Uncertainty favors an induced immune response to infection

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Keywords: Immunity, *Drosophila melanogaster*, bacterial infection, Imd pathway, constitutive defense, mathematical model

Abstract

Organisms can use constitutive or induced defenses against natural enemies, such as pathogens, parasites, and herbivores. Constitutive defenses are constantly on, whereas induced defenses are only activated upon exposure to an enemy. Constitutive and induced defensive strategies each have costs and benefits, which can affect which type of defense evolves for a particular threat. Previous modeling that compared induced and constitutive defenses relied on conceptual models that lacked mechanistic details about host defense, did not consider pathogen proliferation rates, or lacked both features. To address this gap, we developed a detailed mechanistic model of the well-characterized *Drosophila melanogaster* immune response to bacteria with different proliferation rates and environmental distributions. Our model includes a system of differential equations that capture the *D. melanogaster* immune signaling network. We used our model to evaluate the factors favoring the evolution of constitutive and induced defenses by comparing the fitness of each strategy upon stochastic encounters of flies with bacteria. We found that induction is generally preferred in environments where fly-bacteria interactions are less frequent. We further show that the relative fitness of the induced defense depends on the interaction between the bacterial proliferation rate and the spatial distribution of the bacteria. Also, our model predicts that the specific negative regulators that optimize the induced response depend on the bacterial proliferation rate. Finally, we show that uncertainty over bacterial encounters favors the evolution of an induced immune response. Uncertainty in our model can arise from heterogeneous distributions of bacteria, as well as fluctuations in the density or patchiness of the bacterial population. This result provides evidence that environmental uncertainty favors induced defenses.

Introduction

Organisms defend themselves against natural enemies—including pathogens, parasites, and herbivores—using induced and constitutive defenses. Induced responses are produced when an enemy is present or an organism is attacked, whereas constitutive defenses are always on. Induced and constitutive defenses each offer distinct advantages, yet also have limitations. For example, constitutive strategies may be costly because of the resources required to produce the defenses or direct deleterious (immunopathological) effects of defenses on host tissues (Li et al. 2020; Aggarwal and Silverman 2008). It may be more cost effective for an organism to deploy an induced strategy in which defensive molecules are only produced when needed, such as when the host is attacked by an enemy (Richard Karban 2020). However, this induced strategy can create a costly delay in response (R. Karban and Myers 1989), although priming might reduce this delay (Pham et al. 2007). If the cost of defense is low or delay in response is too costly, a constitutive strategy might therefore be favored (Bixenmann et al. 2016).

Mathematical models have revealed insights into the evolution of induced and constitutive defenses. For example, Clark and Harvell (1992) used a minimal model to study resource allocation to growth, reproduction, and defense. They showed that induction is favored when attacks are unpredictable. Later models that solely focused on the cost of defense found that increasing the probability of attacks favors a constitutive defense (Shudo and Iwasa 2001; Kamiya et al. 2016). Other theoretical models have explored how different degrees of uncertainty shape the evolution of constitutive and induced defenses. For example, Adler and Korban (1994) showed that, in the absence of environmental variation (i.e., there is certainty of an attack), a constitutive defense is favored. On the other hand, Hamilton et al. (2008) found that uncertainty about the parasite proliferation rate favors induction. However, none of this work considered the actual biological mechanisms underlying induced and constitutive defenses, including the signaling pathways responsible for induction.

The well-characterized innate immune response of *Drosophila melanogaster* is a good model system for incorporating biological mechanisms into our understanding of the evolution of induced and constitutive defensive strategies. *D. melanogaster* uses a combination of constitutive and induced expression of antimicrobial peptides (AMPs), along with other effectors, to fight off pathogens (Lemaitre and Hoffmann 2007). Induced and constitutive expression of AMPs is under tissue-specific control in *D. melanogaster.* For example, the constitutive expression of AMPs in the *D. melanogaster* salivary gland and ejaculatory duct is under the control of a single transcription factor, Caudal (Ryu et al. 2004).

The *Drosophila* induced immune response depends on the microbial pathogen, as well as which tissue is infected. The immune deficiency (Imd) and Toll pathways are the two major signaling cascades involved in the *Drosophila* induced immune response to bacterial and fungal infection (De Gregorio et al. 2002). The Toll pathway regulates the expression of AMPs upon exposure to glucans found in fungal cell walls or lysine-type peptidoglycans (PG) present in the cell wall of Gram-positive bacteria (El Chamy et al. 2008). The Imd pathway is activated by diaminopimelic acid (DAP)-type PG in the cell walls of Gram-negative bacteria (Kaneko, Golenbock, and Silverman 2005). The Imd pathway is active in the fat body and gut, which are the primary immune organs of flies (Myllymäki, Valanne, and Rämet 2014). Following ingestion, bacteria proliferate inside the gut and release PG, which activates the Imd pathway (Neyen et al. 2012). The Toll pathway, in contrast, is not active in the gut because it relies on cytokines for activation, which are not stable in the low pH environment of the digestive tract (Ryu et al. 2006; Buchon et al. 2009). Because flies in nature are infected by bacterial pathogens mainly by ingestion during feeding (Siva-Jothy et al. 2018), modeling the Imd pathway in response to bacteria is more ecologically relevant.

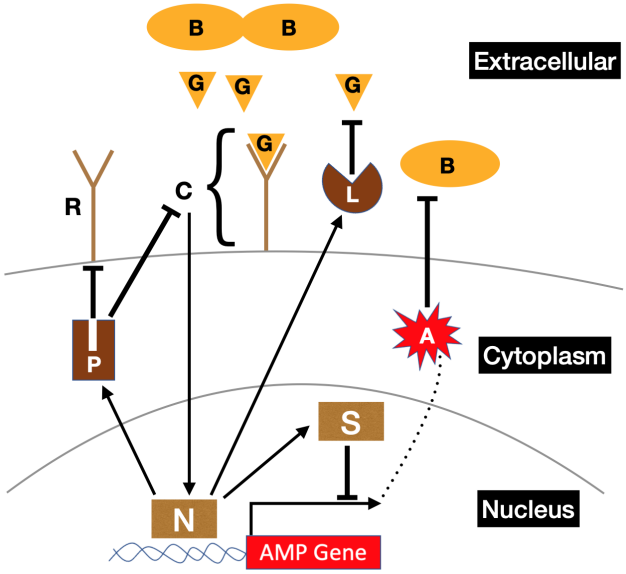

Figure 1. Diagram showing the Imd signaling pathway for induction of antimicrobial peptides (A). The letters in the diagram correspond to the variables in the system of differential equations of the induced model (Eqs. 1-9). Peptidoglycans (G) produced by bacteria (B) bind to cell surface receptors (R), forming a receptor complex (C) that initiates an NF-κB signaling pathway to activate the transcription factor Relish (N). Relish promotes the transcription of AMP genes, which leads to the production of AMP (A). Relish also induces the production of Pirk (P), scavengers of peptidoglycans (L), and at least one gene encoding a component of the repressosome complex (S). AMPs destroy bacteria, and Pirk reduces available cell surface receptors.

We developed a mathematical model of the well-characterized *D. melanogaster* Imd pathway (Figure 1). The first step of Imd pathway activation entails binding of bacterial PG to receptors

(PGRP-LC and -LE) on the surface of *Drosophila* enterocytes (Schmidt et al. 2008; Kaneko et al. 2006). Next, a signaling complex—consisting of the Imd protein, the adaptor protein dFADD, and the caspase protein DREDD—is recruited to the intracellular domain of the receptor (Georgel et al. 2001; Leulier et al. 2002). DREDD cleaves Relish, which is an NF-κB transcription factor (Stoven et al. 2003), and the N-terminal domain of Relish is translocated into the nucleus where it facilitates the transcription of AMP genes (Stöven et al. 2000). Imd signaling concurrently activates negative feedback loops, which protect the host from harmful effects of excessive immune response (Badinloo et al. 2018). For example, scavengers of PG (PGRP-LB) are expressed in a Relish-dependent manner, which reduce Imd signaling by removing PGs from the gut (De Gregorio et al. 2002). Relish also upregulates *pirk*, which encodes a negative regulator of the Imd pathway. Pirk reduces the number of available PGRP-LC receptors on the cell surface (Lhocine et al. 2008; Kleino et al. 2008). Finally, following activation of the Imd pathway, two transcription factors, Jra and Stat92Ea, form a repressosome complex with a high mobility group protein DSP1. The repressosome competes with Relish over binding to promoters of AMP genes, thereby reducing the AMP expression (Kim et al. 2007). The production of the transcription factor Jra has been shown to be regulated by Relish, thus acting as a negative feedback loop to attenuate Imd signaling (De Gregorio et al. 2002).

To investigate the conditions that favor induced versus constitutive defenses, we used a system of ordinary differential equations (ODEs) to model the Imd response to bacteria in *D. melanogaster*. Our model of the Imd signaling pathway goes beyond one previously developed by Ellner et al. (2021) because ours includes mechanistic details of negative feedback loops involved in the pathway. We compared the fitness of this model to a simple model of constitutive defense. We did not consider mixed strategies as it has been shown that immune genes can adopt a purely constitutive or a purely induced strategy (Asgari et al. 2022). Both our induced and constitutive models include features that capture the bacterial proliferation rate and survival in the gut, which has been shown experimentally to vary across bacteria species (Pais et al. 2018; Duneau et al. 2017). We considered stochastic encounters of flies with bacteria in a variety of environments, with different bacterial density and patchiness, to determine the conditions in which induced or constitutive defenses are favored.

Materials and Methods

The Model

We modeled the Imd pathway using a system of ODEs which capture the dynamics of immune response to bacterial infection (Figure 1). Equation (1) describes the change in bacterial density (B) in the gut of an individual fly.

$$\frac{dB}{dt} = f(t) + k_0 B_t - A_t B_t \qquad (1)$$

Under our model, the density of bacteria entering the gut at time t is described by the function $f(t)$. In general $f(t)$ depends on the local concentration of bacteria encountered by the fly as it moves around its environment. For example, we may consider scenarios in which $f(t)$ is a deterministic, oscillating (sinusoidal) function, reflecting an environment in which bacteria occur in regular "patches", as well as scenarios in which $f(t)$ describes the probability of entering or leaving a patch in an environment with randomly distributed colonies of bacteria. After ingestion, bacteria proliferate inside the gut at rate k_0, and are killed by AMPs at a rate $A_t B_t$, where A_t is the concentration of AMP at time t.

The concentration of free bacterial PG at time t (G_t) is described by Equation (2).

$$\frac{dG}{dt} = \alpha k_0 B_t - L_t G_t - R_t G_t + P_t C_t + \lambda_3 C_t - \lambda_1 G_t \qquad (2)$$

The production of free PG is stimulated by the proliferation of bacteria ($k_0 B_t$) at rate α. We assume that free PG is scavenged (i.e., removed from the system) by PGRP-LB (L_t) interacting with G_t (Zaidman-Rémy et al. 2006; Costechareyre et al. 2016). Free PG is also lost when it binds to receptors on the cell surface (R_t). We also assume that PG is released when the PG-receptor complex (produced at a rate C_t) is pulled down by Pirk (produced at a rate P_t) (Kleino et al. 2008). The receptor-PG complex is dissociated at rate λ_3, which produces free PG and free receptors. Finally, PG is degraded at rate λ_1.

Next, we describe the change in concentration of free receptor (R) over time via Equation (3).

$$\frac{dR}{dt} = R_0 + \beta_1 \frac{N_t}{N_t + Z_n} - P_t R_t - R_t G_t + \lambda_3 C_t - \lambda_2 R_t \qquad (3)$$

Here, receptors are produced at a base rate R_0. Receptor expression is positively regulated by the NF-κB transcription factor Relish (N_t) at rate β_1, which is modeled as a Hill function (Liu et al. 2020). Z_n is the binding energy of Relish to the promoter, which is inversely proportional to the probability of binding. Interactions with Pirk and PG reduce the concentration of free receptors. We considered both separate and identical degradation rates for R, N, L, P, S, and A (described below). Equations in the main text have equal degradation rates for R, N, L, P, S, and A (λ_2).

Following from Equation (3), change in the concentration of receptor-PG complex (C) is described by Equation (4).

$$\frac{dC}{dt} = R_t G_t - P_t C_t - \lambda_3 C_t \quad (4)$$

Equation (5) describes the change in the NF-κB transcription factor Relish, which is activated at rate β_2 following formation of the PG-receptor complex (Stoven et al. 2003).

$$\frac{dN}{dt} = \beta_2 C_t - \lambda_2 N_t \quad (5)$$

Equations (6), (7), and (8) describe negative feedback loops mediated by Relish.

$$\frac{dL}{dt} = \beta_3 \frac{N_t}{N_t + Z_n} - \lambda_2 L_t \quad (6)$$

$$\frac{dP}{dt} = \beta_4 \frac{N_t}{N_t + Z_n} - \lambda_2 P_t \quad (7)$$

$$\frac{dS}{dt} = \beta_5 \frac{N_t}{N_t + Z_n} - \lambda_2 S_t \quad (8)$$

PGRP-LB, Pirk, and the repressosome (AP-1 and STAT complex, S_t) are activated by Relish at rates β_3, β_4, and β_5, respectively (Zaidman-Rémy et al. 2006; Kleino et al. 2008; Lhocine et al. 2008; Kim et al. 2007).

