sensitivity analysis of the model indicated that a sea fan’s ability to sustain a long-term immune response is crucial for containing the spread of an Aspergillus sydowii infection. If this is true, it has important consequences for a host’s ability to deal with multiple infections that originated at different locations in the host.

Two simultaneous infections are worse for the host than a single infection (all else being equal) in two distinct but interacting ways. First, the initial pathogen load—the number of hyphae that have successfully penetrated through mucus and coenenchyme into the skeleton—is higher. Second, the host has to fight on two separate “fronts” at once, rather than concentrating its defenses in one region, so each spreading lesion may be less severely limited.

Using the model, we can examine the impacts of these factors separately, by simulating the following thought experiment. For a specified initial total pathogen load $p_0$ (given by the integral of $P$ over the entire host at time 0), we can place the entire load within one small region (as in figs. 4, 5) or divide it evenly across two, three, four, or more separate regions of the host, or, to represent the extreme limit of multiple infection points, we can spread the initial load evenly across the entire host (i.e., at $t = 0$, the pathogen density $P$ is at all locations equal to $p_0$ divided by the area of the host). Comparing model runs with these different initial conditions for the pathogen shows the effects of having to fight on multiple fronts. Then, by varying $p_0$, we can see the effect of initial pathogen load.

Figure 8 illustrates that the effect of multiple versus single infection points can be substantial. After 120 days, a host with two initial infections in equations (7) loses about 70% more tissue than a host with the same initial pathogen load concentrated around a single point (fig. 8A). With the initial pathogen load spread uniformly within the host, the pathogen distribution remains uniform, so host responses cannot focus where the damage is worst. The result is a 2.5- to threefold increase in host tissue loss relative to a single localized infection with the same initial pathogen load. The effect of a higher initial pathogen load is less dramatic; a fivefold increase in $p_0$ increases the host tissue damage by factors of 2.5 or less. There are two reasons for this: pathogen growth is locally self-limiting through consumption of the host, and higher pathogen density leads to a higher fraction of naive amoebocytes converting into GA and AE cells. Combining the two effects, we find that having two infections is more than twice as bad for the host as having a single infection of initial size (assuming that each infection has the same initial pathogen load). When we average across the simulations in figure 8A, the damage from two infections is 2.4 times as high as the damage from a single infection.

Two local infections cannot be represented in the radial model (eqq. [8]) because of its radial-symmetry assumption, so we compared only the extreme cases, a single infection point versus uniform initial pathogen. Using the default parameters with higher pathogen virulence ($q = 0.05$), we find again a very substantial increase in damage to the host if the pathogen is initially uniform rather than localized. When the pathogen is less virulent ($q = 0.02$), the effect of initial pathogen distribution is smaller.

Pathogen distribution affects the outcome of infection through its effect on the pathogen’s spatial covariances with host tissue and immune cells. Let $\langle Y(t) \rangle$ denote the spatial average of $Y(r, t)$ in model (8) or $Y(x, t)$ in model
Figure 8: Effect of initial pathogen distribution on disease progression. A, Curve 1 shows the fraction of host skeleton tissue consumed after 120 days if the infection is initialized with one localized infection centered at $x = 0.5$ in the linear spatial model (eqs. (7)), as a function of initial pathogen load $p_c$. Curve 2 shows the outcome when the same initial pathogen load is divided between two localized infections centered at $x = \pm 0.5$. Curve $U$ shows the outcome when the initial pathogen load is spread uniformly across the entire host. These model runs used the default parameters with higher pathogen virulence $q_c$ except that we set $q = 0.04$ so that the two initially separate infections would not meet and merge within 120 days. B, C As in A for the radial model with more and less virulent pathogen parameters ($q = 0.05, 0.02$), respectively. D, Time course of the spatial coefficients of covariation $V$ between pathogen and immune cells (solid curves) and between pathogen and unconsumed host skeleton tissue (dashed curves). Lighter and heavier curves are for the less and more virulent pathogen parameters, respectively. Granular amoebocytes and axis epithelial cells have the same spatial distribution for our default parameters and therefore have the same coefficient of covariation with the pathogen.

