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# Modeling host-associating microbes under selection

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The concept of fitness is often reduced to a single component, such as the replication rate in a given habitat. For species with complex life cycles, this can be an unjustified oversimplification, as every step of the life cycle can contribute to reproductive success in a specific way. In particular, this applies to microbes that spend part of their life cycles associated to a host, *i.e.* in a microbiota. In this case, there is a selection pressure not only on the replication rates, but also on the phenotypic traits associated to migrating from the external environment to the host and vice-versa. Here, we investigate a simple model of a microbial population living, replicating, migrating and competing in and between two compartments: a host and its environment. We perform a sensitivity analysis on the global growth rate to determine the selection gradient experienced by the microbial population. We focus on the direction of selection at each point of the phenotypic space, defining an optimal way for the microbial population to increase its fitness. We show that microbes can adapt to the two-compartment life cycle through either changes in replication or migration rates, depending on the initial values of the traits, the initial distribution of the population across the compartments, the intensity of competition, and the time scales involved in the life cycle versus the time scale of adaptation (which determines the adequate probing time to measure fitness). Overall, our model provides a conceptual framework to study the selection on microbes experiencing a host-associated life cycle.

**Keywords:** microbial life cycle; host association; sensitivity analysis; selection gradient;

**Competing interests:** the authors declare no competing interest.

## 23 1 Introduction

24 Fitness is a central concept in evolutionary biology, of particular importance for the theory of natural selection.  
25 Fitness measures how well a phenotype performs in terms of reproductive success, *i.e.* in terms of its ability to  
26 survive and reproduce. Natural selection, acting through reproduction and inheritance of the phenotypic traits,  
27 then leads to an increase in the population of the genotypes producing high fitness phenotypes [1].

28 In any system, fitness emerges mechanistically from birth and death events [2]. However, when it comes to  
29 the study of particular experimental systems or models, the question of how to measure fitness is often delicate,  
30 and fitness is often defined from the outset, as a phenomenological parameter. For example, fitness may be  
31 quantified as a net replication rate measured over a limited period of time in fixed laboratory conditions, or as a  
32 proportion of habitats successfully colonized. But none of these fitness components alone provides a holistic view  
33 of what fitness encompasses in natural conditions. Indeed, in nature, individuals undergo complex life cycles to  
34 produce new offspring, which makes fitness a multivariable function of all the life-history traits characterizing  
35 that organism's life cycle. In addition to offspring production, this may include, for example, the ability of that  
36 offspring to migrate or disperse to the appropriate environments, or the ability to find mates in the case of  
37 sexual reproduction.

38 Life cycle complexity has been repeatedly shown to be important for the characterization of fitness. Histor-  
39 ically, age-structured models have been developed to study human demography [3]. In the context of species  
40 conservation, or, at the other end of the spectrum, pest management, the focus has been on finding the "Achilles  
41 heels" of species life cycles to design efficient strategies to act upon them, in order to shape and preserve biodiver-  
42 sity [3]. This idea has further been developed theoretically, within the conceptual framework of metapopulation  
43 dynamics [4, 5]. Finally, life cycle complexity is also a concept central to the study of the onset of multicellularity,  
44 to understand why and how group replication can be selected for [6, 7].

45 The question of how life cycle components contribute to fitness is of particular relevance for the study of  
46 microbial communities that associate with hosts – microbiotas. Intricate life cycles are common in nature, where  
47 microbes can for example use hosts as vectors between different habitats [8, 9]. Having a living host as a habitat  
48 adds complexity to the assessment of fitness, given that the presence of the microbes may impact the host fitness  
49 and vice-versa. It is in fact the whole life cycle of host-associating microbes that is intertwined with the one  
50 of their host. Research has often been biased towards the host perspective, and has focused on how microbes  
51 can contribute to host fitness by extending the host functional repertoire, *e.g.* performing digestive or immune  
52 tasks [10, 11, 12]. An exception is epidemiology and parasitology, that have specifically addressed the impact  
53 of the host fitness on the pathogen, in the form of trade-offs between transmission and within-host virulence  
54 [13, 14, 15, 16]. But what about commensal relationships, where bacteria do not have a negative impact on the  
55 host fitness? In this context, what are the factors that determine a microbial population's fitness?