Equation (9) describes AMP expression, which is upregulated by Relish and repressed by the repressosome with a single rate of β_6. The repressosome competes with Relish for binding to promoters of AMPs (Kim et al. 2007). The binding energy for the repressosome is Z_s.

$$\frac{dA}{dt} = \beta_6 \frac{N_t}{N_t + Z_n + \frac{Z_n S_t}{Z_s}} - \lambda_2 A_t \quad (9)$$

Finally, in order to contrast the Imd pathway with a constitutive immune response, we modeled constitutive expression by simply assuming that A_t is a constant ($A_t = A$) in Equation (1).

Simulation of the bacterial environment

In order to capture the effect of patchiness of bacteria colonies on immune response, we simulated bacterial populations on a 2-dimensional lattice (100×100) using a simple random walk algorithm with periodic boundary conditions (Figure 2). We placed bacterial colonies at positions on the lattice which were determined according to a random walk. We initialized the walk at a random starting coordinate $[i_0, j_0]$, and the subsequent direction of movement was

specified to be left $[i_0 - p, j_0]$, right $[i_0 + p, j_0]$, up $[i_0, j_0 + p]$, or down $[i_0, j_0 - p]$, with equal probabilities ($\frac{1}{4}$), where p describes the (constant) step size. A bacterial colony was then placed on the lattice at the updated coordinate [i,j] . The process was repeated until a predetermined number of colonies had been added to the lattice. If a lattice point was already occupied by a bacterial colony, the lattice was not updated, the colony count remained unchanged, and another step was taken. The number of colonies added to the lattice determined the density of bacteria ($d = \frac{\#colonies}{100\times100}$) in the environment. The value of the random walk step size, p, determines the patchiness of the bacterial distribution; low values of p create a more heterogeneous distribution of bacteria, while high values create uniform distributions (Figure 2).

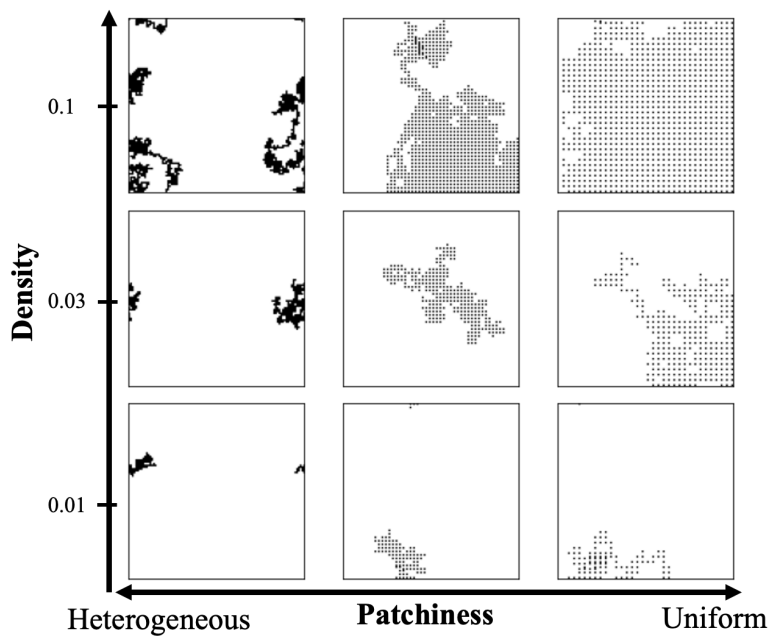

Figure 2. Examples of simulated environments with different numbers (Density) and patchiness of bacterial colonies. Each dot represents a bacterial colony on a 100×100 lattice.

Movement of the fly through the environment

We used both a deterministic and stochastic approach to model fly movement through bacterial environments. In the deterministic approach, we considered an oscillating model in which $f(t) = \omega\, sin(t\,\Phi)^2$ such that the fly experiences a deterministic oscillating bacterial environment. For the deterministic oscillating model, we solved the system of ODEs using the scipy python package .

In the stochastic method, we assessed the efficacy of constitutive defense and induced immune response by comparing their efficacy over the course of a large number of sojourns through a

simulated bacterial environment (Figure 2). To those ends, we simulated the behavior of a fly within a given bacterial environment as a random walk across a lattice seeded with a bacterial distribution as described above. The random walks were performed as described above for seeding the lattice, but all fly walks used step size p =1. When a fly landed at a given coordinate [i,j], the immune response was determined by the input function $f(t)$ (Eq. 1), such that, if a bacterial colony was present $f(t)$=1, and $f(t)$=0 otherwise. Thus as the fly moves through the environment $f(t)$ takes values 1 or 0 depending on the presence or absence of bacteria at the current lattice position. This process simulates stochastic fly-bacteria interactions. We solved the system of ODEs using the Runge-Kutta Method (RK4) algorithm for the random walk model. All simulations of bacterial and fly random walks were performed using python code available from https://github.com/danialasgari/Uncertainty-favors-an-induced-immune-response-to-infection-.

Two parameters in our induced model define the interaction between the bacteria that flies encounter and their immune system during their deterministic oscillations and random walks: the rate at which PG is released upon bacterial proliferation (α) and the degradation rate of PG (λ_1). We considered four values for α ($0.2,\ 1,\ 2,\ 4$), and two values for λ_1 (0.01 and 0.05). However, our results focus on $\lambda_1 = 0.01$ for reasons described in the Results. In both the induced and constitutive models the efficacy of the immune response also depends on the bacterial proliferation rate (k_0). We tested both constitutive and induced defenses against three different bacterial proliferation rates ($k_0 = 0.1$, $k_0 = 0.2$, and $k_0 = 0.5$).

Calculating fly fitness

After obtaining a vector containing values at every time point for each variable in the model ($B_t,\ G_t,\ R_t,\ C_t,\ N_t,\ L_t,\ P_t,\ S_t,\ A_t$), we calculated fitness as an exponentially decreasing function of the summed time averaged values of each of the model variables, as shown in equation (10) for an induced response and in equation (11) for constitutive defenses.

$$F_{Induced} = e^{-(\overline{B}+\overline{N}+\overline{L}+\overline{P}+\overline{S}+\overline{A})} \quad (10)$$

$$F_{Constitutive} = e^{-(\overline{B}+A)} \quad (11)$$

In these calculations (Eqs. 10-11), $\overline{B}$ is the time averaged bacterial concentration in the gut, $\overline{N}$ is the time averaged production of Relish, and so on. This method assumes that fitness declines exponentially with the average bacterial load $\overline{B}$ and with the strength of the immune response due to the cost of production.

For each environment with a particular bacterial density and patchiness, we simulated 1,000 fly random walks each consisting of 100,000 steps, and calculated the arithmetic mean fitness for the constitutive and induced defenses across simulations. The fitness difference (ΔF) between the induced and constitutive immune responses was calculated by subtracting the average fitness for the constitutive defense ($\overline{F}_{constitutive}$) from the average fitness of the induced response ($\overline{F}_{induced}$) using equation (12).

$$\Delta F = \overline{F}_{induced} - \overline{F}_{constitutive} \tag{12}$$

Parameter optimization

The model for induced response has 11 parameters that describe the attributes of the Imd pathway ($\lambda_2, \lambda_3, R_0, \beta_1, \beta_2, \beta_3, \beta_4, \beta_5, \beta_6, Z_n, Z_s$). To find the parameters that maximize the fitness of the induced response, we simulated random walks within the 11-dimensional landscape consisting of the 11 parameters, which we refer to as "optimization walks". We used the Muller (1959) method to generate random points in an 11-dimensional space, where step size = 0.01. First, we found the position vector of a point (u) in an 11-dimensional sphere by generating 11 independent normal deviates (equation 13).

$$u_i = N(0, 0.01);\ i = 1,\ldots, 11 \tag{13}$$

The length of the vector is calculated using equation (14).

$$w = \sqrt{\sum_{i=1}^{11} u_i^2} \tag{14}$$

The updated value for each variable (X') is found by summing the current value (X) with the direction cosines ($\frac{u_i}{w}$) multiplied by the radius of the sphere (0.01), using equation (15).

$$X_i^{'} = \left| 0.01\left(\frac{u_i}{w}\right) + X_i \right| \tag{15}$$

We used equation (10) to calculate the fitness of induced defense ($F_{Induced}$) using the 11 parameters as input to equation (10). At each step in the optimization walk, the 11 parameters are simultaneously updated. If $F_{\lambda'_2,\lambda'_3,\ldots,Z'_s} > F_{\lambda_2,\lambda_3,\ldots,Z_s}$, the new set of parameter values is accepted; otherwise, the set is rejected. We used 10,000 steps for the lower bacterial proliferation rates (

$k_0 = 0.1$ and $k_0 = 0.2$), and 20,000 steps for the higher proliferation rate ($k_0 = 0.5$) because it took longer for fitness to plateau for the higher proliferation rate. When we considered different degradation rates (λ_2) for R, N, L, P, S, and A, we used a larger step size (0.03) with 20,000 steps because optimizing the model with more parameters demands a broader search of the fitness landscape.

Random walks within a 11-dimensional landscape are computationally intensive if $f(t)$ is stochastic. To perform a more efficient search, we calculated fitness at each step of our random walk using a deterministic oscillating input with different frequencies to the system of ODEs instead of a stochastic one ($f(t) = sin(t\,\Phi)^2$). We optimized the induced model using different values of Φ, i.e., the frequency of the sinusoidal input. Higher Φ describes a higher density of bacteria. Using a sinusoidal input is more efficient than stochastic simulations of bacterial distributions at each step of the walk through parameter space, and it also dispenses with the need for simulating random walks of flies among bacteria at each step. Different local optima might be reached when starting from different points in the landscape. Therefore, we considered a large and a small starting value for each parameter and ran a random walk for every combination of large and small parameter values. This makes a total of 2048 walks for the purpose of parameter optimization ($11^2 = 2048$).

To optimize the constitutive defense, the amount of constitutively expressed AMP (A) was changed over a range of 0.01 to 2 (step size = 0.01), and the value of A that confers the highest fitness is chosen. The Euler method was used (h = 0.01) to solve equation (1), where A_t is a constant ($A_t = A$). Due to the simplicity of the model for constitutive defense, the model was optimized in stochastic environments. The optimum fitness for the constitutive defense was calculated in each combination of bacterial density and pathiness and then compared to the fitness of different optimizations of the induced defense (i.e., induced defenses optimized with different values of Φ).

Calculation of time to response to bacterial infection for the induced defense

We considered the time to response (Δt) to be the interval between the first ingestion of the pathogen at time t_0 to the earliest time point (t_1) at which bacterial concentration is reduced due to the action of AMPs (Eq. 16).

$$\Delta t = t_1 - t_0 \;\mid\; B_{t_{min}} > 0 \text{ for } t_{min} = t_0 \text{ and } B_{t'_{min}} < 0 \text{ for } t'_{min} = t_1 \qquad (16)$$

Simulating fluctuation across multiple environments

To simulate fluctuation in density and/or patchiness of bacterial populations, we randomly selected j environments with different densities and/or patchiness of bacteria and assumed that flies encounter those environments with equal probabilities. Sampling was done without replacement in order to maximize fluctuations in the density and/or patchiness of the bacterial population. To estimate the fitness of flies encountering j bacterial environments with equal probabilities, we calculated the arithmetic mean of the fitness values across j environments (Eq. 17).

$$F_{induction} = \sum_{i=1}^{j} \frac{F_i}{j} \tag{17}$$

In each of the j environments, the fly stochastically encounters bacteria. Simulations were performed for induced defenses that were optimized with different values of Φ.

To find the optimum fitness for the constitutive defense when flies inhabit j environments with equal probabilities (j^{-1}), we calculated the fitness in each environment for constitutive defenses ranging from 0.01 to 2 units of AMP production (i.e., $0.01 \leq A \leq 2$). Next, we calculated arithmetic means of fitness values across j environments (the dashed lines in supplemental figure 1) and chose the maximum value (the arrows in supplemental figure 1). We measured the proportion of times induction outperforms constitutive defense by repeating the process of random selection of environments and calculation of the relative fitness (ΔF) 10,000 times.

We also calculated the relative fitness of the constitutive and induced defenses when the fly inhabits 2 environments with probabilities q and $1 - q$. This differs from the previous analysis, where $q = j^{-1}$. The fitness of the induced response is the weighted average of fitness values across the two environments (Eq. 18).

$$F_{induction} = q\,F_1 + (1 - q)\,F_2 \tag{18}$$

We used the following approach to find the optimum fitness for the constitutive defense when flies inhabit two environments with probabilities q and $1 - q$. First fitness was calculated in each of the two environments for constitutive defenses ranging from 0.01 to 2 units of AMP production ($0.01 \leq A \leq 2$). The optimum fitness of constitutive defense is the maximum value (arrows in supplemental figure 2) of the weighted averages of fitness values (dashed lines in supplemental figure 2). The proportion of times induction outperformed constitutive defense for 10,000 combinations of environments was calculated for each value of q, as explained above.