(7), where $Y$ is a state variable or function of the state variables. The spatial average of advection and diffusion terms is 0 because of the zero-flux boundary conditions, so averaging over space in either model, we have

$$\dot{P} = qP \langle T \rangle - \mu_\nu P - m_c(P) \langle G + 0.5A \rangle + q \text{Cov} \langle P, T \rangle - m_c \text{Cov} \langle P, G + 0.5A \rangle,$$

where Cov is the spatial covariance, $\text{Cov} \langle X, Y \rangle = \langle XY \rangle - \langle X \rangle \langle Y \rangle$. The first line in equation (9) is the mean field model in which spatial variation is ignored, and the second line shows how the mean field dynamics is modified by spatial covariance. So the relative importance of the covariance terms relative to the corresponding term in the mean field model is given by

$$V(X, Y) = \frac{\text{Cov} \langle X, Y \rangle}{\langle X \rangle \langle Y \rangle},$$

which is a spatial coefficient of covariation.

If the pathogen distribution is nonuniform, the covariance between pathogen and effector immune cells is positive because immune cells chase the pathogen, using
the signal gradient as their cue. This covariance is initially very high because conversion to GA and AE cells occurs only where the pathogen is present. It decays as the pathogen spreads out within the host but remains high enough to be very important: a value of \( V = 2 \) means that the host cell’s spatial tracking of the pathogen triples the fungal mortality rate inflicted by the cells. The pathogen tries to create a positive covariance between itself and its resource \( T \) by moving toward high-\( T \) areas. The pathogen is less successful than the host immune cells, and \( V(\bar{P}, T) \) remains relatively small because the movement of the pathogen through the host skeleton is slower than that of the immune cells through the coenenchyme (recall that recruitment of distant amoebocytes to a new infection is observed within 24 h, while at least several months are required for the pathogen to spread throughout the host).

The model runs in figure 8 all involve infections that start simultaneously and are therefore equal in size. Another kind of interaction between infections is that an established and spreading lesion will “distract” the host from battling a subsequent new infection at another location, as shown in figure 9. After the first infection occurs, host immune cells concentrate around the point of infection, and within about 10 days, the pathogen growth rate is greatly reduced. But when the second infection occurs, the immediate local immune response is weaker, and most immune cells remain near the first infection because the signal gradient over most of the host attracts them toward the older, larger infection. The second infection therefore grows more rapidly than the first until it “catches up” in size. After that, host responses are divided between the two infections, so the growth rate of the first infection quickly accelerates. The two infections then grow in parallel, at a rate much higher than that of a single lesion facing an undivided host immune response.

**Discussion**

The focus of our model is on the fine-scale interactions of fungal pathogen spread and host response. Although many details of our model are specific to infection of *Gorgonia ventilina* by the fungus *Aspergillus sydowii*, it provides a general modeling framework for studying the role of within-host spatial dynamics in fungal infections. Spatial expansion of lesions and the corresponding loss of host tissue are also the modes of progression for many of the diseases currently afflicting corals, not just fungal pathogens (e.g., yellow band on *Montastraea* in the Caribbean, which appears to be bacterial; fig. 10; Cervino et al. 2004).

