Title: Evolution of constitutive bacteriocin production and release

Abstract

The production of the bacterial anti-competitor toxins, called bacteriocins, is widely described as spite, because the production of the toxins is costly and often requires cell death for release. Given these costs, it reasonable to think that bacteriocin production should be induced by the presence of unrelated competitor strains. Nonetheless, recent evidence in the insect-pathogenic bacterium *Xenorhabdus koppenhoeffri* showed that bacteriocin production occurs regardless of the presence of unrelated competitors. Could the constitutive production of bacteriocins be favored by natural selection? Here we use a mathematical model to examine this question, both within and between subpopulations. The model assumes that bacteriocin production is constitutive and costly, and that toxin release only occurs during natural cell death. We found that constitutive bacteriocin producers can outcompete non-producer sensitive strains within populations, and that it can spread in a metapopulation provided there is local competition for patches. Hence, the evolution of bacteriocin production does not require the detection of competitors or "suicidal" release of toxins.

Introduction

The evolution and maintenance of spiteful behaviors that reduce the fitness of both the actor and the recipient pose a long-standing evolutionary puzzle (Hamilton, 1964, 1970). How can a trait that reduces the fitness of the actor be favored by natural selection? Yet, one of the clearest examples of spite in nature, bacteriocin production, is a trait that is ubiquitously expressed by almost all known lineages within the bacterial kingdom (Klaenhammer, 1988; Riley & Chavan, 2006). Bacteriocins are proteinaceous toxins with diverse mechanisms of action, that can kill closely related local competitors without killing clone-mates (Reeves, 1965; Riley & Chavan, 2006). Bacteriocin production is considered spiteful because these toxins both kill their targets and impose fitness costs of production, thus reducing the fitness of both recipients and actors (Gardner et al., 2004; West et al., 2007). In addition to the costs of DNA replication, maintenance, and protein synthesis, the release of these large, proteinaceous toxins often requires cell lysis, particularly in gram-negative cells that lack a cell wall (Riley & Wertz, 2002; Riley & Chavan, 2006; Wloch-Salamon et al., 2008) .

Theory predicts that the competitive environment is key for the maintenance of costly bacteriocin production (Chao & Levin, 1981; Gardner et al., 2004). Bacteriocin-production is predicted to be most favored when competitors occur at comparable frequencies to the producing lineage (Gardner et al., 2004; Inglis et al., 2009). Further, a recent meta-analysis revealed that bacteriocin production is more likely to be associated with competition-related stressors such as starvation or cellular damage, than abiotic stressors such as heat or osmotic stress(Cornforth & Foster, 2013). This led to the proposition of the ‘competition sensing hypothesis,’ which posits that bacterial populations have evolved the ability to sense and respond to competition in their

environment by upregulating bacteriocin production (Cornforth & Foster, 2013). However, the gram negative bacteria, *Xenorhabdus koppenhoeffri*, was found in vitro to produce and release the same levels of bacteriocins in both the presence and absence of non-self competitors (Bhattacharya et al., 2018). Why do *X koppenhoeffri* produce and release bacteriocins in the absence of competitors?

Here we address this question by building a mathematical model to ask whether the constitutive production of bacteriocins can be selected in natural bacterial populations. The model examines competition between bacteriocin-producer and bacteriocin-sensitive cells when bacteriocin production is both constitutive and costly. We assume that toxins are only released upon natural cell death. Our results demonstrate that constitutive bacteriocin-producers can outcompete faster growing sensitive cells within patches, and that they increase in frequency in structured populations, if there is some degree of local competition.

Model

We develop a model that tracks competition between two pathogenic bacterial strains: a bacteriocin producing strain and a sensitive strain that is killed by the producer's bacteriocins. The strains compete for a finite amount of a non-renewable resource within a shared host. We assume that the production of bacteriocins is constitutive and costly. In other words, we assume that bacteriocin production is not a plastic response to the presence of competitors and that it imposes a fitness cost by reducing the birth rate of producers. Finally, we assumed that the release of bacteriocins only occurs during natural cell death, independent of whether competing

cells are present. Importantly, the producer death rate is fixed and independent of competitor densities enabling the test of inducibility being necessary for the maintenance of costly bacteriocin production. First, we ask whether constitutive bacteriocin production could be favored by selection within hosts. We then examine whether constitutive bacteriocin production could increase when rare in a metapopulation in which there is between-host competition.