Population based model

We used a single locus biallelic population model to further compare the relative fitness of constitutive and induced immune responses in fluctuating environments. Our model treats each strategy as a single trait controlled by a single locus (i.e., the two alleles in our model are induced and constitutive). Although in nature flies are diploid and sexual, we used a haploid asexual model because recombination can be ignored with a single locus (Ewens 2010). The frequency of the induced allele at generation t is m_t, and the frequency of the constitutive allele (n_t) is $1 - m_t$. At each generation, the fly inhabits 2 environments with probabilities q and $1 - q$. The fitness of the induced response is the weighted average of fitness values across the two environments (Eq. 18). This is done for induced responses that are optimized with different values of Φ. The optimum fitness for a constitutive defense is calculated as explained above (supplemental figure 2).

The mean fitness of the population was calculated using equation (19).

$$\overline{W} = m_t[q\ W_{I1} + (1 - q)W_{I2}] + (1 - m_t)[q\ W_{C1} + (1 - q)W_{C2}] \tag{19}$$

Here, fitness of the induced allele in environment j is W_{Ij} , and fitness of the constitutive allele is W_{Cj}. The frequency of the induced allele in the next generation (m_{t+1}) was calculated using equation (20).

$$m_{t+1} = \frac{m_t[q_1 W_{I1} + (1-q)W_{I2}]}{\overline{W}} \tag{20}$$

The frequency of the constitutive allele in generation $t + 1$ (n_{t+1}) was calculated using equation (2).

$$n_{t+1} = 1 - m_{t+1} \tag{21}$$

We tested if the frequency of the constitutive or induced allele increased over time as an indication of the allele that confers a higher fitness.

Results

Induction is favored when bacteria are at low/intermediate density and have heterogeneous distributions

In order to compare the fitness of induced and constitutive defenses, first we found parameter values in our model that maximize fitness for each strategy in a variety of environments. Constitutive defense was optimized by finding the value of constitutively expressed AMP (A) that produces the highest fitness within each environment, where the environment consists of a specified density (d) and patchiness (p) of bacteria. To optimize the induced response, we simulated random walks in a 11-dimensional landscape corresponding to the 11 parameters in our model, and we identified the combination of 11 parameter values that maximize fitness in a specified environment. We optimized the induced response across a variety of environments with different frequencies of bacterial encounters using a sinusoidal function, where Φ corresponds to the frequency of bacterial exposure. Higher values of Φ correspond to environments with a higher density and more uniform distribution of bacteria, where fly-bacteria interactions are more likely. Lower values of Φ, in comparison, correspond to environments with low density or more heterogeneous bacterial distributions, where bacterial exposure is lower.

We evaluated how the rates of bacterial PG production and natural degradation of PG affect the performance of an induced immune response across different frequencies of bacterial exposure. We optimized induced defenses for four rates of bacterial PG production (α = 0.2, 1, 2, and 4; supplemental figure 3A). Our results were qualitatively similar across different α values (supplemental figure 3B), and we only focus on results for α = 2 for the remainder of the manuscript. We chose a small value for the natural degradation rate of PG (λ_1= 0.01) because the natural degradation rate is much slower compared to degradation by PGRPs (Filipe, Tomasz, and Ligoxygakis 2005). We found that when λ_1= 0.01, the parameter controlling production of PGRP-LB (β_3) is larger than λ_1 (supplemental figure 4A), consistent with more degradation by PGRP-LB than the natural degradation rate. However, when when λ_1 = 0.05, β_3 is smaller (supplemental figure 4B). Therefore, setting $\lambda_1$to 0.01 is biologically realistic because it captures the important role that PGRP-LB plays in degradation of PG. By setting α = 2 and λ_1= 0.01 we found that increasing the frequency of fly-bacteria encounters (Φ) reduced the optimal fitness of the induced response, regardless of the bacterial proliferation rate inside the fly (k_0) (supplemental figure 5). Our results therefore suggest that induced responses are less fit in environments with a higher frequency of fly-bacteria interactions.

We compared the fitness of the induced and constitutive defenses under a sinusoidal mode of encounter with bacteria ($f(t) = \omega\, sin(t\,\Phi)^2$). We found for most optimizations of the induced

defense, an induced response is favored for at least some values of amplitude (ω), when the frequency of encounter with bacteria (Φ) is intermediate (supplemental figure 6). The exception occured when induction was optimized with Φ = 0.1. In this case, the induced defense performs poorly when compared to a constitutive defense for most frequencies and amplitudes of exposure. A limitation of this analysis is that fly-bacteria encounters are predictable when there is a sinusoidal input of bacteria into the model.

To model unpredictable encounters, we compared the fitness of induced and constitutive defenses upon stochastic encounters with bacteria. To those ends, we simulated stochastic fly-bacteria interactions using random walks of a fly in a 100×100 grid with differing densities and patchiness of bacteria (Figure 2). For each combination of bacterial density and patchiness values, we report the average relative fitness of induced versus constitutive defenses across 1,000 random walks for each value of Φ and k_0 used to optimize the induced defense.

First, we tested an induced response with equal degradation rates for all proteins involved in the Imd pathway against a constitutive defense. For most values of Φ, induced defenses had higher fitness values than constitutive defense in environments with a heterogeneous distribution and low to intermediate density of bacteria (Figure 3). For the highest bacterial proliferation rate (k_0 = 0.5) and $\Phi < 0.1$, induction had the highest relative fitness when bacterial density was lowest and distributed with maximal heterogeneity (Figure 3). For other bacterial proliferation rates (k_0 = 0.1 and k_0 = 0.2) and $\Phi < 0.1$, the relative fitness for induction was highest in environments with an intermediate density of bacteria and a heterogeneous distribution (Figure 3). The only exceptions to these patterns was when the induced response was optimized with Φ = 0.1 and tested against bacteria with an intermediate (k_0 = 0.2) or a high (k_0 = 0.5) proliferation rate (Figure 3). For these parameter combinations, induction performed best at higher bacterial densities. The poor performance of the induced defense optimized with Φ = 0.1 in the stochastic model is consistent with results from the sinusoidal model of fly-bacteria encounters (supplemental figure 6). In general, induction almost always performed best, relative to constitutive defense, in environments with heterogeneous bacterial distributions. However, the effect of bacterial density on the relative fitness of induced and constitutive defenses depended on the values of Φ and k_0 used to optimize the induced defense.

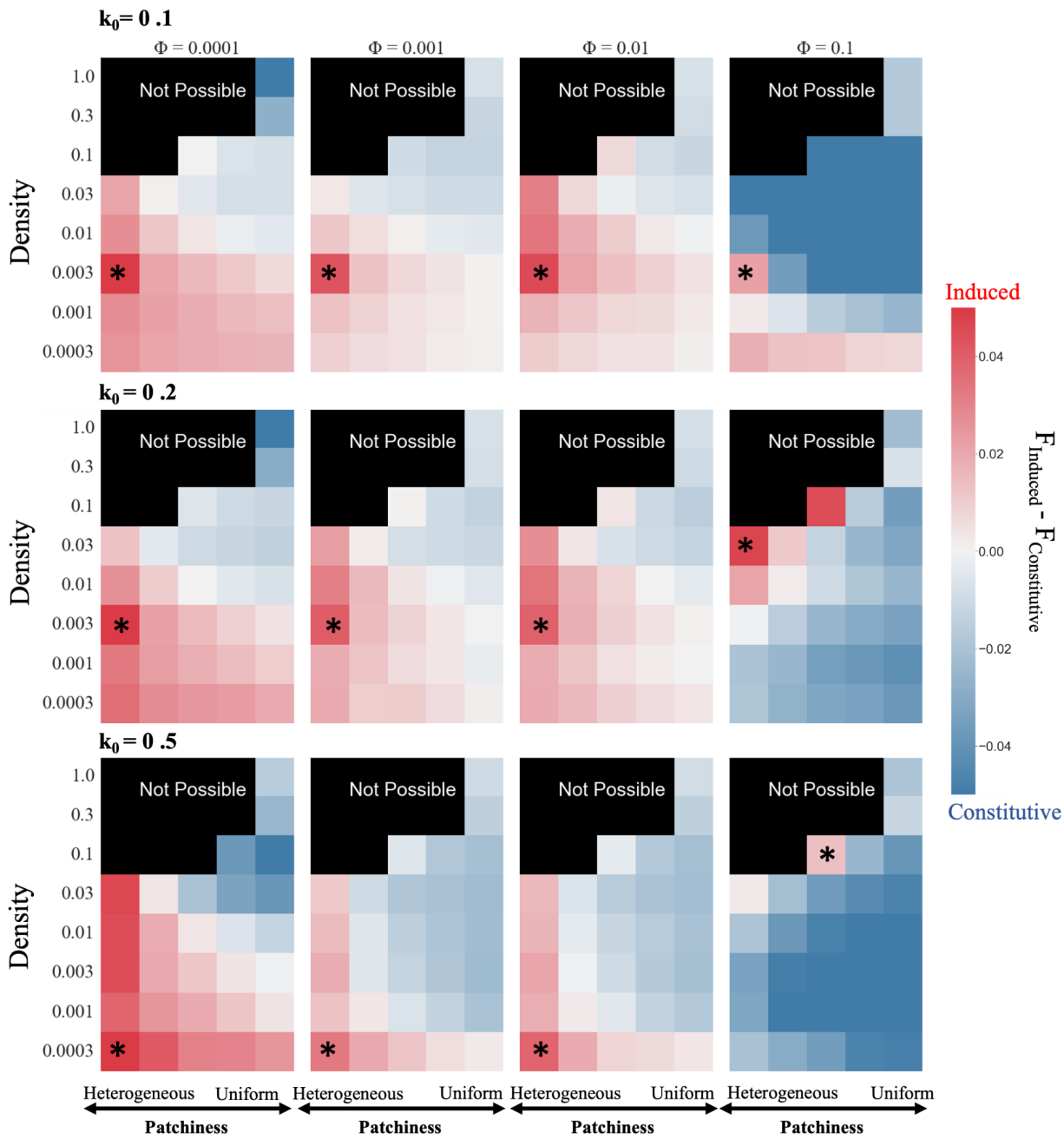

Figure 3. Induced responses tend to outperform constitutive defenses when flies inhabit environments with low densities and heterogeneous distributions of bacteria. The relative fitness of induced versus constitutive strategies are shown as heatmaps for environments with different densities (*d*) and patchiness (*p*) of bacteria. Patchiness values (*p*) for each cell are reported in Supplemental Table 1. Red cells indicate that the fitness of the induced response ($F_{Induced}$) is higher than fitness of the constitutive response ($F_{Constitutive}$), and blue cells indicate that $F_{Constitutive} > F_{Induced}$. Heatmaps in the same column show induced responses that were optimized with the same frequency of the sinusoidal input of bacteria (Φ), and heatmaps in each row show the results for the same proliferation rate of bacteria (k_0). Asterisks show the environment in which the induced defense has the highest relative fitness.

The analysis above assumes identical degradation rates for Imd signaling pathway proteins (it is an 11-parameter model), and we tested the effects of this assumption. To that end, we optimized a model of induction with different parameters for degradation rates (16-parameter model). We found that the induced response has the highest performance when compared to constitutive defense under the same conditions for both the 11-parameter and 16 parameter models. Consistent with our previous results (Figure 3), when bacterial proliferation rate is low ($k_0 = 0.1$ or $k_0 = 0.2$), induction performs best when bacterial density is intermediate and the distribution is patchy for the 16-parameter model (supplemental figure 7). When bacterial proliferation rate is high ($k_0 = 0.5$), induction performs best when bacterial density is low and distribution is patchy under both models (Figure 3 and supplemental figure 7). Compared to the 16-parameter model, the 11-parameter induced model has a higher performance against a constitutive defense across more environments. We hypothesize that the better performance of the 11-parameter model occurs because, with fewer parameters, the optimization algorithm is more efficient at finding local maxima when searching through the fitness landscape. Because we aim to compare the best induced response against constitutive defenses, we proceed with the 11-parameter model that has a single parameter for protein degradation rates (λ_2).

Induction is most favored in environments containing rapidly replicating bacteria with low density and heterogeneous distributions

We used two approaches to further examine the effects of the bacterial density, patchiness, and proliferation rate on the performance of the induced and constitutive defenses. In the first approach, we calculated the proportion of environments with different combinations of density and patchiness in which induction outperforms constitutive defense for a given bacterial proliferation rate and optimization of the induced response. This was done by calculating the fraction of red cells out of the total number of cells for each heatmap in Figure 3 for the stochastic model, and supplementary figure 6 for the sinusoidal model, which we refer to as the "proportion of induced wins" (PIW). Under the stochastic model, induction performed best (i.e., highest PIW) when bacterial proliferation rates were low or medium, unless induction was optimized with $\Phi = 0.1$ (Figure 4A). As proliferation rates increased, constitutive defense performed bettermost environments (i.e., PIW decreased). The exception to this rule was when $\Phi = 0.0001$, in which case PIW remained fairly constant regardless of the bacterial proliferation rate.