56 Here, we propose a framework to assess the selection gradient acting upon the life history traits of a microbial  
57 population with a life cycle including host association. The gradient of selection determines the direction in the  
58 phenotypic space that evolution is expected to follow to maximize fitness. Our general aim is to provide a tool to

59 compare the relative importance of the different life-history traits of a microbial population, starting only from  
60 the equations that describe the population dynamics experienced throughout the life cycle. We explore a simple  
61 continuous-time two-compartment model that allows microbes to migrate between a host and its environment.  
62 We use the method of sensitivity analysis [3] to infer how strongly the population growth rate depends on the  
63 traits we are considering. In the baseline version of the model, we consider unconstrained growth. Subsequently,  
64 we extend our framework numerically to include population size constraints. We define the local direction of the  
65 selection gradient as the optimal strategy for a microbial population to adapt to its life cycle, starting from  
66 the local values of the traits. We show the existence of defined regions of different optimal strategies in the  
67 phenotypic space in which it is either more beneficial to optimize growth or transmission. The boundaries of  
68 these regions are driven by modeling assumptions such as competition, and the probing time chosen to measure  
69 fitness.

## 70 2 Model

71 We focus on a single microbial type and ask how the growth rate of its whole population is affected by its life  
72 history traits. We study the population in two compartments corresponding to communicating habitats: the  
73 host and its environment. Let us write  $n_H$  for the number of host-associated microbes and  $n_E$  for the number of  
74 environmental ones. We define the life history traits of the microbial population as the rates at which individuals  
75 of the compartmental populations reproduce and die, compete and migrate from one compartment to another  
76 (Figure 1A). The net replication rates in the environment and within the host are  $r_E$  and  $r_H$ , respectively.  
77 They could encompass both offspring production and death, and thus could be negative. The migration rates  
78 from the host to the environment and from the environment to the host are  $m_E$  and  $m_H$ , respectively. We  
79 start with exponentially growing populations. We later introduce a competition of intensity  $k_{ij}$  experienced  
80 by the microbes of compartment  $i$  due to the abundance of microbes in the compartment  $j$ . We assume that  
81 the number of microbes is large enough to be described by differential equations and assume that all rates  
82 introduced above are constant.

This leads to the general equations

$$\frac{\partial n_H}{\partial t} = r_H n_H + m_H n_E - m_E n_H - k_{HE} n_H n_E - k_{HH} n_H^2 \quad (1)$$

$$\frac{\partial n_E}{\partial t} = r_E n_E + m_E n_H - m_H n_E - k_{EH} n_E n_H - k_{EE} n_E^2.$$

83 In the following, we first consider unconstrained growth, where there is no competition ( $k_{EE} = k_{HH} = k_{EH} =$   
84  $k_{HE} = 0$ ), before adding global competition ( $k_{EE} = k_{HH} = k_{EH} = k_{HE} = k$ ), competition limited to one of  
85 the compartments ( $k_{EH} = k_{HE} = 0$  and  $k_{EE} \neq 0$  or  $k_{HH} \neq 0$ ), and finally, equal competition in each of the  
86 compartments ( $k_{EH} = k_{HE} = 0$  and  $k_{EE} = k_{HH} = k$ ). While in nature it is likely that none of the  $k_{ij}$  vanishes  
87 and that a wide range of values are possible, the study of these limit cases gives powerful insights into what is



88 to be expected in a wide range of situations.