Within-Host Competition

Let B_t be the "birth" number per sensitive cell at time t. We assumed B_t was both density dependent and resource dependent, as follows:

$$B_t = b\left[1 - \frac{N_t}{N_t + R_t}\right] \quad (1)$$

where b is the intrinsic number of new cells produced per bacterial cell per time step, N_t is the total number cells (producer + sensitive) in the population at time t, and R_t is the number of resource units remaining in the population at time t. The variable b was set to 1 in our simulations. We also assumed that each cell division requires one unit of the resources available at time t. Similarly, the death rate, D_t, was also density-dependent and calculated as:

$$D_t = d\left[\frac{N_t}{N_t + R_t}\right] \quad (2)$$

where d is the intrinsic death rate. The number of producer cells at time step, t+1, is then given by

$$P_{t+1} = P_t[1 + B_t(1 - c) - D_t] \quad (3)$$

where P_t is the number of producer cells at time step t, and c is the birth-rate cost of bacteriocin production.

Toxin molecules were released only upon the natural death of producer cells. The total number of toxin molecules in the system at time t+1 was

$$X_{t+1} = (1 - z)X_t + xD_tP_t + S_t[1 - \exp\left(-\frac{\beta X_t}{S_t}\right)] \quad (4)$$

Where, X_t is the number of toxin molecules in the system at time t; z is the loss probability of toxin molecules; x is the number of toxin molecules released by each dying producer cell; and β is the contact probability between toxin molecules and sensitive cells, where S_t is the number of sensitive cells at time t. The first term on the right-hand side (RHS) of eq. (4) thus gives number of toxin molecules carried over from the previous time step. In the present study we set z equal to 0.05 to give a 5% decay rate. The second term on the RHS gives the number of new toxin molecules released into the system resulting from producer deaths. The third term on the RHS of (4) gives the number of sensitive cells that were killed by contact with toxin, where X_t/S_t gives the mean number of toxin molecules per sensitive cell. Note that the exponential term $[\exp(-\frac{\beta X_t}{S_t})]$ gives the probability of the zero class in a Poisson distribution; hence $[1 - \exp\left(-\frac{\beta X_t}{S_t}\right)]$ gives the probability that a sensitive cell would contact at least one toxin molecule, thus killing the sensitive cell. Thus, the third term on the RHS gives the number of toxin molecules removed from the system, assuming each toxin-induced death removes a single toxin molecule from the system.

The number of sensitive cells at time step t+1 was calculated as:

$$S_{t+1} = S_t[1 + B_t - D_t - (1 - \exp(-\beta X_t/S_t)] \quad (5)$$

where S_t is the number of sensitive cells at time t. After every time step, the number of resource units remaining in the system were calculated as:

$$R_{t+1} = R_t - P_tB_t(1 - c) - S_tB_t \quad (6)$$

where R_t is the number of resource units at time t. The model thus assumes that the birth of every cell requires one resource unit.

The recursion equations above were iterated using R (R Core Team. 2019). The code is given in Supplementary Files 1 and 2. The simulation was initiated with 10 producer cells and 90 sensitive cells in a single host containing 10^8 resource units. We ran the simulation for 100 time steps, which was more than enough for the resources to be depleted to zero. The intrinsic birth rate was set to 1 (b = 1), and the intrinsic death rate was set to 0.01 (d = 0.01). The cost of toxin production was set to 0.05 (c = 0.05). We ran the model for a range of parameters for β and x to explore the effects of the contact probability between toxin and sensitive cells, and the number of bacteriocin particles released following the death of producer cells.

Between-Host Competition

For bacteriocin production to be favored by natural selection, producers not only have to "win" within hosts, but they also require an advantage under some level of between-host competition. Such an advantage likely depends on the degree of soft selection and migration. Here we used Frank's "scale-of-competition" method to examine bacteriocin evolution in a metapopulation (Frank, 19**). The method uses the variable 'a' to represent the probability of competition with individuals derived from the same patch. The variable can also be seen as a combination of soft selection (s) and migration (m)(van Dyken, 2010), where

$$a = s + (1 - s)(1 - m)^2 \tag{7}$$

The model assumes a metapopulation comprising of a large number of hosts where each host is colonized by a fixed initial number of bacteria (N = 100). One host in the metapopulation is colonized by a mixture of 10 producer cells and 90 sensitive cells. This is called the "local" host. All remaining hosts are colonized by 100 sensitive cells.