Results under the sinusoidal oscillating model were consistent with the stochastic model in three ways. First, the performance of the induced defense optimized with $\Phi = 0.0001$ was relatively unaffected by the bacterial proliferation rate under both models (Figure 4). Second, under both models, when optimized with $\Phi = 0.1$, induction performs poorly against a constitutive defense

(Figure 4). Finally, for intermediate values of Φ ($\Phi = 0.001$ and $\Phi = 0.01$), higher proliferation rates reduced PIW under both models (Figure 4).

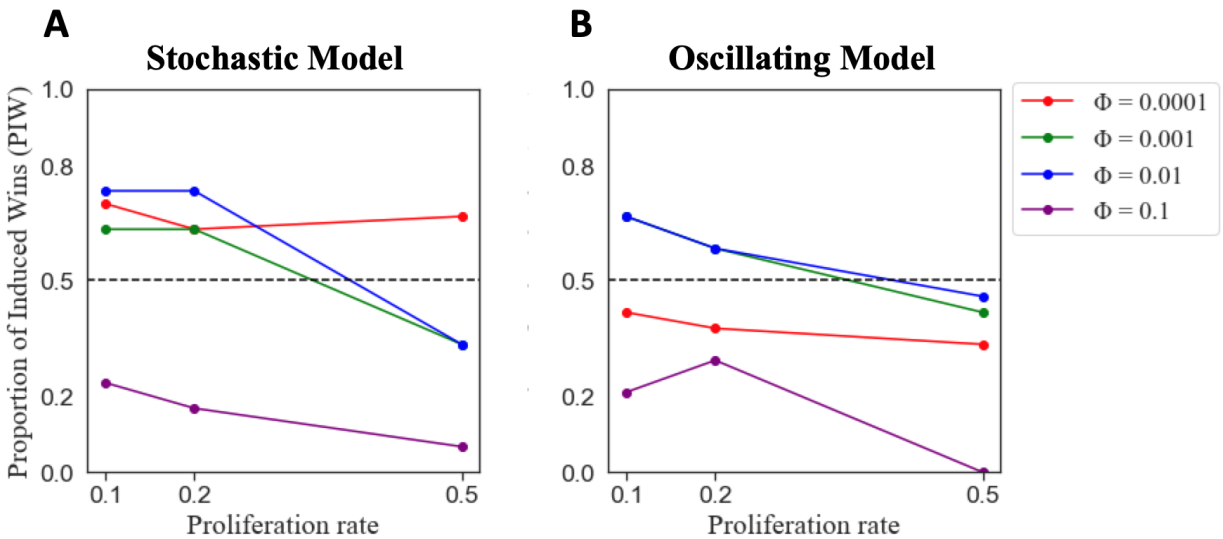

Figure 4. Proportion of induced wins (PIW) (Y-axis) for different proliferation rates (k_0) of bacteria (X-axis) under the stochastic (A) and sinusoidal oscillating models (B). PIW measures the proportion of environments in which an induced response outperforms constitutive defense. The results are shown for induced response optimized in environments with varying frequencies of encounter with bacteria (Φ). The dashed line at 0.5 shows equal performance of induced and constitutive strategies.

Our analysis is unlikely to be biased by the parameters we selected in Figures 3 and 4. We explored specific values for bacterial density and patchiness in the stochastic model; amplitude and frequency in the sinusoidal model; and proliferation rate in both models. If we had sampled more environments with a high bacterial density, PIW would be lower. However, the observed trends in figure 4 are unaffected by our parameter values because we are comparing the relative performance of induced and constitutive defenses across different values of Φ and k_0 using fixed values of density and proliferation rate. The relative performance (i.e., change in PIW) across proliferation rates should be robust to the parameters selected in the heat maps (Figure 3). Because our results are consistent under both stochastic and sinusoidal models, we will proceed only with the stochastic model. The stochastic model allows us to consider fitness differences between the induced and constitutive defenses when fly-bacteria encounters are unpredictable.

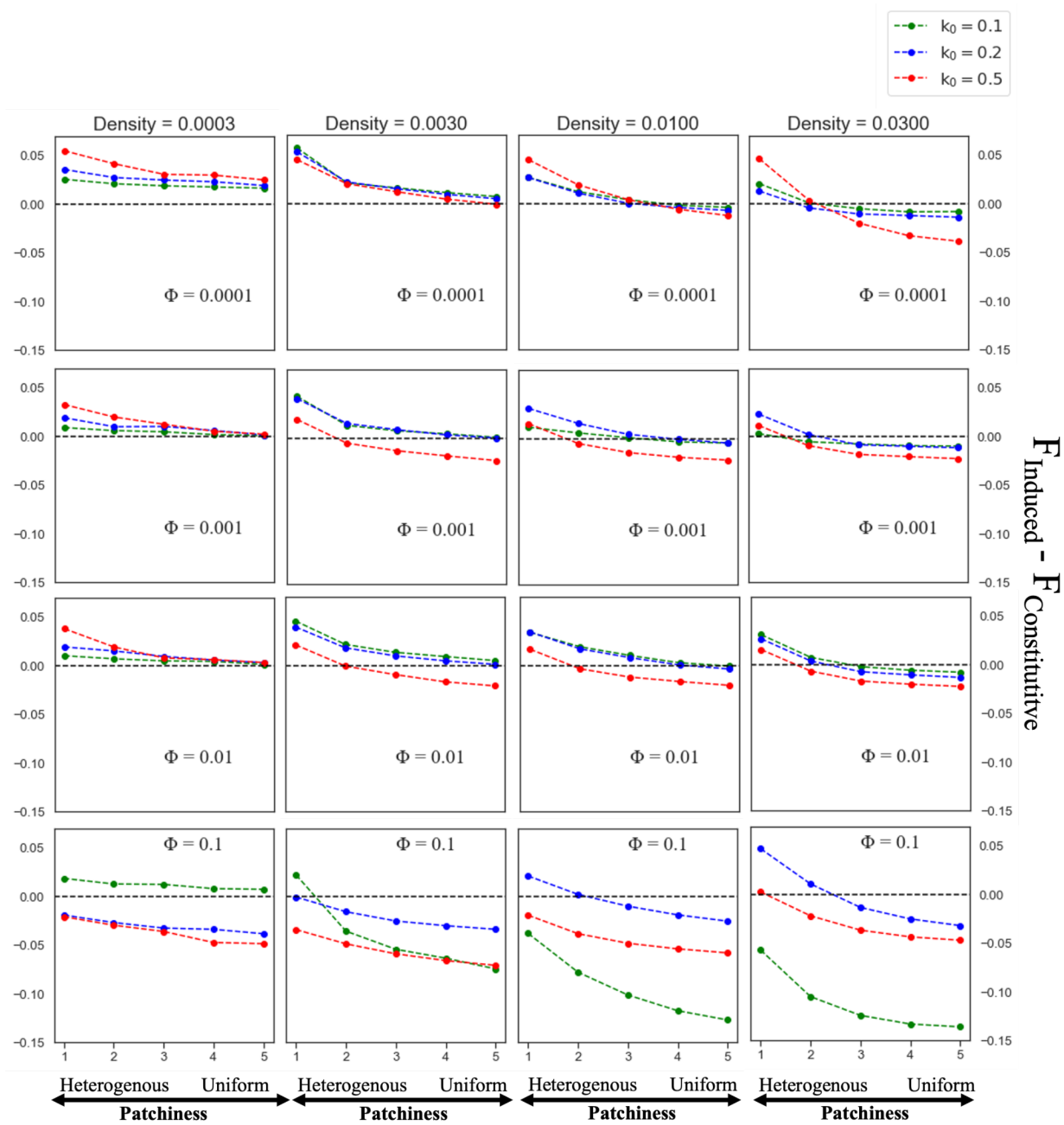

Figure 5. Effect of proliferation rate (k_0), bacterial density (d), and patchiness (p) of bacteria on the relative performance of induced and constitutive defenses. Parameter values for induced response are optimized with different frequencies of encounter with bacteria (Φ). The Y axis is the difference in fitness of induced and constitutive responses ($F_{induced} - F_{constitutive} = \Delta F$). The X axis shows the patchiness (p) of the environment. Graphs in a column have the same bacterial density, and graphs in a row have the same Φ value. Induction outperforms constitutive defense above the dashed line (positive values), and constitutive defense performs better below the dashed line (negative values).

In the second approach, we compared ΔF (Eq. 12) across different bacterial patchiness and proliferation rates for each combination of bacterial density and Φ. If $\Delta F > 0$, induction is favored, and constitutive defense is favored if $\Delta F < 0$. Induction generally outperformed constitutive defense in environments with heterogeneous bacterial distributions (Figure 5). In these environments, however, induction optimized with $\Phi = 0.1$ only outperformed a constitutive defense when either the bacterial proliferation rate was low ($k_0 = 0.1$) and bacterial density was low (d = 0.0003 and 0.003), or when bacterial proliferation rate was intermediate ($k_0 = 0.2$) and density was high (d = 0.1 and 0.3). Induced defenses optimized with lower Φ outperformed a constitutive defense in heterogeneous environments regardless of the density and bacterial proliferation rate. We also found that, for low bacterial density (d = 0.0003) with a heterogeneous distribution, selection for induced responses was stronger (ΔF is larger) when the bacterial proliferation rate was highest (k_0 = 0.5) and induction was optimized with lower frequencies of bacterial input (Φ = 0.0001,Φ = 0.001, and Φ = 0.01) (Figure 5). Induction was most favored (largest ΔF) when the bacterial proliferation rate was high ($k_0 = 0.5$) and induction was optimized with the lowest frequency of bacterial input (Φ = 0.0001).

Negative regulators of Imd signaling affect the fitness of induction depending on the bacterial proliferation rate

We compared the time of response to bacteria for induced defenses optimized with different values of Φ (Eq. 16). This is motivated by our observation that the fitness of induced responses depend on the Φ value used to optimize the model parameters (Figures 3, 4, 5). We compared induced responses optimized with $\Phi = 0.0001$, Φ = 0.01, and Φ = 0.1. We chose the first two values because models optimized with $\Phi = 0.0001$ offer the best defense against bacteria with a high proliferation rate (highest PIW), and models optimized with Φ = 0.01 are the best defense against bacteria with lower proliferation rates (Figure 4). We included induced responses optimized with Φ = 0.1 in our comparisons because they were outliers in most of our comparisons up to this point.

We found that induction optimized with the highest frequency of encounter with bacteria ($\Phi = 0.1$) responded faster to infection than other induced defenses (supplemental figure 8). This, however, comes with a higher cost of investment in the production of proteins involved in Imd signaling (supplemental figure 9). For example, induced defenses optimized with Φ = 0.1 produced more proteins involved in the negative regulation of the Imd signaling pathway (PGRP-LB, Pirk, and repressosome complex) compared to Φ = 0.0001 and Φ = 0.01 (Figure 6). This was true for simulations in environments with heterogeneous or uniform distributions of bacteria (supplemental figure 10). We found that induced defenses optimized with Φ = 0.0001 produced more respressosome complexes than those optimized by Φ = 0.01, while induced defenses optimized with Φ = 0.01 produced more PGRP-LB and Pirk (Figure 6). Thus, our

model predicts that investing in negative regulators that reduce the input to the signaling pathway (PGRP-LB and Pirk) is beneficial against bacteria with low proliferation rates, whereas investing in negative regulators that reduce the transcription rate of AMPs (repressosome) is beneficial against bacteria with a high proliferation rate.

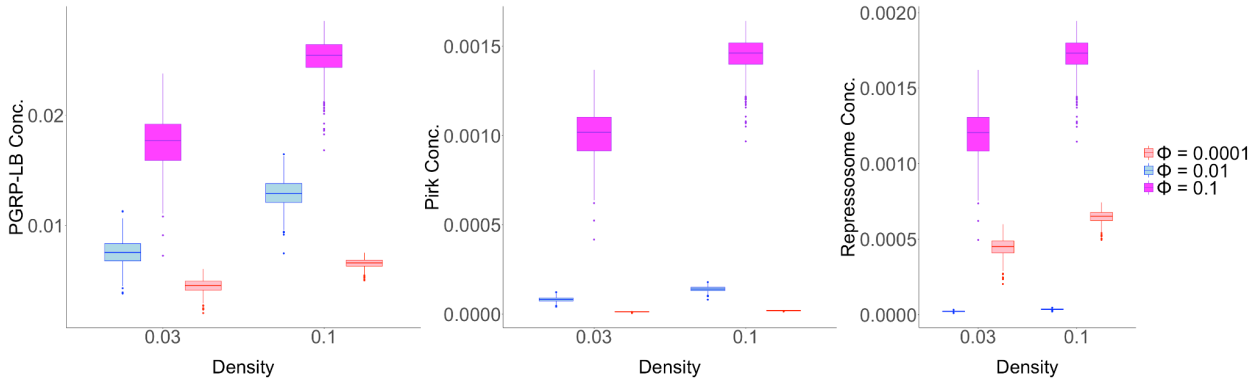

Figure 6. The box plots show the concentration of proteins involved in negative regulation of the Imd signaling pathway (Y axis) in environments with two different densities (*d*) of bacteria (X axis) across 1,000 simulations. Induced defenses were optimized with different frequencies of bacterial encounter (Φ). The distribution of bacteria in all analyses is heterogeneous ($p = 1$).