## 89 3 Results

### 90 3.1 Baseline model: no competition

91 We start by assuming no competition and consider unconstrained growth in each of the two compartments. In  
92 this case, the equations describing our model become linear and can be rewritten in matrix form [3] as

$$93 \begin{pmatrix} \frac{\partial n_H}{\partial t} \\ \frac{\partial n_E}{\partial t} \end{pmatrix} = \underbrace{\begin{pmatrix} r_H - m_E & m_H \\ m_E & r_E - m_H \end{pmatrix}}_{\text{projection matrix}} \begin{pmatrix} n_H \\ n_E \end{pmatrix}. \quad (2)$$

94 The dominant eigenvalue  $\lambda$  of the above-defined projection matrix gives the asymptotic growth rate of the  
95 whole population. This quantity is an appropriate measure of fitness [3] insofar as it measures reproductive  
96 success and recapitulates the effects of all the life history traits. The dominant right eigenvector represents the  
97 stable distribution in the two compartments of an exponentially growing population. The value of  $\lambda$  can be  
98 calculated at each point of the phenotypic space defined by the ranges of possible values that could be taken by  
99 the life-history traits  $r_E, r_H, m_E$ , and  $m_H$ . The dependence of  $\lambda$  on these traits tells us at which points of the  
100 phenotypic space fitness is maximized and how it can be increased at all other points.

101 From the projection matrix, we calculate the dominant eigenvalue as

$$102 \lambda = \frac{1}{2} \left( \sqrt{(r_E + r_H - m_E - m_H)^2 - 4(r_E r_H - r_E m_E - r_H m_H)} + r_E + r_H - m_E - m_H \right). \quad (3)$$

103 Note that if microbes replicate at the same rate in the host and in the environment, *i.e.* if  $r_E = r_H = r$ ,  $\lambda$   
104 simplifies to  $r$ , regardless of the migration rates  $m_H$  and  $m_E$ . When there is an asymmetry between the two  
105 replication rates however, which is very likely to be the case in nature, then the migration rates also affect the  
106 population growth rate. In the following sections, we study this effect compared to the effect of the replication  
107 rates. We arbitrarily set  $r_H \leq r_E$ , and  $r_E > 0$  – otherwise the population gets extinct. In biological terms, this  
108 corresponds to the situation where the microbial population is initially more adapted to the environment than  
109 to the host and thus grows faster in the environment. But mathematically, in this model, host and environment  
110 are symmetrical, *i.e.* they only differ by the rates defined above. Thus, the chosen direction of this inequality  
111 does not carry any strong meaning, and there is no loss of generality in making this choice. In particular, one  
112 can access the opposite biological situation where microbes replicate faster in the host than in the environment  
113 – as is the case for viruses, that can only replicate in the host ( $r_H > 0$ ) but decay in the environment ( $r_E < 0$ )  
114 – by a single switch of the index  $E$  and  $H$ .

115 Let us first study the case where the migration rates from and towards the environment are equal, *i.e.*  
116  $m_E = m_H = m > 0$ . Let us denote  $\rho = \frac{r_H}{r_E} \leq 1$  the ratio of the replication rates. Then, setting  $r_E = 1$  to scale  
117 time (and thus, measuring all other rates in units of the replication rate of the microbe in the environment),  $\lambda$

118 reduces to

$$119 \quad \lambda_{sym} = \frac{1}{2} \left( 1 + \rho - 2m + \sqrt{(1 - \rho)^2 + 4m^2} \right). \quad (4)$$

120 For any fixed positive value of  $m$ ,  $\lambda_{sym}$  is a strictly increasing function of  $\rho$ , which reflects the fact that increasing  
121  $\rho$  allows for additional growth within the host. We will limit ourselves to the study of  $\rho \geq -1$ , which guaranties  
122 a positive value for  $\lambda_{sym}$ . For any fixed value of  $\rho$ ,  $\lambda_{sym}$  is a decreasing function of  $m$ , which reflects the  
123 fact that for increasing  $m$ , microbes are increasingly lost towards the host, where growth is slower than in  
124 the environment. Figure 1B shows the value of  $\lambda_{sym}$  on the reduced phenotypic space defined by  $\rho$  and  $m$ .  
125 The maximum possible value for  $\lambda$  is 1 (in units of  $r_E$ ). This value is achieved either by increasing the ratio  
126 of replication rates between host and environment, so that both microbial populations grow at the same rate  
127 (strategy I), or by reducing migration between host and environment (strategy II). This second strategy allows  
128 microbes to spend a longer time in the environment on average. Note however, that this strategy is limited,  
129 since setting  $m$  to zero decouples the two compartments completely, in which case the two subpopulations grow  
130 independently at different rates.