The relative fitness of bacteriocin producers (w_{prod}) was calculated as (Frank, 1998):

134 $$w_{\text{prod}} = \frac{W_{\text{prod}}}{a\,\bar{W}_{\text{local}} + (1-a)\bar{W}_{\text{global}}} \quad (7)$$

135 where, W_{prod} is the number of producer cells at time t (P_t) divided by the number of colonizing
136 producer cells ($W_{\text{prod}} = P_t/P_1$) where P_1 =10; $\bar{W}_{\text{local}}$ is the number of cells per colonizing cell
137 in the local host (i.e., (P_t + S_t)/100) containing the producer cells; and $\bar{W}_{\text{global}}$ is the number of
138 cells per colonizing cell in the non-focal hosts at dispersal, i.e., S_{tot}/100, where S_{tot} is the total
139 number of sensitive bacteria in non-local hosts at dispersal.
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142 Results
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144 Within-Host Competition
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146 The model tracks the densities of bacteriocin-producer cells, bacteriocin-sensitive cells, and
147 resource units within the host under varying contact probability between toxin molecules and
148 sensitive cells, β, and varying levels of the number of bacteriocin particles released per dying
149 producer cell, X (Figure 1). Each simulation begins with 10 producer cells and 90 sensitive cells
150 within a shared host. When X is set to 0 (Fig 1 a-c) the densities of bacteriocin producers never
151 exceed the densities of bacteriocin sensitive competitors, demonstrating that producers are
152 unable to outcompete sensitive cells. However, bacteriocin-producing cells outcompete
153 bacteriocin-sensitive competitors when X is non-zero (Fig 1 e-i). Producers outcompete sensitive
154 cells faster at higher levels of contact probability, β, and with higher numbers of bacteriocin
155 particles released per dying producer cell, X (Fig 1).

156

157 Between-Host Competition

158 Relative fitness of bacteriocin producers is examined across a metapopulation of hosts under
159 initial conditions of $\beta = 0.1$, varying levels of X, and three difference scales of competition (Fig
160 2). Relative fitness measures greater than 1 indicate invasion of the metapopulation by
161 producers despite being rare initially. Our results demonstrate that the relative fitness of
162 bacteriocin producers can exceed 1 under many conditions (Fig 2 d-i) despite conservative
163 assumptions about bacteriocin potency ($\beta = 0.1$; Fig 2). Invasion occurs faster and is favored
164 more strongly at more local scales of competition i.e., higher values of 'a', and with higher
165 numbers of bacteriocin particles released per dying producer cell, X.

166

167 **Discussion**

168

169 Bacteriocin production is an example of spiteful behavior, meaning that both the actor and the
170 recipient pay fitness costs (Riley & Chavan, 2006). Bacteriocin production is frequently
171 considered a 'suicide mission' where producer cells sacrifice themselves to deploy these anti-
172 competitor toxins (West et al., 2007; Nedelcu et al., 2010). Inducible mechanisms that allow
173 bacteriocin production to be increased by the competitive environment are predicted to be
174 favored by selection (Gardner et al., 2004; Cornforth & Foster, 2013). Yet, contrary to
175 expectations, bacteriocin production in *X koppenhoeffri* was found to occur even in the absence
176 of non-self competitor cells (Bhattacharya et al., 2018). Our goal here was to understand why.
177 We built a mathematical model to examine if and how, despite considerable costs of production
178 and release, bacteriocin-producing lineages like *X. koppenhoeffri* persist.