Inhabiting multiple types of environments favors induction

We next evaluated the performance of induced and constitutive defenses when a fly inhabits multiple environments. We were specifically interested in whether experiencing multiple types of environments (i.e., different bacterial densities and patchiness) favors induction, even when each individual environment favors constitutive defense. For that reason, we only sampled from environments in which constitutive defense outperforms induction (blue cells in Figure 3), and then we measured the relative fitness of induced and constitutive defenses when flies inhabit multiple environments that were each sampled with equal probabilities. We calculated PIW when flies experience multiple bacterial environments for induced strategies that were optimized with different frequencies of bacterial input (Φ), across different numbers of possible environments, and at three different bacterial proliferation rates ($k_0 = 0.1, 0.2, 0.5$).

We found that inhabiting multiple environments favored induction over a constitutive defense. PIW increased (and eventually reached its maximum) as the number of environments increased for induced defenses optimized with lower frequencies of bacterial input ($\Phi = 0.0001$, $\Phi = 0.001$, and $\Phi = 0.01$), regardless of the bacterial proliferation rate (Figure 7, first three rows). In addition, the rate of increase of PIW was greater for the lower proliferation rates (k_0 = 0.1 or 0.2) than the higher proliferation rate (k_0 = 0.5). Notably, PIW neared is maximum value when the number of environments exceeded 3-7 for most combinations of proliferation rates (k_0) and Φ values (Figure 7). In contrast, when induction was optimized with a high frequency of bacterial

input ($\Phi = 0.1$), PIW remained low for both low and high bacterial proliferation rates (k_0 = 0.1 or 0.5).

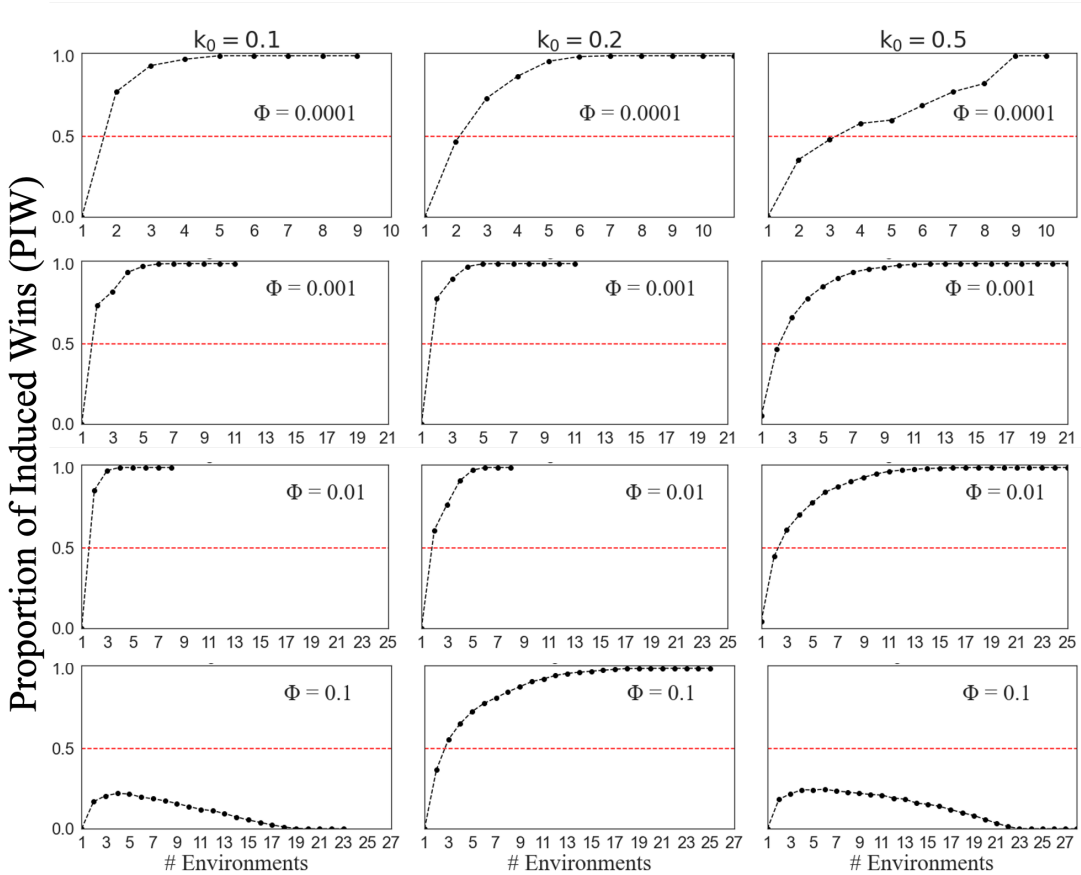

Figure 7. Induction outperforms constitutive defense as the number of different environments a fly inhabits increases. The proportion of times induction outperforms constitutive defense, PIW (Y-axis), is plotted against the number of possible environments that are sampled (without replacement). Graphs in a column have the same bacterial proliferation rate (k_0), and graphs in a row have the same Φ value used to optimize the induced model. Induction outperforms constitutive defense above the dashed line (PIW>0.5), and constitutive defense performs better below the dashed line (PIW<0.5).

Next, we explored how the frequency of time the fly spends in two different environments affects the relative fitness of induced and constitutive defenses. As above, we calculated PIW when we sampled from individual environments where constitutive defense has a higher fitness than induction. We found that PIW was greatest when the two environments occur at equal frequencies ($q = 0.5$), regardless of the frequency of the bacterial input used to optimize the induced response (supplemental figure 11). PIW decreased to 0 as the frequency of time spent in one environment increased.

Population simulations confirm the predictions from differential equations

To evaluate the robustness of the results above, we performed population simulations. We simulated evolution in an asexual, haploid population of flies with two alleles: constitutive and induced. We started each simulation with a population of flies with equal frequencies of induced and constitutive alleles ($m_0 = 0.5$), and we considered induced strategies that were optimized with different frequencies of bacterial input (Φ). Similar to above, each population evolved in two environments with different frequencies of each environment (q and $1-q$).

Our population simulations produced similar results as the analysis calculating PIW. The induced allele was more likely to reach fixation when $q \approx 0.5$, but whether the induced allele reached fixation depended on the bacterial proliferation rate and Φ (supplemental figure 12). For example, when the induced strategy was optimized with an intermediate frequency of bacterial input ($\Phi = 0.01$), equal frequencies of both environments ($q = 0.5$) resulted in fixation of the induced allele regardless of the bacterial proliferation rate (supplemental figure 12). However, when the induced strategy was optimized with a low frequency of bacterial input ($\Phi = 0.0001$), equal frequencies of both environments ($q \approx 0.5$) resulted in fixation of the induced allele only when the bacterial proliferation rate was low (k_0 = 0.1, 0.2). In addition, when induction was optimized with the highest frequency of bacterial input ($\Phi = 0.1$), equal frequencies of two environments only resulted in fixation of the induced allele when there was an intermediate bacterial proliferation rate (k_0 = 0.2), consistent with the analysis of PIW (Figure 5). When there was only one environment ($q = 0$ or $q = 1$), the constitutive allele reached fixation regardless of the bacterial proliferation rate or the optimization method (supplemental figure 11).

Discussion

We modeled constitutive and induced immune defenses of *D. melanogaster* (Figure 1), and we compared their relative fitness in environments with different densities and patchiness of bacteria (Figure 2). Our model predicted that induction is generally favored in environments with low density and heterogeneous distributions of bacteria (Figure 3). This result is robust across a range of bacterial proliferation rates, and under most approaches for optimizing the parameters of the induced response (Figures 4 & 5). We also found that induction is favored when flies experience multiple bacterial environments with different densities and patchiness, even when those individual environments favor a constitutive defense in isolation (Figure 7, supplemental figure 11, and supplemental figure 12). In general, our results support the hypothesis that uncertainty over encountering bacteria favors an induced immune response, whereas certainty favors constitutive defense, consistent with previous theoretical models (Adler and Karban 1994;

Hamilton, Siva-Jothy, and Boots 2008). However, we observed multiple other factors that affect the benefits of an induced response, which were previously uncharacterized.

Uncertainty favors an induced immune response

Uncertainty of bacterial exposure can come from multiple sources, and we find that the interactions of these different sources can affect the extent to which different types of uncertainty favor induction. For example, certainty of encountering bacteria can be due to high bacterial density and a uniform bacterial distribution, which ensures frequent encounters with bacteria. We found that environments with high bacterial densities and uniform distributions do indeed favor a constitutive defense (top right of heat maps in Figure 3). Uncertainty in our model can come from inhabiting multiple environments with different combinations of bacterial densities and patchiness. A key finding of our model is that induction is favored when flies experience multiple environments, even when a constitutive defense is favored in each individual environment (Figures 7). In particular, a high level of uncertainty for choosing between 2 environments, i.e., equal probabilities of each environment, is especially favorable for induction (supplemental figure 11).

We identified one important exception to the rule that certainty favors a constitutive defense. When bacterial density is low, fly-bacteria encounters are rare, which creates high certainty of no exposure. In contrast to certainty of bacterial exposure (which favors constitutive defense), certainty of no exposure favors an induced defense. This can be seen in environments with low bacterial density and heterogeneous distributions, which favor induction because of a low frequency of fly-bacteria encounters (bottom left of heat maps in Figure 3). We interpret this to mean that, when there is high certainty of no exposure, a constitutive defense is costly without much benefit, which favors induction.

Bacterial proliferation rate affects the benefits of induction

We also find that bacterial proliferation rate affects the benefit of an induced response in uncertain environments. Hamilton *et al.* (2008) showed that induction is favored when the bacterial proliferation rate is unknown. Other work showed that a higher pathogen growth rate favors constitutive defense over induction (Shudo and Iwasa 2001; Kamiya et al. 2016). However, previous work ignored structural differences in bacterial distributions (here modeled as pachiness and density) when calculating effects of bacterial proliferation on the relative performance of the induced and constitutive defenses. One of the benefits of our model is that it allows for separate and combined study of the effect of bacterial proliferation rate and uncertainty on the relative performance of induced and constitutive defenses.

Our model shows that the effect of bacterial proliferation rate on the relative fitness of induced and constitutive defenses depends on the bacterial density and distribution. For example, we found that induced defenses outperform a constitutive defense in most types of environments

when the proliferation rate is lower (Figure 4). However, we found that a higher bacterial proliferation rate strongly selects for an induced defense if the bacterial density is low and the distribution is heterogeneous (Figure 5, first panel on the top left). The latter result makes intuitive sense because, in environments with low bacterial densities and heterogeneous distributions, fly-bacteria interactions are rare, and an immune response is not needed at most times. However, if the bacterial proliferation rate is high, upon ingestion of bacteria, a high level of AMP production is needed to eliminate the pathogen. In this situation, low constitutive AMP expression is not effective because it cannot efficiently eliminate the bacteria. High constitutive expression of AMP, on the other hand, can eradicate a bacterial infection, but the cost of constitutive production of AMP outweighs the benefits because AMPs are not needed at all times. An induced defense, however, which produces large amounts of AMPs upon demand, is optimal for rare encounters with bacteria that have a high proliferation rate.

Our model also predicts that bacterial proliferation rate affects the benefits of producing different negative regulators of the Imd pathway. Specifically, we found that production of more negative regulators that reduce the input to the Imd signaling pathway (PGRP-LB and Pirk) increases the relative performance of an induced response against bacteria with low proliferation rates (figure 6). On the other hand, production of more repressosome complexes—which compete with Relish for binding to the promoter of AMP genes—increases the performance of induction against bacteria with a high proliferation rate. Previous theoretical work that did not simultaneously model the multiple negative feedback loops of the Imd signaling pathway and bacterial proliferation rates could not have predicted these results. For example, Ellner et al. (2021), who provided a detailed model of the Imd pathway, did not explicitly model the action of three negative feedback loops (PGRP-LB, Pirk, and the repressosome complex), and therefore could not observe their different effects.