131 How strong is the selection on these traits? This question can be approached by inferring how strongly the  
132 population growth rate depends on the traits we are considering. One standard approach to measure this is  
133 sensitivity analysis [3]. One defines the sensitivity of the population growth rate  $\lambda$  achieved by the phenotype  
134 described by the vector  $\mathbf{x} = (x_1, \dots, x_N)$  in the trait space to its  $i^{th}$  life-history trait as

$$135 \quad s_i(\mathbf{x}) = \left. \frac{\partial \lambda}{\partial x_i} \right|_{\mathbf{x}}. \quad (5)$$

136 This quantity gives the change in the value of  $\lambda$  that results from a small increment of the trait  $i$ . It is a local  
137 property that can be calculated for each point  $\mathbf{x}$  of the trait space. The vector of the sensitivities at point  $\mathbf{x}$  gives  
138 the direction of the selection gradient on the fitness landscape. In other words, to achieve efficient phenotypic  
139 adaptation, the population should move in the trait space following the direction of this gradient.

140 If the population can invest in phenotypic adaptation only by tuning one of its life-history traits at a  
141 time, then it should act upon the trait that has the largest (absolute) sensitivity at the current position of  
142 the population in the trait space. This reasoning allows to divide the trait space into regions of distinct  
143 optimal strategies, as shown in Figure 1B. In the regime of high migration rates (*i.e.* when the switch between  
144 the compartments is so rapid that the population is almost experiencing a habitat having average properties  
145 between the host and the environment), strategy I (increasing  $\rho$ ) becomes almost always optimal, except for  
146 small replication ratios, where there is almost no replication in the host. In summary, migration rates are  
147 important when replication in the host is slow compared to the environment, and when migration itself is slow.  
148 These conclusions remain qualitatively unchanged with asymmetric migration rates, as discussed in more detail  
149 in the electronic supplementary material (ESM) section A.1.