179 Our results demonstrate that a bacteriocin-producing lineage can outcompete a faster
180 growing sensitive lineage, if bacteriocins are released only upon natural cell death (Fig 1 and 2).
181 The time taken for the producing lineage to outcompete the sensitive lineage decreases with
182 increasing transmission efficiency, β, and higher numbers of bacteriocin particles released per
183 dying producer cell, x (Fig 1). These results challenge the assumption that producer cells must
184 incur additional cell deaths by 'suicide' to be effective competitors, and they suggest that natural
185 cell death may suffice to confer a competitive advantage to bacteriocin production.
186 Further, the results show that bacteriocin producers when rare can invade a
187 metapopulation of sensitive cells, provided some degree of local competition (Figure 2).
188 Consistent with previous work on the scale of competition in general (Durrett & Levin, 1997;
189 Gardner & West, 2004; Gardner et al., 2004), our results show that increasing local competition
190 favors the invasion of bacteriocin production (Figure 2b). These results demonstrate that, despite
191 cell deaths being necessary for bacteriocin release, inducible or higher-than-natural rates of cell
192 death for 'suicidal' bacteriocin release are not required for bacteriocin-producing lineages to
193 persist and increase in frequency when rare.
194 The assumptions about costs of bacteriocin production and bacteriocin potency (number
195 of particles released per dying cell) in our models are probably conservative. The cost of
196 bacteriocin production is set at 5% reduction in fitness ($c = 0.05$), which imposes a considerable
197 growth disadvantage on the producer strain relative to the sensitive competitor. It has been
198 previously argued that the fitness costs of bacteriocin production may be much less steep than
199 commonly assumed (Dykes & Hastings, 1997). Empirical estimates in *E coli* that produce the
200 most well-studied group of bacteriocins called colicins, suggest that every lysing producer cell is
201 capable of releasing bacteriocin particles on the order of thousands of particles per cell (Gordon

202 & Riley, 1999). Our model examines a wide range of this parameter ranging from 0-1000
203 particles of bacteriocin per dying producer cell, remaining highly conservative. Thus, our results
204 demonstrate that the competitive benefits of bacteriocin production may be realized without
205 invoking inducible mechanisms or 'suicide' despite high costs and low potencies of toxins
206 produced.

207 It is important to note that these results do not preclude the possibility of plastic
208 bacteriocin production or release. Indeed, recent work in *E coli* (Mavridou et al., 2018; Granato
209 & Foster, 2020) provide evidence for increased toxin production in response to attack from a
210 neighboring competitor. However, bacteriocin production in *E coli* has also been reported to
211 occur in the absence of competitors, typically at late growth stages (Eraso et al., 1996).
212 Additionally, recent work using models to compare various mechanisms of bacteriocin
213 production and release found that toxin sensitivity to be the preferred mode of bacteriocin
214 production and release over unregulated mechanisms (Niehus et al., 2021) . Our goal here is not
215 to claim that bacteriocin production cannot be inducible, rather to demonstrate that non-inducible
216 mechanisms of bacteriocin release can maintain bacteriocin production. We aim to caution
217 against the generalization of bacteriocin production as a 'suicide' mission and to encourage
218 additional direct investigation of the inducibility of this spiteful, yet ubiquitous bacterial trait.
219

220 To summarise, the model described in this study demonstrates, using conservative
221 assumptions, that inducible mechanisms of bacteriocin expression aren't necessary to explain the
222 maintenance of bacteriocin production in nature. Further, these results challenge the common
223 assumption that bacteriocin production and release is a 'suicide mission.' Our models provide an
224 alternative mechanism to explain the maintenance of spiteful bacteriocin production through

canalized, constitutive expression instead of inducible production. We do not claim that canalized spite is the only, or even the most common, mechanism of bacteriocin production. However, we do offer a previously underexamined mechanism to explain the early evolution of bacteriocin production, and how spiteful bacteriocin production may be maintained in some species.

Conflict of Interest: The authors declare no competing interests.