Our strategy for optimizing parameter values against different frequencies of bacterial inputs (Φ) is also informative of the relative fitness of induced and constitutive defenses across bacterial proliferation rates. Our results agree with previous findings which demonstrated that the evolution of induction is more likely when the frequency of host-pathogen interactions is low (Hamilton, Siva-Jothy, and Boots 2008). Therefore, we would expect induction to be more effective if it is optimized in environments where host-pathogen interactions are not frequent. Consistent with this hypothesis, we found a high relative fitness for the induced defense when optimized with a low frequency of bacterial input (i.e., low Φ), across a range of bacterial proliferation rates (Figure 4). As discussed above, we found that uncertainty favors induction across most optimization methods. However, our model predicts that for low and high proliferation rates, if the induced response is optimized with a high frequency of bacterial input, fluctuation in density and/or patchiness of bacteria does not favor induction. Instead, when the induced response is optimized for a high frequency of bacterial input, uncertainty only favors induction for an intermediate proliferation rate of bacteria. This effect of the optimization

parameters on the performance of the induced response suggests that optimization of an induced response under a regime of frequent bacterial exposures creates an induced response that is ineffective in an environment with uncertain bacterial exposure. In general, optimizing induction with a high frequency of bacterial input and a high proliferation rate creates an ineffective induced defense.

Conclusions

Our model predicts that uncertainty favors induction in ways that were not previously recognized, and we identified multiple important caveats to that rule that were also not characterized in prior work. Importantly, we find that induction outperforms constitutive defense when flies inhabit multiple environments, even when constitutive defense has a higher fitness in each of those individual environments. Moreover, by modeling mechanistic complexities of the induced signaling pathway in the fly along with properties of the bacteria that the fly encounters (bacterial density, patchiness of the distribution, and bacterial proliferation rate), we were able to identify how bacterial properties can affect the extent to which uncertainty favors induction. For example, we found that the relative fitness of induction is higher when the fly encounters a low density of bacteria with a high proliferation rate that are distributed heterogeneously. Also, our detailed model predicts that investing in negative regulators that reduce the input to the signaling pathway is beneficial against bacteria with low proliferation rates, while investing in negative regulators that reduce the transcription of AMP genes is beneficial against bacteria with high proliferation rates. Although we modeled the Imd pathway in *D. melanogaster,* our results provide valuable insight as to why some immune genes are induced whereas others are constitutively expressed in other systems. Future work could test if interactions between pathogen proliferation and distribution affect the benefit of induction in uncertain environments for fly-bacteria and other host-pathogen systems.

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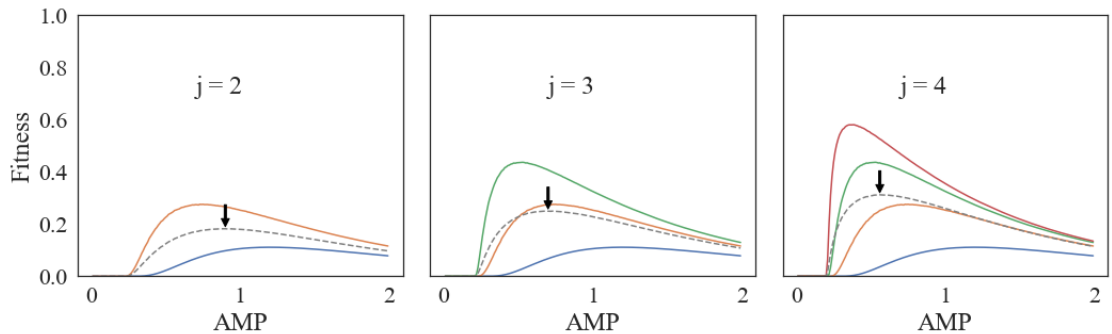
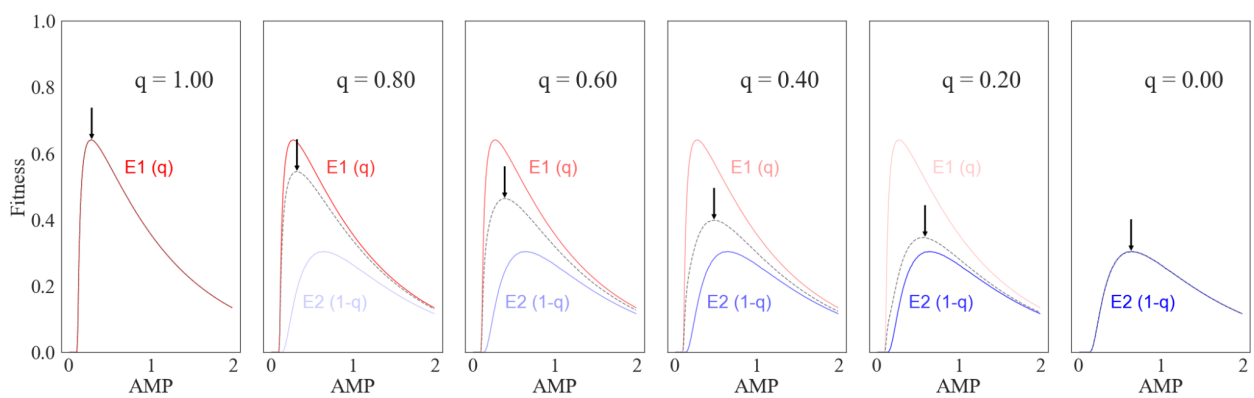
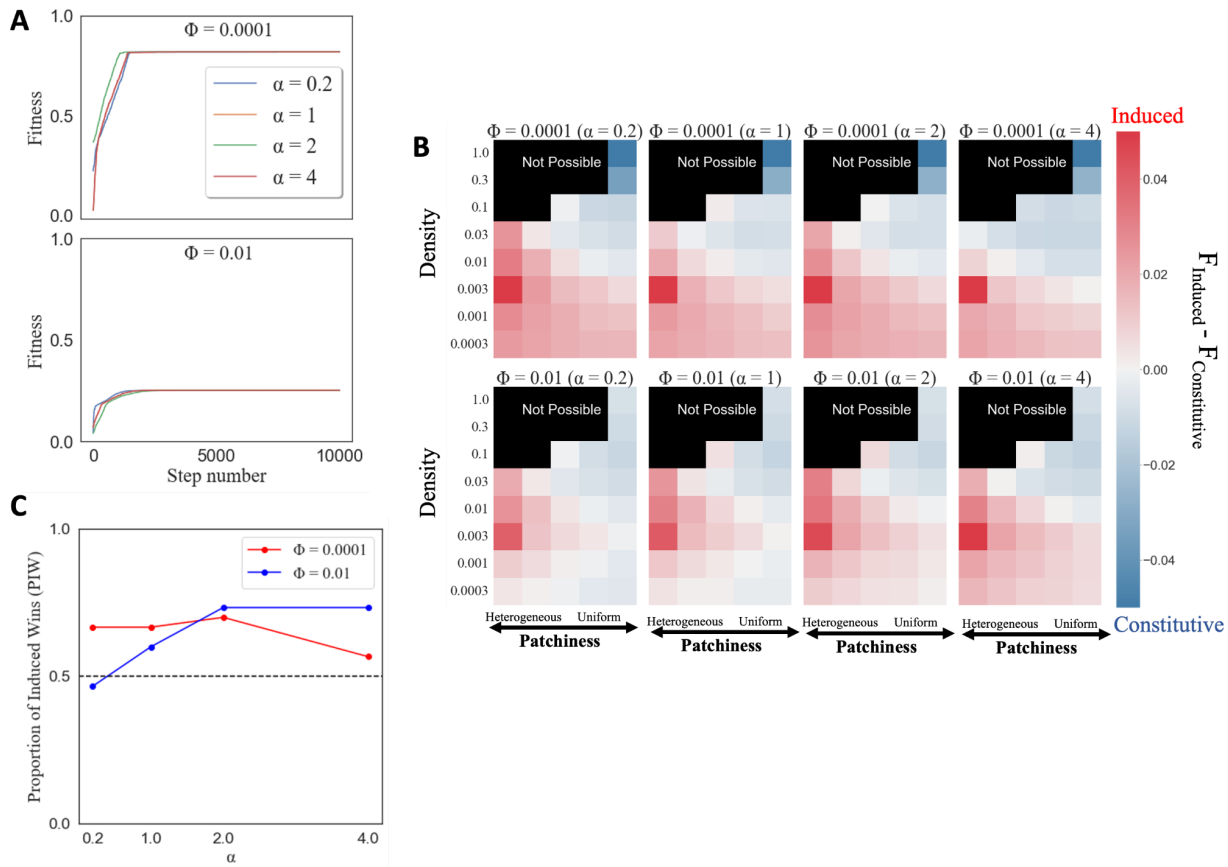


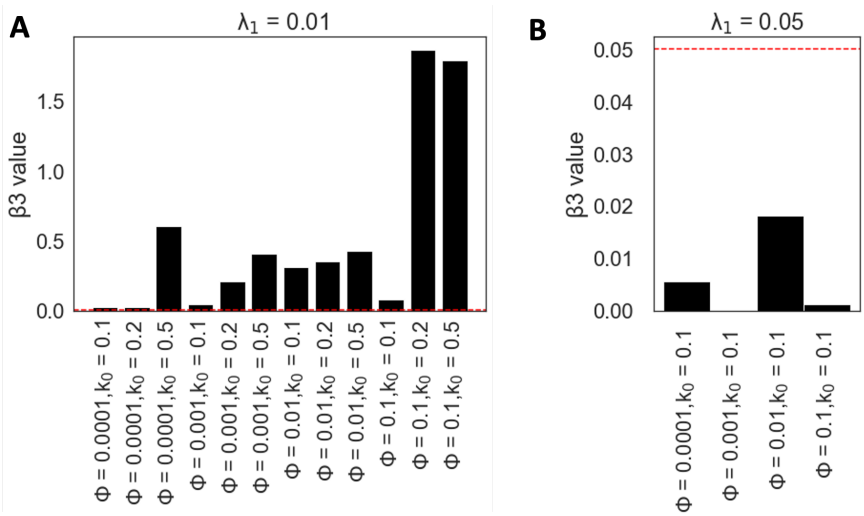
Supplemental figure 1. Finding the best constitutive defense when a fly inhabits j environments, with different density and patchiness of bacteria, with equal probability. Fitness is shown on the Y-axis in environments with different density and/or patchiness of bacteria (different colors) for varying levels of constitutive AMP expression (X-axis). The dashed line represents the fitness if the fly randomly encounter j environments each with a probability $\frac{1}{j}$. The arrow shows the optimum fitness upon random encounter with j environments.



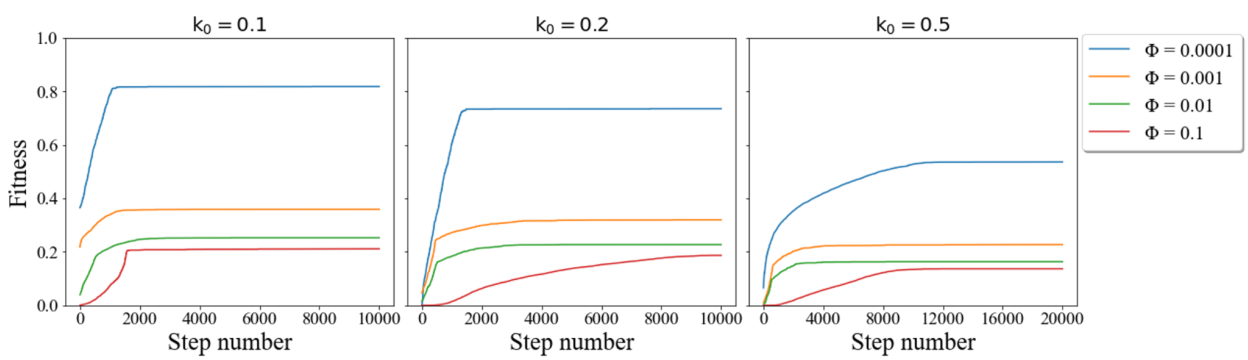
Supplemental figure 2. Optimum fitness of constitutive defense (shown with an arrow) when flies inhabit two environments, E1 and E2, with probability q or $1 - q$, respectively. Red lines represent the fitness in E1 and blue lines represent the fitness in E2. The dashed line is the fitness when the fly inhabits both environments with different probabilities. The optimum fitness is shown with an arrow.