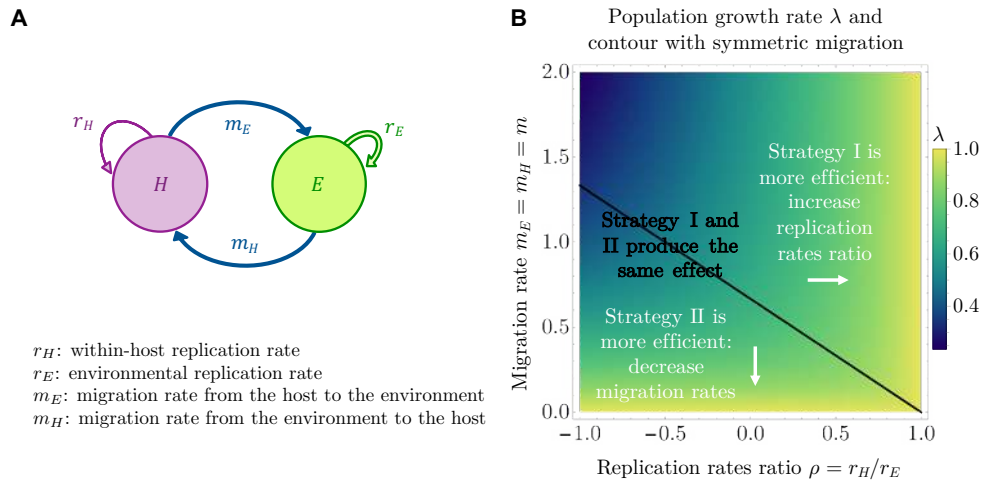


Figure 1: **Optimal strategies in the baseline model (no competition).** (A) Schematic diagram and definition of the rates for a microbial population migrating between a host and its environment and replicating in each compartment. For  $r_E > 0$ , the population increases exponentially and we ask how the exponential growth rate can be increased by changing the parameters of the model. (B) Population growth rate  $\lambda$  (color scale) on the trait space determined by  $\rho = r_H/r_E$  and  $m = m_H = m_E$ . The population growth rate  $\lambda$  is maximized for small  $m$  or for large  $\rho$ . In addition, we focus on sensitivities, which capture how strongly the population growth rate depends on the two traits. The contour line shows the line of the traits space that equalizes the absolute values of the two sensitivities derived analytically from equations 4 and 5,  $|s_m|/|s_\rho| = 1$ , delimiting the regions of optimality of the two different strategies. Note that we take the absolute values of the sensitivities, because in the baseline model the sensitivity of  $\lambda$  to increase in  $m$  is always negative, while it is always positive to increase in  $\rho$ . When  $|s_m|/|s_\rho| < 1$ , the optimal strategy is to increase the replication rates ratio (strategy I). When  $|s_m|/|s_\rho| > 1$ , the optimal strategy is to decrease the migration rate (strategy II).

### 150 3.2 Model with global competition between all microbes

151 In the baseline model, there are no constraints on population growth. In nature, however, microbial populations  
 152 do face limits to their growth. Since the equations above are linear and can only give rise to exponential growth  
 153 or exponential decay, they can only describe the dynamics of a population over a limited period of time. In  
 154 order to account for saturation and competition during growth, we thus need to introduce non-linear terms to  
 155 the equations 1. The study of this kind of systems often focus on long term dynamics, yet it can be of high  
 156 practical relevance to study the transient optimal strategies, as shorter timescales are often relevant in the real  
 157 world – whether it be due to experimental constraints or to ecological disturbances and perturbations [17].  
 158 Since we are going to consider some out-of-equilibrium dynamics, in particular in the section with competition  
 159 limited to one of the compartments, and because we are also interested in transient properties, we will adopt a  
 160 numerical approach based on population sizes [18, 19].

161 In this section we study the case of a microbial population limited in size by global competition occurring  
 162 at rate  $k = k_{HH} = k_{EE} = k_{EH} = k_{HE}$ . This situation could correspond to a microbiota living in direct contact  
 163 with an external environment, *e.g.* on the surface of an organism. Alternatively, what we call the “environment”  
 164 in our model could represent another host compartment in direct contact with the other, like the gut lumen and  
 165 the colonic crypts. In that case, microbes living in association with the host are in direct contact with those in  
 166 the environment and can mutually impact each other’s growth. This is of particular relevance if both microbial  
 167 subpopulations rely on and are limited by the same nutrients for growth.

168 From the microbial abundances in the different compartments obtained by numerically solving the equations,  
169 one can build a proxy for the population growth rate. To remain coherent with the previous section, we define

$$170 \quad \Lambda(\mathbf{x}) = \frac{1}{t_{max}} \log \left( \frac{n_E(t_{max}) + n_H(t_{max})}{n_E(0) + n_H(0)} \right), \quad (6)$$