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Figure 1

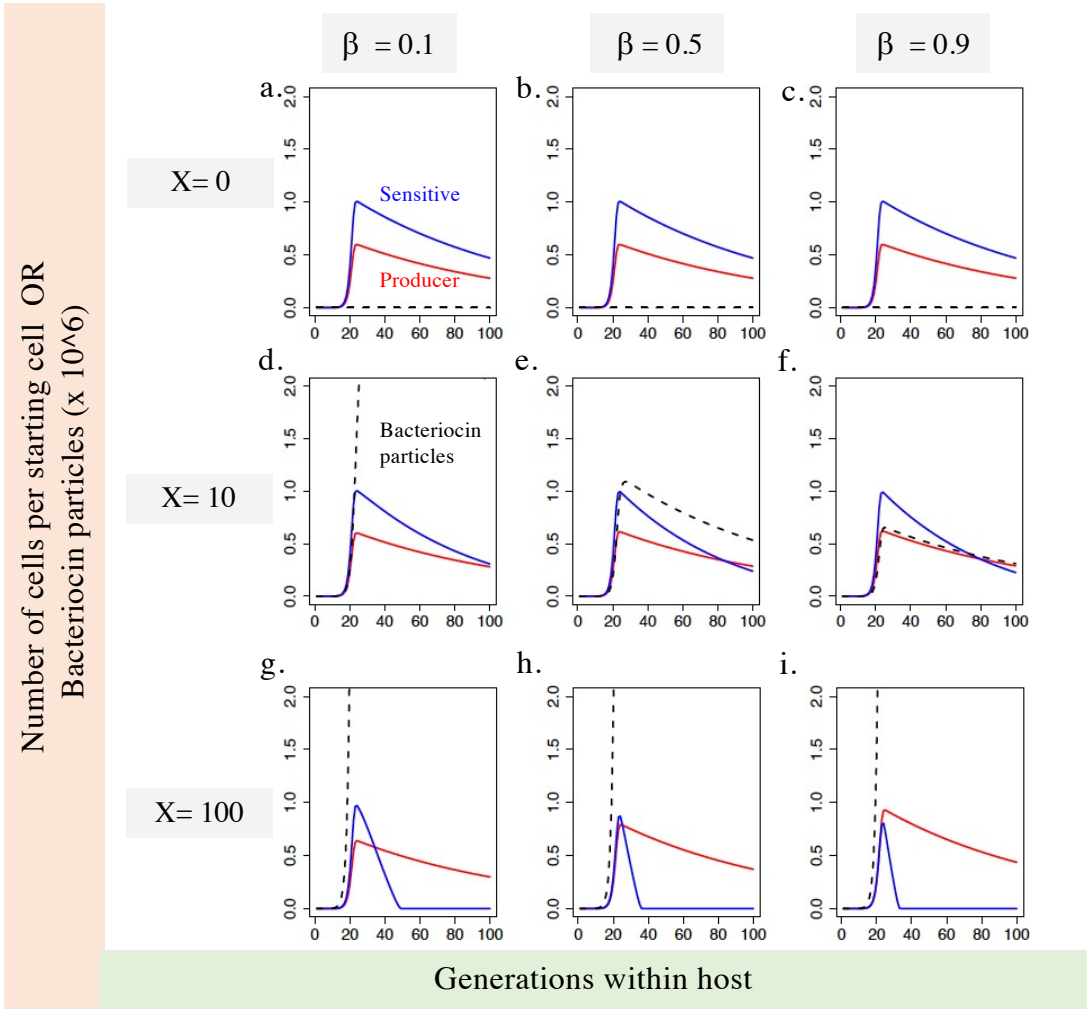


Figure 1: Within-host competitive dynamics of bacteriocin producing cells (red) and bacteriocin sensitive competitors (blue). Red and blue lines represent the number of producer or sensitive bacterial cells, respectively, relative to the initial number of cells of each type. Variable X represents the number of bacteriocin particles released by every dying producer cell. Variable β represents the contact probability between bacteriocin particles and sensitive cells. Black dashed lines represent the total number of unused bacteriocin particles in the environment. Panels a-c represent competitive dynamics when no bacteriocin is not effective at killing sensitive cells. Here sensitive cells outcompete producer cells every time and the slower