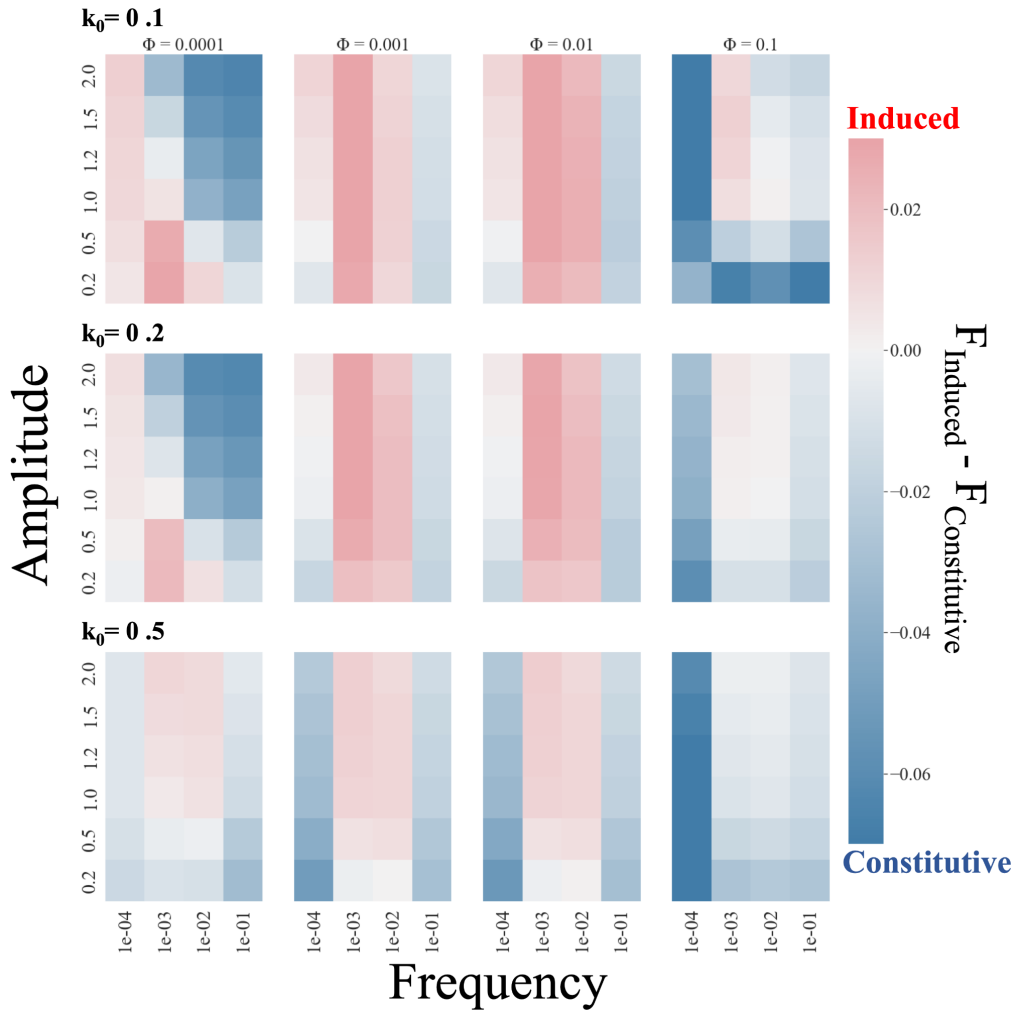
Supplemental figure 3. Results for optimization of induced defense for four values of α (0.2, 1, 2, and 4). **A:** The fitness value (y axis) at every step of optimization (x axis) for optimization that resulted in highest fitness amongst 2048 simulations. Optimizing induced defense for different values of α does not change the optimum fitness value for two frequencies of input to the system ($\Phi = 0.0001$ and $\Phi = 0.01$). **B:** The relative fitness of induction vs constitutive defense (color bar) in environments with different patchiness (x axis) and bacterial density (y axis). Induction has a higher fitness in red colored cells than the best constitutive defense. Induction is prefered in heterogeneous environments with low bacterial density regardless of the value of α. **C :** Comparing the proportion of induced wins (y axis) across different values of α (x axis) for two values of Φ. The proportion of induced wins is highest for $\alpha = 2$ regardless of the value of Φ.



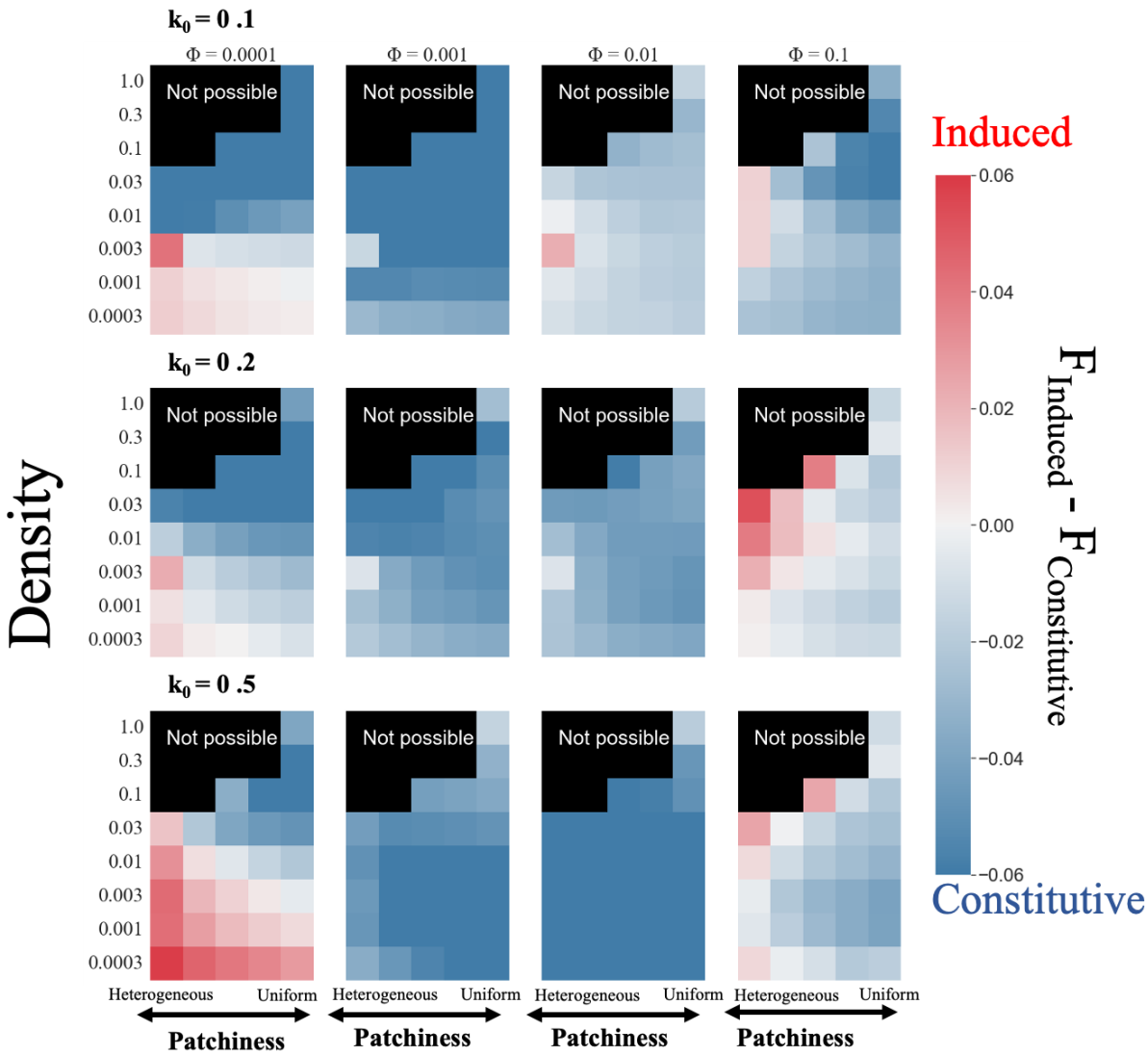
Supplemental figure 4. Optimization of the induced defense with two values of λ_1. The x axis are the parameters affecting the production of PGRP-LB (β_3), and the y axis is the value of β_3 in the optimized model. **A:** Optimization of the induced defense with $\lambda_1 = 0.01$ using different values of Φ and k_0. The red dashed line is the value of λ_1 (0.01). **B:** Optimization of the induced defense with $\lambda_1 = 0.05$ using different values of Φ. The red dashed line is the value of λ_1 (0.05).



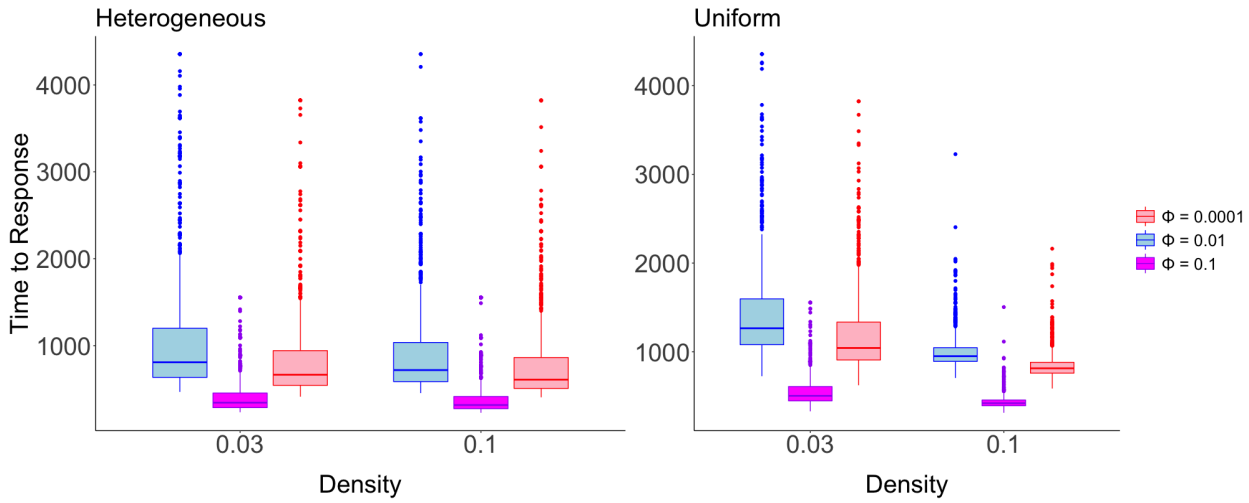
Supplemental figure 5. Optimization of the induced response using an oscillating deterministic input (sinusoidal) with different frequencies (Φ) for three different proliferation rates of bacteria inside the fly (k_0) and $\alpha = 2$. Optimization is performed by taking 10,000 steps in the fitness landscape. The X axis is the number of steps taken in the fitness landscape.



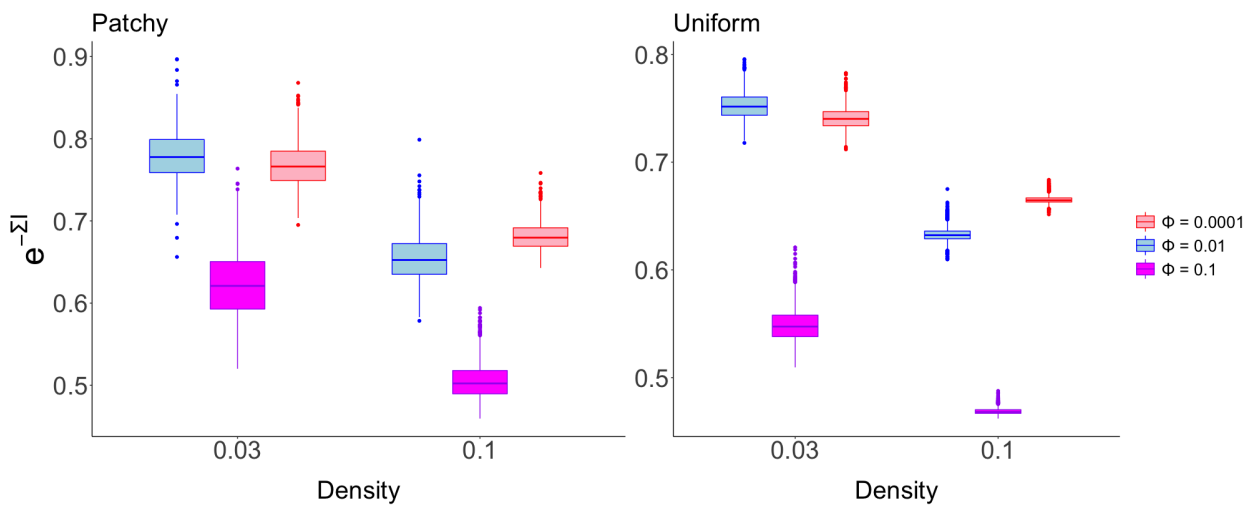
Supplemental figure 6. Induced responses tend to outperform constitutive defenses when flies encounter bacteria with intermediate frequencies. The relative fitness of induced versus constitutive strategies are shown as heatmaps for environments with different amplitudes and frequencies of sinusoidally oscillating encounters with bacteria. Red cells indicate that the fitness of the induced response ($F_{Induced}$) is higher than fitness of the constitutive response ($F_{Constitutive}$), and blue cells indicate that $F_{Constitutive} > F_{Induced}$. Heatmaps in the same column show induced responses that were optimized with the same frequency of the sinusoidal input of bacteria (Φ), and heatmaps in each row show the results for the same proliferation rate of bacteria (k_0).