The dynamics of host-fungal interactions is poorly known for deep-tissue fungal infections. These are regarded as especially difficult infections to eradicate and are often thought to be beyond the reach of host immunity. To our knowledge, our model is the first attempt to model the within-host spatial dynamics of a fungal pathogen. Previous models for host-fungus interactions have oper-
ated at larger spatial levels: susceptible-infected-recovered-type models for transmission of a fungal pathogen between different host individuals (e.g., Gibson et al. 1999; Hall et al. 2006) or between different leaves or roots on a growing plant (e.g., Segarra et al. 2001; Bailey and Gilligan 2004) and spatial models for spread of a fungal pathogen from plant to plant within the rhizosphere (reviewed by Otten and Gilligan [2005]) or from one agricultural field to another (e.g., Parnell et al. 2006). The dynamics of host responses to pathogen attack has not been included in any of these models or in the spatially explicit models for expansion of fungal colonies on growth medium (Edelstein-Keshet and Ermentrout 1989; Davidson 1998; Stacey et al. 2000; Boswell et al. 2002, 2003). Even for human immune response to pathogens, the development of spatial immune models (e.g., for macrophage-bacteria interactions) has begun only very recently (Gammack et al. 2004; Segovia-Juarez et al. 2004; Funk et al. 2005).

Fungal infections in sea fans are an interesting model system for deep-tissue fungal infections where the fungus resides primarily in the proteinaceous host skeleton. Skeletal infections are rapidly walled off by melanizing amoeocytes that block pathogen spread into adjacent tissues. The success of this defense depends on the ability of the host to sustain an immune response that limits pathogen spread within the skeleton. The prophenoloxidase-mediated cascade that produces melanin is the primary antifungal mechanism in invertebrate innate immunity and is highly conserved, present from cnidarians to arthropods.

Our model predicts that the pathogen’s potential virulence \( q \) has an overriding effect on the amount of host tissue loss (figs. 6, 7). This was not a foregone conclusion or an inevitable result forced by model assumptions because in a spatial setting, the pathogen’s success is jointly determined by its local proliferation rate, governed by \( q \), and its potential rate of expansion into unoccupied territory, governed by \( \eta_p \). The relatively small effect of \( \eta_p \) on disease severity says that pathogen spatial expansion is fast enough not to be a strongly limiting factor, even though it is much slower than the movement of host immune cells. Even at the more virulent default parameter set, the pathogen’s spatial expansion is fast enough to create a positive spatial correlation between pathogen density and available host tissue (fig. 8D).

The high sensitivity to \( q \) provides some possible explanations for the high between-host and spatial variability in disease severity and prevalence, noted in the introduction to this article. First, it constitutes a mechanistic link whereby disease severity can be strongly affected by increased water temperature and other anthropogenic stresses. Fungal growth rate increases with temperature, even in the temperature range where the host becomes adversely affected by increasing temperature (Alker et al. 2001). The severity of fungal and bacterial infections in corals was increased by experimental nitrogen additions (to mimic water pollution) that were probably too small to affect the host (Bruno et al. 2003; Voss and Richardson 2006). Increase in pathogen growth rate due to the removal of nitrogen limitation is thus the most likely mechanism for increased disease severity in response to small nutrient additions (Bruno et al. 2003).

A second possible mechanistic link to variability in disease severity involves the host’s noncellular responses. These are not explicitly represented in our model; rather, the value of \( q \) incorporates any decrement to pathogen virulence resulting from the noncellular responses. Consequently, any stress (such as temperature) that compromises the host’s noncellular innate responses would increase the potential virulence of an established infection, and our model predicts that this could substantially increase disease severity. Similarly, relatively small variability among hosts in the magnitude or effectiveness of their noncellular responses could be magnified into substantial between-host variability in disease severity. Kim and Harvell (2004) argued that the waning of the aspergillosis epizootic was driven primarily by natural selection in the host population, leaving alive only individuals with stronger immune responses. The results from our model show that a moderate selection response in the host population, leading to a reduction in \( q \), could indeed cause major population-wide changes in disease impact.