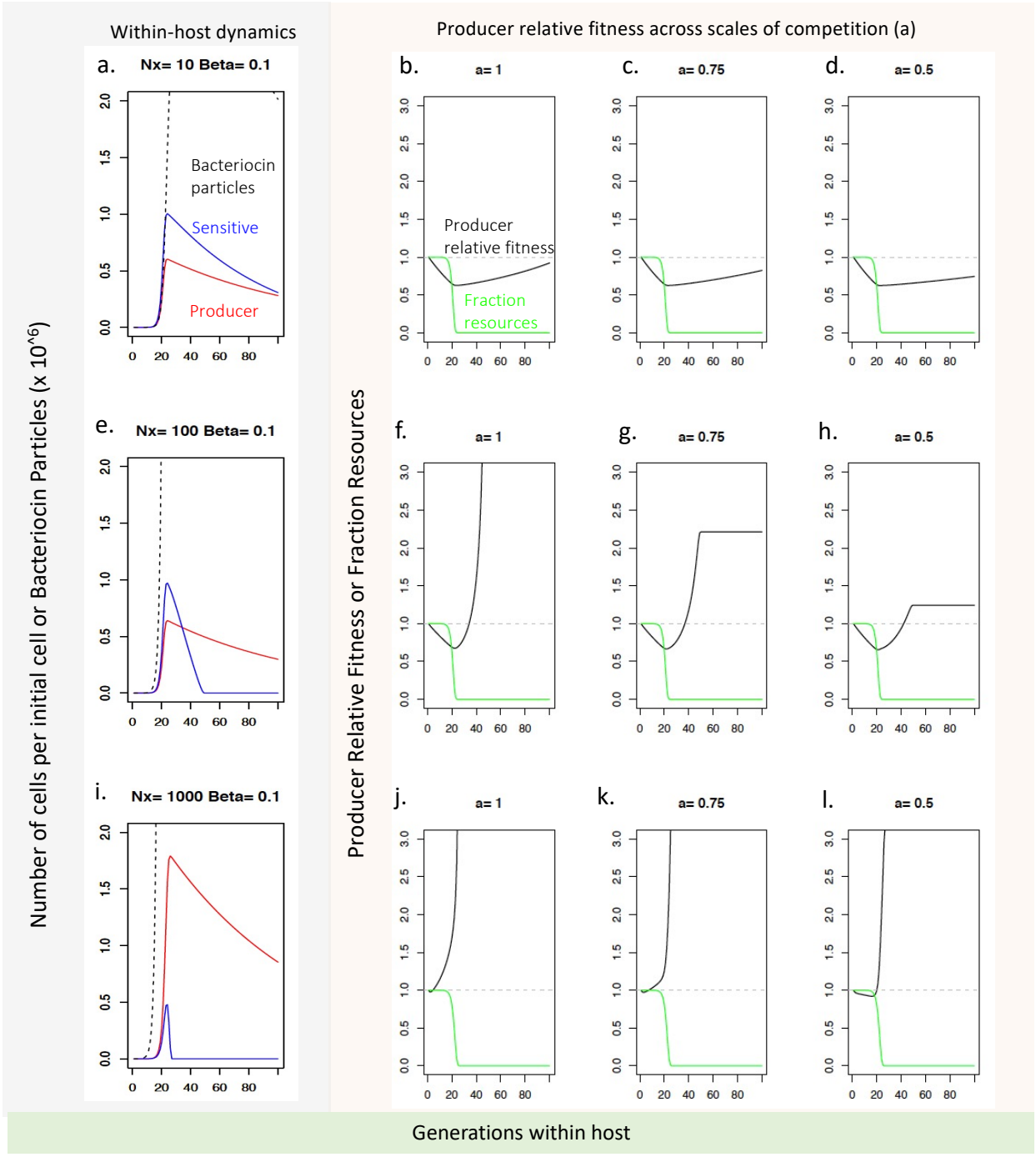
291 producer growth represents the growth cost of bacteriocin production incurred by producer cells.

292 Producer cells can outcompete sensitive cells when $X > 0$ and do so faster with increasing

293 contact probability β between bacteriocins and sensitive cells (panels e – i) .

294

295 Figure 2



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297 Figure 2: Within-host dynamics and relative fitness of bacteriocin producer cells across the

298 metapopulation of hosts. Fig 2 a, e and i represent the within-host growth of bacteriocin

299 producers and sensitive competitors for varying number of bacteriocin particles released per

dying producer cell (X). Panels 2 b-d, f-h, j-l show the relative fitness of producer cells across the metapopulation of hosts for varying values of scale of competition, ‘a’ (Frank, 1994). Scale of competition, a can range from completely local (a = 1) to completely global (a =0).