171 *i.e.* the effective exponential growth rate of the whole microbial population – which captures the individual level  
172 reproductive success, since we consider a homogeneous population – over the chosen period of time  $[0, t_{max}]$ .  
173 There are several fundamental differences between the effective exponential growth rate  $\Lambda$  in a non-linear  
174 system and the population growth rate  $\lambda$  in a linear system, the dominant eigenvalue of the projection matrix  
175 as defined in the baseline model. First,  $\Lambda$  only provides a measure of growth for the whole population, but  
176 does not correspond to the asymptotic growth rate of each subpopulations as it was the case with  $\lambda$  in the  
177 baseline model. In fact, it is not either the asymptotic growth rate of the whole population: in the case of global  
178 saturation, replication stops when the carrying capacity is reached, and the asymptotic growth rate for the whole  
179 population is thus zero. Therefore, the choice of the probing time  $t_{max}$  has an impact on  $\Lambda$ , which we will see in  
180 more detail below. Second, the choice of the exact form of  $\Lambda$  now implies biological assumptions on the selection  
181 pressure felt by the population: choosing the effective exponential growth rate over the whole population as we  
182 do implies that selection is acting on the whole population evenly. There may be some situations, for example  
183 experiments in which the population of one of the compartments is artificially selected for, where it would make  
184 more sense to define  $\Lambda$  as the effective exponential growth rate over just this subpopulation. This may lead to  
185 different conclusions, in particular at the transient scale. One must thus adapt  $\Lambda$  to the specifics of the modeled  
186 system. In addition, the choice of  $t_{max}$  itself has a biological meaning, and should in particular not exceed the  
187 time upon which the dynamics of the system are accurately described by the set of equations. This may also  
188 be determined by experimental times.

189 We now calculate the sensitivity of  $\Lambda$  in the direction of the trait  $i$  at the point  $\mathbf{x}$  of the phenotypic space as

$$190 \quad S_i = \frac{\Lambda(x_1, x_2, \dots, x_{i-1}, x_i + \delta x_i, x_{i+1}, \dots, x_N) - \Lambda(x_1, x_2, \dots, x_N)}{\delta x_i} \quad (7)$$

191 with  $\delta x_i$  the discretization interval, and  $N$  the number of traits defining a phenotype  $\mathbf{x}$ .

192 For the numerical approach, additional choices need to be made. First, the trait space needs to be discretized.  
193 Then, to calculate Eq. 7, one needs to choose a set of initial conditions and a probing time at which to measure  
194 the population sizes, as exposed in details for the linear case in [17]. Finally, we need to choose the discretization  
195 interval  $\delta x_i$ . In the following, we always choose  $\delta x_i$  sufficiently small for convergence, *i.e.* so that it does not  
196 significantly impact the numerical values of the sensitivities, and focus on the choices of the other parameters  
197 (probing time and initial conditions) and the influence of the competition intensity  $k$ . One strategy to explore  
198 the possible impact of initial conditions is to use “stage biased vectors” [17], *i.e.* extreme distributions of the  
199 population. This corresponds to initial conditions where microbes either exist only in the host or only in the  
200 environment.