The model predicts that the effectiveness of the host’s cellular immune response is determined primarily by its ability to sustain a long-term response by quickly replenishing the supply of naive amoebocytes that can differentiate into GA and AE cells. Virtually nothing is known about the dynamics of amoebocyte replenishment, but it is a reasonable and testable hypothesis that the amoebocyte replenishment rate will be compromised under stress. Movement of host immune cells to a point of infection is so fast relative to pathogen spread that even substantial changes in their advection rate have essentially no effect. In fact, faster diffusion of signal compounds—which would accelerate the recruitment of distant amoebocytes to a newly detected infection—is counterproductive and leads to an increase in tissue loss. In the long run, the faster initial response is more than offset by the fact that higher signal diffusion decreases the precision within which immune cells can “track” the pathogen and thus decreases the spatial covariation between pathogen abundance and host response. Spatial covariation between threat and response is (in the model) one of the host’s most potent weapons, doubling or tripling the average mortality imposed by each immune cell relative to a spatially uniform response (fig. 8D). Host response is therefore compromised by the presence of multiple infections,
and the response to new, small infections is compromised by the presence of older and larger infections (fig. 9).

Localization of fungal infections is therefore very important for the host. The primary mechanism for this may be high levels of defense in the host tissue and its surface mucosal covering. For the pathogen to reach the skeleton may require an open wound exposing the skeleton or a localized compromise of host surface defenses. Such small breaks in host defense should result in infections being localized initially. Although our model suggests that rapid immune response is not important for the control of established lesions, it may be crucial for “nipping in the bud” new infections before they can become established in the skeleton.

Predictions about between-lesion interactions should be tested experimentally because of confounding factors in observational field data on lesion growth rates. A particular host may have more lesions and faster-growing lesions because that individual has a weak immune defense, not because of between-lesion interactions. Alternatively, hosts with more lesions might have slower-growing lesions (contrary to our predictions) because only those hosts with strong defense can remain alive to be sampled if they have multiple lesions. Experimental lesion inductions using randomly selected fans are necessary to control for potential effects of between-host variability.

To complete our understanding of the factors determining disease prevalence, the model should be expanded to include the host’s noncellular innate responses and the effects of the host and its environment on the effectiveness of the surface mucus layer in excluding pathogens (Ritchie 2006). There are also some potentially important aspects of established infections that we have omitted for this initial study, as discussed below.

**Autotoxicity.** The cellular response is not a direct interaction between immune cells and pathogen; rather, it is mediated by chemicals released by immune cells. Consequently, the cellular response is also cytotoxic to the host and leads to mortality of host coenenchyme within the spreading lesion and eventual loss of all tissue within the lesion (see fig. 1C). As with the signal chemicals, there is a trade-off between speed and precision: a rapidly diffusing chemical will reach the pathogen quickly but will also be less concentrated on its target and may do more damage to the host. A mechanistic model for release and diffusion of antifungal compounds by amoebocytes could also incorporate effects of local tissue properties that our model omits, including coenencyhyme loss and melanin deposition, but given our present knowledge, a model at this level of mechanistic detail would be highly speculative.

**Inhomogeneity.** Our model treats host tissue as homogeneous and isotropic, but it is really heterogeneous, with older tissue having weaker immune responses, and it is really a network. Infections that reach a major axis of the skeleton may be able to spread more rapidly and infect a large fraction of the host area.

**Mechanics of fungal spread.** Paraphrasing slightly Anderson and May (1992, p. 9): to those familiar with the manifold complexities of real fungal infections in real tissues, our pathogen model may seem oversimplified to the point of lunacy. By modeling the actual processes of fungal colony development (Boswell et al. 2002, 2003) and the physics of fungal expansion and movement, as in biomechanical models for tumor growth (e.g., Lubkin and Jackson 2002; Araujo and Mcllwain 2004), we could generate the pathogen’s tissue consumption, colony growth, and advection rates as emergent properties of the underlying processes. But the fact that spatial expansion is not the limiting factor for the pathogen’s net consumption of host tissue provides some retrospective support for our phenomenological modeling of colony expansion. Any other model for colony spread would also be parameterized to match the pathogen’s observed ability to spread through an entire host within a year or less, so that spatial expansion is not limiting to the pathogen. As a result, the predictions of disease severity and the conclusions about the factors limiting disease severity should be fundamentally the same.