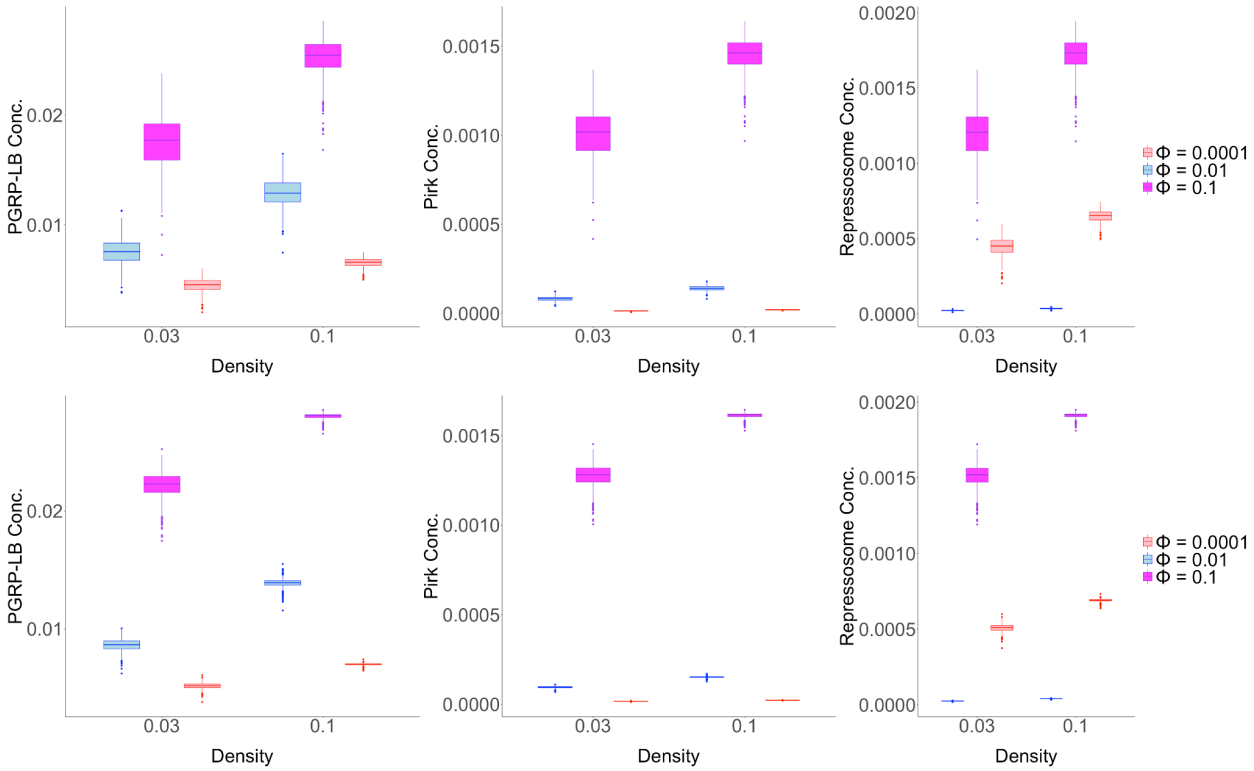
Supplemental figure 7. Comparing the performance of the Induced model with different degradation rates for immune proteins against constitutive defenses. optimized tend to outperform constitutive defenses when flies encounter bacteria with intermediate frequencies. The relative fitness of induced versus constitutive strategies are shown as heatmaps for environments with different densities and patchiness of bacteria. Red cells indicate that the fitness of the induced response ($F_{Induced}$) is higher than fitness of the constitutive response ($F_{Constitutive}$), and blue cells indicate that $F_{Constitutive} > F_{Induced}$. Heatmaps in the same column show induced responses that were optimized with the same frequency of the sinusoidal input of bacteria (Φ), and heatmaps in each row show the results for the same proliferation rate of bacteria (k_0).



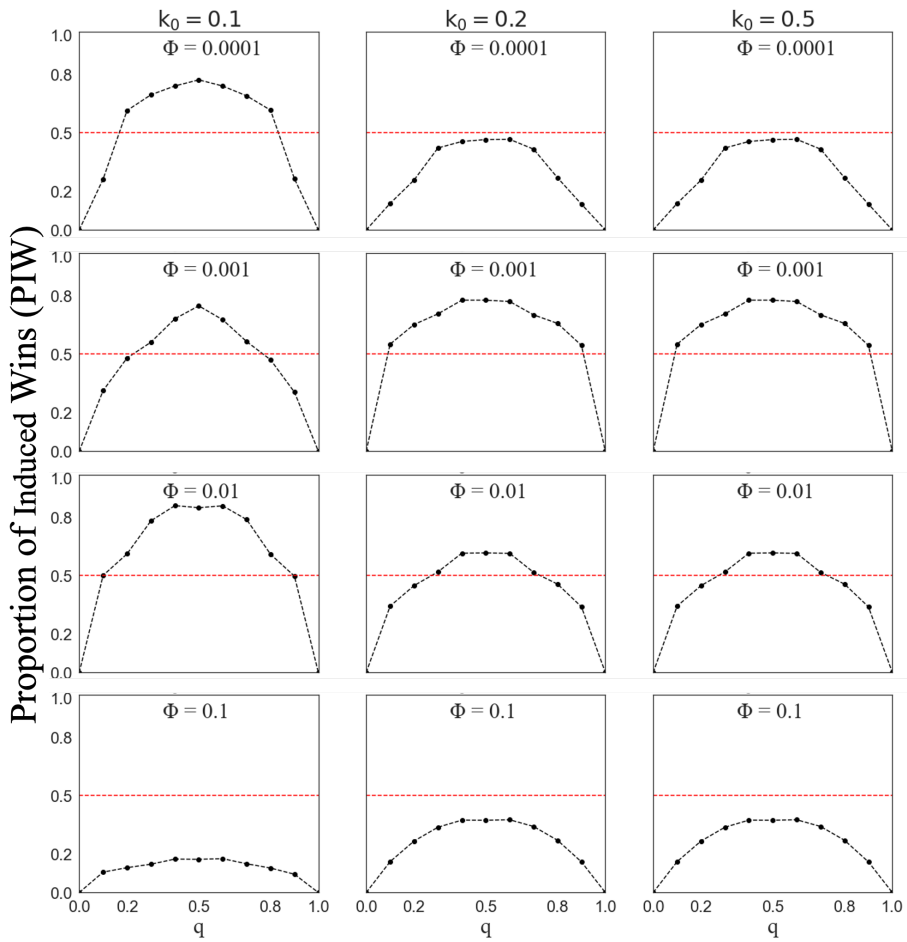
Supplemental figure 8. The time to response for different optimizations of the induced defense (different values of) across 1,000 simulations for two bacterial densities (0.03 and 0.1) and patchiness (heterogenous ($p = 1$) and uniform ($p = 3$)).



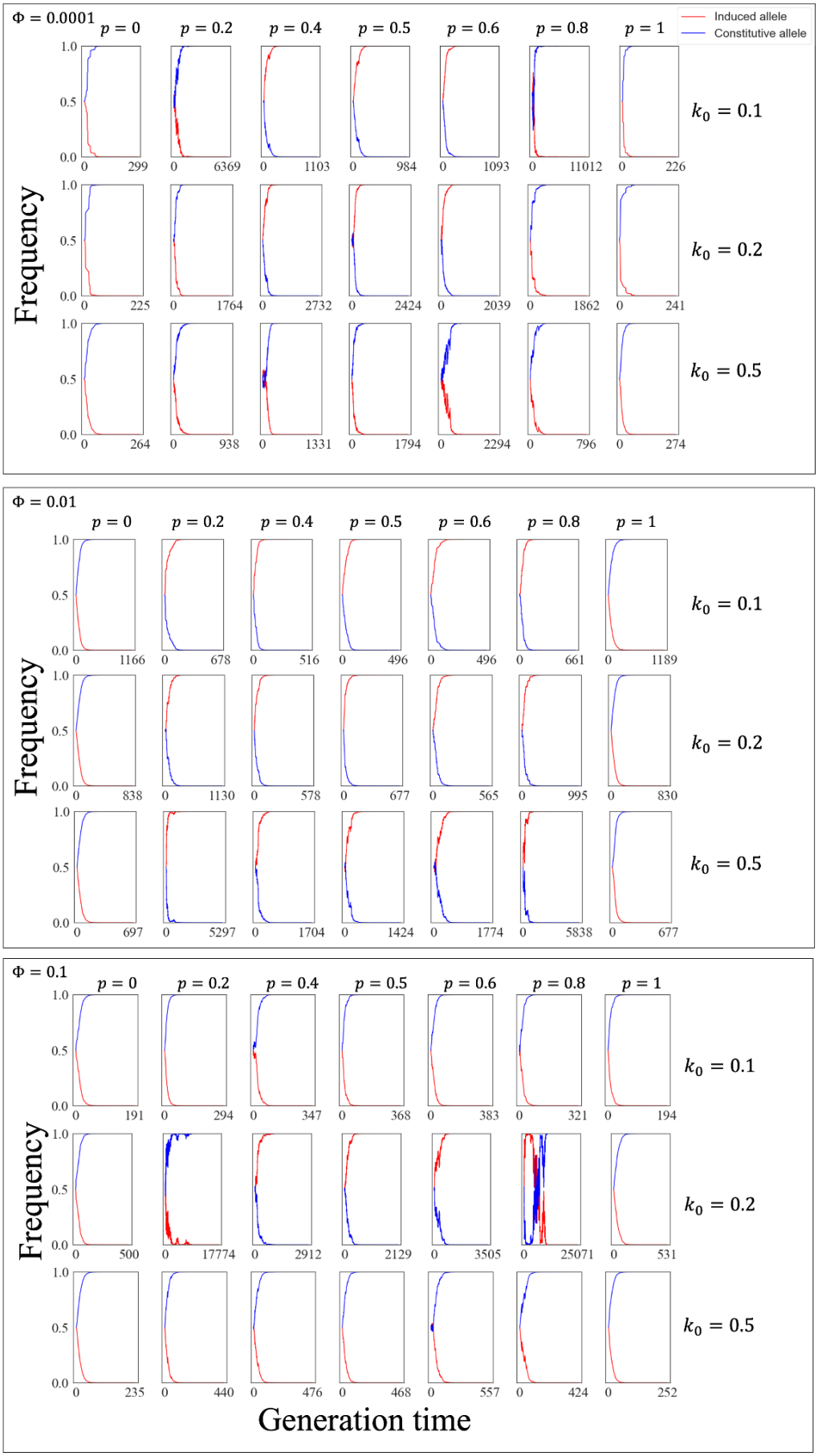
Supplemental figure 9. The energetic cost of production of proteins involved in the immune response. ∑I is the sum of the concentration of all proteins. The results are shown for different optimizations of the induced defense (different values of) across 1,000 simulations for two bacterial densities (d = 0.03 and 0.1) and patchiness (heterogenous [$p = 1$] and uniform [$p = 3$]).



Supplemental figure 10. Concentration of proteins involved in negative regulation of the Imd pathway for different optimizations of the induced defense (different values of Φ) across 1,000 simulations for two bacterial densities (0.03 and 0.1). The top row shows the results for heterogeneous ($p = 1$) distribution of bacteria and the bottom row for uniform ($p = 3$) distribution of bacteria.



Supplemental figure 11. Equal frequencies in two environments favors an induced response. The proportion of times induction outperforms constitutive defense, PIW (Y-axis), is plotted against the probability of inhabiting one of the environments (X-axis). Graphs in a column have the same bacterial proliferation rate (k_0), and graphs in a row have the same Φ value used to optimize the induced response. Induction outperforms constitutive defense above the dashed line (PIW>0.5), and constitutive defense performs better below the dashed line (PIW<0.5).


Φ = 0.0001
Φ = 0.01
Φ = 0.1
p = 0
p = 0.2
p = 0.4
p = 0.5
p = 0.6
p = 0.8
p = 1
Induced allele
Constitutive allele
k0 = 0.1
k0 = 0.2
k0 = 0.5
Frequency
Generation time

Supplemental figure 12. Simulation of a population of flies with either induced (red) or constitutive (blue) allele. Flies randomly encounter 2 environments with probability p or $1 - p$. For induced responses that are optimized with relatively low frequencies of bacterial input ($\Phi = 0.0001$ and $\Phi = 0.01$), higher uncertainty (p close to 0.5) results in fixation of the induced allele regardless of the proliferation rate of bacteria. For the induced defense optimized with high frequency of bacterial input ($\Phi = 0.1$), the induced allele is only fixed when uncertainty is high and proliferation rate is intermediate ($k_0 = 0.2$).

A

Not possible				23
				24
		11	17	25
1	6	12	18	26
2	7	13	19	27
3	8	14	20	28
4	9	15	21	29
5	10	16	22	30

B

Cell #	*p*	*d*
1	1	0.03
2	1	0.01
3	1	0.003
4	1	0.001
5	1	0.0003
6	2	0.03
7	2	0.01
8	2	0.003
9	2	0.001
10	2	0.0003
11	1	0.1
12	3	0.03
13	3	0.01
14	3	0.003
15	3	0.001
16	3	0.0003
17	2	0.1
18	4	0.03
19	4	0.01
20	4	0.003
21	4	0.001
22	4	0.0003
23	1	1.0
24	1	0.3
25	3	0.1
26	5	0.03
27	5	0.01
28	5	0.003
29	5	0.001
30	5	0.0003

Supplemental table 1. The numerical value of density (d) and patchiness (p) used to simulate bacterial population. Table B contains d and p values for cells with different cell numbers (cell #), based on table A.