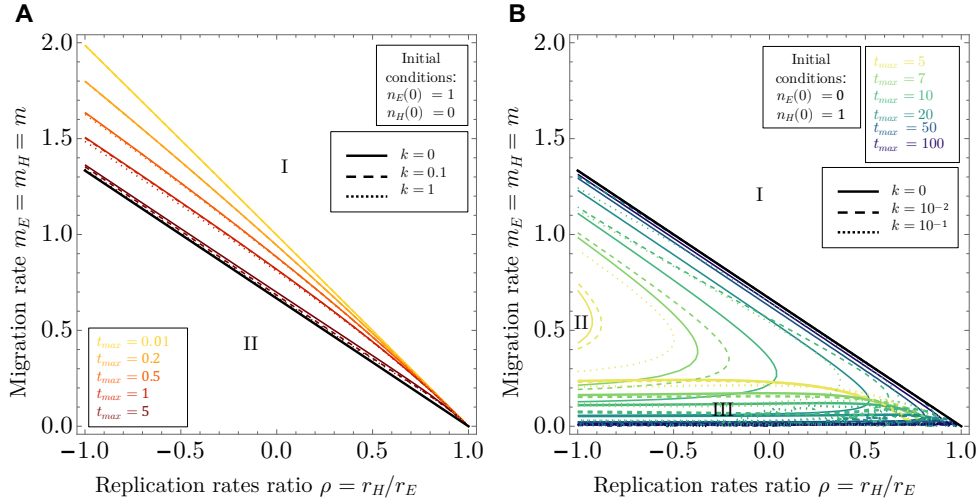


Figure 2: **Optimal strategies in the model with global competition.** (A) Change in the contour line delimiting the regions of optimality of the two strategies (strategy I: increasing the replication rates ratio; strategy II: decreasing migration) with  $t_{max}$ , the time chosen to measure the final population size, measured in units of  $1/r_E$ . Initially all the microbes are in the environment. Because in this model all the microbes are equally impacted by competition, with  $t_{max}$  large enough, one recovers the contour line of the baseline model calculated analytically (black line). Continuous lines:  $k = 0$ , *i.e.* no competition. Dashed lines: increasing values of  $k$  (competition intensity). (B) Extension of A with all the microbes in the host initially. In this case the convergence to the case without competition appears to be slower, while increasing the value of  $k$  seems to accelerate it. A third optimal strategy (III: increasing migration) appears around  $m = 0$ , delimited from strategy I by thicker lines.

201 In Figure 2, we show how the contour lines delimiting the two optimal strategies change with the final  
 202 time  $t_{max}$  chosen to measure the population growth rate and with the intensity of competition  $k$ , for these two  
 203 extreme cases:  $n_E(0) = 0, n_H(0) = 1$  and  $n_E(0) = 1, n_H(0) = 0$ . In all cases, with sufficiently long  $t_{max}$ , the  
 204 contours converge to the contour plot of the baseline model in the previous section. This is expected, since  
 205 competition here affects all the microbes in the same way, so that the equilibrium distribution is the same as  
 206 the asymptotic distribution of the baseline model (given by the dominant eigenvector). Mathematically, global  
 207 competition can be seen as a modification of the baseline projection matrix by subtracting an identity matrix  
 208 times a scalar depending on time. This does neither affect the eigenvectors nor the dependence of the dominant  
 209 eigenvalue on the traits.

210 In the case where all the microbes are initially in the host (Figure 2B), the convergence to the baseline case  
 211 requires higher values of  $t_{max}$  than in the case where all the microbes are initially in the environment (Figure  
 212 2A). Intuitively, this corresponds to the fact that convergence to the baseline distribution requires population  
 213 growth, and growth is slower if all the microbes are initially in the host compartment – where replication is  
 214 slower. There is thus a time delay between these two situations, corresponding to the time it takes for migration  
 215 to carry microbes into the environment – where replication is faster. When all the microbes are initially in the  
 216 host (Figure 2B), we also observe the appearance of a third optimal strategy around  $m = 0$ : increasing the  
 217 migration rate. In this unfavorable condition ( $m = 0$  and an initially empty environment), increasing the  
 218 microbial flux towards the environment becomes more important than limiting the flux of microbes leaving it  
 219 (which is nonexistent when  $m = 0$ ). At large  $t_{max}$  there is a direct transition between strategy II and strategy

220 III when increasing  $m$  from zero, thus the two contour lines overlap.

221 Finally, we observe that the intensity of competition has only a small effect on the contours when all the  
222 microbes are initially in the environment but a larger effect when all the microbes are initially in the host.  
223 In both cases, adding competition ( $k > 0$ ) appears to accelerate convergence to the baseline contour. This  
224 is because during the transient dynamics, the distribution balances to the expected asymptotic distribution  
225 from the initial conditions. In most cases, this equilibrium distribution is a mixed state, where a part of the  
226 population lives in the environment and another in the host. To reach this balance quickly from a pure biased  
227 state necessitates immigration to and replication in the initially empty compartment, while immigration to and  
228 replication in the other compartment remain slow. Competition limits the growth in the compartment that is  
229 not initially empty, and thus helps this balancing process. This effect is even stronger if the initially empty  
230 compartment happens to be the environment, where microbes replicate faster. This explains why the effect of  
231  $k$  is stronger in this case, see Figure 2B.