Although these and other aspects of the model should be refined and more data-rich parameter estimates should be developed, we have been able to draw some specific conclusions about the relative importance of different processes for the outcome of an infection. Among these are the role of cellular immunity in localizing infections and the compounding effect of multiple lesions on host immune compromise. Mechanistic explanations for the high individual- and population-level variation in disease prevalence and severity could include localized factors, such as elevated temperature and nutrients, that cause an increase in fungal growth rate, which produces a disproportionate increase in disease severity (i.e., the rate of host tissue loss). Our conclusions can be understood intuitively in terms of the relative timescales for different processes, so they are likely to be robust and may generalize to other coral diseases, whose long-term affects often result from gradual pathogen spread across the host. Integrating within-host spatial immune dynamics with conventional infectious disease models that focus on between-host transmission will be essential for predicting the impacts of these diseases at the population level and for understanding the links between environmental drivers and disease. Our model is a useful example of modeling within-host spatial immune dynamics that should be adaptable to many other pathogens in colonial organisms such as corals and to both deep-tissue and surficial fungal infections in a variety of organisms.
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APPENDIX A

Parameter Values and Ranges

Here we discuss the rationale for our default parameter sets and for the ranges of values considered in global sensitivity analysis.

Pathogen

Lesion growth has been quantified from photographs of marked fans at several locations near Akumal, Mexico, that were photographed each summer from 2002 to 2005 (J. Bruno and I. Vu, unpublished data). Although lesions differ greatly in their rate of growth, the average rate of increase in the fraction of area infected on a given fan, between one annual census and the next, was <1% day\(^{-1}\) in all cases. Over a year, however, the area of infected tissue on a host can increase nearly 20-fold (J. Bruno and I. Vu, unpublished data). If we assume that this represents an infection with very low immune response from the host, no background mortality, and food halfway consumed on average (\(T = 0.5\)), we can estimate \(q \approx 2 \log (20)/365 \approx 0.02\). To represent the relatively weak immune response with the immune cell densities being roughly \(O(1)\), we chose \(m_c = 0.002\). Alternatively, observed lesion growth rates may represent the outcome of a strong immune response to a pathogen with a potentially high growth rate. At the height of the epizootic, it appeared that individual sea fans could be completely consumed by the pathogen within months (C. D. Harvell and K. Kim, unpublished observations). To represent this situation, we chose \(q = 0.05\), which allows pathogen density to increase 90-fold in 6 months at \(T = 0.5\), and set \(m_c = 0.02\) so that the lesion growth can be greatly reduced by effective immune response.

Lesion histology shows that the pathogen can consume virtually the entire thickness of skeleton at any location, which (in our model) requires \(\mu_v \ll q\) so that pathogen growth is not slowed by local resource limitation until \(T\) is near 1. As a default value, we chose \(\mu_v = q/10\) and for sensitivity analysis considered \(\mu_v\) ranging from \(q/20\) to \(q/2\), at which pathogen spread is severely limited by resource scarcity in the absence of host response. The velocity of local pathogen advection is the product of \(\eta\) and the local resource gradient \(\partial T/\partial x\). Relatively localized infections (5%–10% of the total fan area) have been observed to spread across nearly the entire fan in a year (J. Bruno and I. Vu, unpublished data) and may have spread even faster at the epizootic peak. A spreading infection can generate a sharp resource gradient from \(T \approx 0\) to \(T \approx 1\) within a small fraction of the total host area. So assuming that the resource gradient is \(O(5)\), we need \(\eta \approx 1/(5 \times 365) \approx 5 \times 10^{-4}\) for the pathogen to spread from the center of the fan to its margin in 1 year. We took this as our default value and considered values larger or smaller by a factor of 10 for global sensitivity analysis.

Signal

Phagocytosis by local amoebocytes (thereby releasing antifungal toxins) occurs within 2 h of infection (Olano and Bigger 2000), while recruitment of nonlocal cells to regions surrounding a new lesion site occurs within 24 h. This suggests that \(\delta_c\) and \(\delta_w\) should be \(O(1) - O(10)\) day\(^{-1}\), while the values of \(\eta\) and \(D_s\) should be such that a period between 0.1 and 1.0 days is required for spread of the signal and chemotactic response by undifferentiated amoebocytes. We therefore posit a signal that can set up a non-localized gradient within 12 h. As a very rough guide to the development of the signal gradient over the first 12 h, we can ignore the boundary conditions (which do not have much effect initially), ignore signal degradation, and assume a point release of signal at the center of the host at time 0.25. This creates a Gaussian concentration profile centered at the point of infection and having standard deviation \(\sigma = (D_s/2)^{1/2}\) at time \(t = 1/2\), which is the distance from the origin at which the signal gradient is strongest. This suggests, to allow nonlocal recruitment of amoebocytes, the range of values \((D_s/2)^{1/2} = 0.1–0.5\), so \(D_s = 0.02–0.5\), with default value 0.1. Numerical solutions of the signal equation in the radial model, with a constant source term in a small disk about the origin, support the ranges suggested by the rough approximation: at \(D_s = 0.02\), a strong signal gradient develops to about \(r = 0.1\) by \(t = 0.5\), while for \(D_s = 0.5\), there is a more gradual signal gradient present to about \(r = 0.4\). The signal loss rate \(\mu_s\) is the inverse of the mean persistence time of signal compounds before degradation to some inefficient form. Little is known about signal degradation rate, so we considered mean persistence times of from 1 to 100
days ($\mu_s = 0.01–1$), with default value $\mu_s = 0.1$ (mean persistence = 10 days).

**Granular Amoebocytes and Axis Epithelial Cells**

The occurrence of antipathogen toxin production within 2 h after infection (Olano and Bigger 2000), presumably by differentiated immune cells, implies relatively rapid differentiation into GA and AE cells. We considered the range $\delta_s = \delta_s = 0.5–10$, which gives between (roughly) 8% and 80% differentiation within 2 h, and a default value of 1, which gives about 15% differentiation within 2 h. Recruitment of nonlocal amoebocytes in response to the signal occurs within 24 h. Assuming a typical signal gradient of $O(0.1)$, based on numerical solutions of the signal equation at the default values, 0.1$\eta$, is roughly the fraction of the fan length from which amoebocytes can converge onto a new infection within 1 day, suggesting a plausible range of $\eta = 0.5–10$. Because of the relatively slow pathogen growth, it hardly matters whether amoebocytes reach an infection point within 1 day rather than 5 days, so there is no need to consider high values of $\eta$. Low values are worth exploring, however, as a possible cause of poorer host defense. We therefore explored the range $\eta = 0.2–2$, with 1 as the default value.

**APPENDIX B**

**Numerical Solution Methods**

We solved the model numerically using the method of lines, which discretizes space but leaves time continuous, producing a system of ordinary differential equations (ODEs) for the state variables at a set of spatial grid points. Values at the grid points are used to approximate spatial derivatives appearing in the model. For derivative estimation, we used Chebyshev interpolation (Trefethen 2000; Boyd 2001). The grid points $x_i = -\cos (i\pi/N)$, $i = 0, 2, \ldots, N$ are linearly rescaled onto the interval of interest. Each state variable is approximated by the unique polynomial of degree $N$ that interpolates the values at the grid points, and the spatial derivatives of the approximating polynomial are then used in the numerical solution of the model. This approach is computationally feasible because the interpolate-differentiate process is a linear operation represented by a matrix that can be computed once and for all and that applies to all state variables. The resulting ODE system is often stiff (Trefethen 2000), so we used an adaptive ODE solver that can deal with stiff systems, "lsoda" in the "odesolve" package (Setzer 2005) in R, version 2.3 or higher (R Development Core Team 2006).

As an example, consider pathogen spreading through an undefended host in the linear model:

\[
\begin{align*}
\dot{T} &= -qPT, \quad (B1a) \\
\dot{P} &= qPT - \mu_P P - \frac{\partial}{\partial x} \left[ P \eta \frac{\partial T}{\partial x} \right]. \quad (B1b)
\end{align*}
\]

Let $T(t) = T(x, t)$ and $\tilde{T}(t) = \{T_1(t), \ldots, T_n(t)\}$ and let $P(t)$ and $\tilde{P}(t)$ be defined similarly. Let $D$ be the matrix representing interpolation and differentiation. The system of ODEs for equation (B1b) is

\[
\begin{align*}
\frac{d\tilde{T}}{dt} &= -q\tilde{P} \circ \tilde{T}, \quad (B2a) \\
\frac{d\tilde{P}}{dt} &= q\tilde{P} \circ \tilde{T} - \mu_P \tilde{P} - D(\tilde{P} \circ \tilde{P} \circ D \tilde{T}). \quad (B2b)
\end{align*}
\]

where $\circ$ denotes the element-by-element (Hadamard) product of two vectors or matrices.

For the radial model, the grid points $r_0, r_1, \ldots, r_N$ are defined by linearly rescaling the $x_i$ defined above so that $r_0 = 1, r_1 > 0, r_i = -r_i < 0$. In particular, the two leftmost points sit symmetrically on opposite sides of 0. This makes it easy to impose the boundary condition that radial derivatives must equal 0 at $r = 0$ by holding the value of each state variable at $r_0$ equal to the value at $r_1$ at all times. The centered-difference estimate for the radial derivative at $r = 0$ is then 0. For advective terms in the radial model, we first use the product rule

\[
\frac{1}{r} \frac{\partial}{\partial r} [rf(r)] = \frac{f(r)}{r} + \frac{\partial f}{\partial r}, \quad (B3)
\]

where $f(r)$ is the advective flux. Then when we set $R^{-1} = (r_0^{-1}, r_1^{-1}, \ldots, r_N^{-1})$, the numerical approximation for equation (B3) is $R^{-1} \circ f + Df$, where $D$ is the differentiation matrix for the grid $\{r_i\}$.

Solutions to advection-dominated problems such as ours tend to develop spurious high-frequency wiggles at roughly the spatial scale of the spatial grid. A standard remedy is to apply a low-pass spatial filter at each time step (Boyd 2001, chap. 11). Hesthaven and Kirby (2003) have shown that filtering is approximately equivalent to modifying the differential equations by adding diffusion with a spatially varying diffusion coefficient in the form of a parabola that falls to 0 at the endpoints of the domain. We added this artificial diffusion to all variables with advection. Amoebocytes required less artificial diffusion (diffusion coefficient $\leq 5\%$ of the advection coefficient) than the pathogen (diffusion coefficient $\leq 10\%$ of the advection coefficient), presumably because amoebocytes add in response to the signal gradient, which is smoothed by diffusion. The addition of artificial diffusion necessarily
changes model solutions, but the main effect (apart from eliminating numerical artifacts) is to slightly smooth out the sharp interface between infected and uninfected tissue.

In practice, we found that it was advantageous for the artificial diffusion to remain nonzero at the boundaries because this reduced the amount of artificial diffusion needed to eliminate spurious wiggles (e.g., diffusion coefficient proportional to $1 - 3.8(r - 0.5)^2$ in the radial model). As a result, the boundary conditions at $r = 1$ or $x = 1$ must be modified for advecting variables so that the total flux across the boundary is 0. We imposed this by finite difference, setting the value at the boundary equal to that at its neighboring grid point at all times. This zeros out the diffusive flux without changing the fact that the advective flux is 0. Boundary conditions involving spatial derivatives are often imposed using spectral differentiation (i.e., setting $Y$ at the boundary so that the corresponding entry of $DY$ is 0; Boyd 2001, sec. 2.4). We used finite difference instead because it is robust against grid-scale wiggles in the interior. Chebyshev grid points cluster near the boundaries, so a boundary grid point is very close to its neighbor (e.g., within 0.003 for $N \geq 30$), and finite difference is acceptably accurate at the boundary.

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