### 232 3.3 Model with competition within one of the compartments only

233 In this section we consider competition happening inside one of the compartments only (*i.e.*  $k_{EH} = k_{HE} = 0$   
234 and  $k_{EE} \neq 0$  or  $k_{HH} \neq 0$ ). We will start by considering competition in the host only, as it seems likely, from  
235 the biological point of view, that resources may be more limited in the host than in the environment. However,  
236 in a second step we also look at the case with competition limited to the environment. Even if this situation  
237 may seem less likely at first sight, one should bear in mind that it also covers the case of competition limited to  
238 a host where replication is faster than in the environment ( $r_H > r_E$ ), provided a switch of the  $H$  and  $E$  index.

239 In the case where competition is limited to only one of the compartments, we do not expect an equilibrium  
240 population size to be reached for all traits combination of the phenotypic space. If migration is not sufficiently  
241 important, the subpopulation in the unconstrained compartment keeps growing exponentially faster than the  
242 other subpopulation, which contribution to the global population thus becomes rapidly negligible. At sufficiently  
243 high migration rates however, an equilibrium is expected, because the microbes switch habitats sufficiently  
244 rapidly for competition to be globally effective, although it directly affects only one of the compartments.

#### 245 3.3.1 Competition in the host only (slow-replicating compartment)

246 When there is competition in the host only, there is no (positive) equilibrium for all  $m < 1$ . In this case,  
247 replication inside the host should have less importance because the host subpopulation size becomes negligible  
248 compared to the one of the environment. On this region of the phenotypic space we thus expect the sensitivity  
249 of parameter  $\rho$  to tend to zero with increasing probing times  $t_{max}$ , and the contour lines to be shifted to  
250 increase the area of optimality of strategy II, whatever be the other parameters (initial conditions, intensity of  
251 competition).

252 Figure 3 verifies this verbal argument: as expected, for a fixed  $t_{max}$ , we recover the shape of the fitness  
253 landscape of the baseline model for small values of  $k$ . Strategy I (increasing the replication rates ratio) however,

254 sees its area of optimality reduced with increasing values of  $k$  (Figure 3A). The values of  $\Lambda$  also become smaller  
255 overall: growth is slower due to competition.

256 As expected, for a fixed value of  $k = k_{HH}$ , the contours delimiting the two optimal strategies shift to reduce  
257 the area of optimality of strategy I with large values of  $t_{max}$  (Figure 3B-C). The disappearance of the contours  
258 from the region where  $m < 1$  takes place in two steps. First, with small  $t_{max}$  values, the effect of competition is  
259 not yet apparent due to the low initial abundance of microbes, and the contours start by getting closer to the  
260 reference contour of the baseline model, just as was observed in Figure 2. In the second step, with larger  $t_{max}$ ,  
261 the effect of competition becomes apparent and the contours are shifted out of the  $m < 1$  region, ultimately  
262 reaching a close-to horizontal limit which can be calculated analytically by performing sensitivity analysis on  
263 the equilibrium population sizes.

264 Additionally, when initially the microbes are in the host (Figure 3C), for the same reasons as in the previous  
265 section, we can again observe the appearance of the third strategy, increasing the migration rate, around  $m = 0$ .

266 The impact of increasing competition  $k$  at fixed  $t_{max}$  on the contour lines delimiting the two optimal  
267 strategies is clear in Figure 3D and E. We see that increasing  $k$  and increasing  $t_{max}$  have very similar effects:  
268 with small values of  $k$ , the baseline case is recovered, as expected. When increasing  $k$  sufficiently, the contour  
269 line is shifted out of the  $m < 1$  region with strategy I, *i.e.* increasing the replication rates ratio, until reaching  
270 the equilibrium limit. This is because increasing growth in the host can only have a limited effect when growth  
271 in the host is limited by competition, which makes strategy II comparatively more efficient.



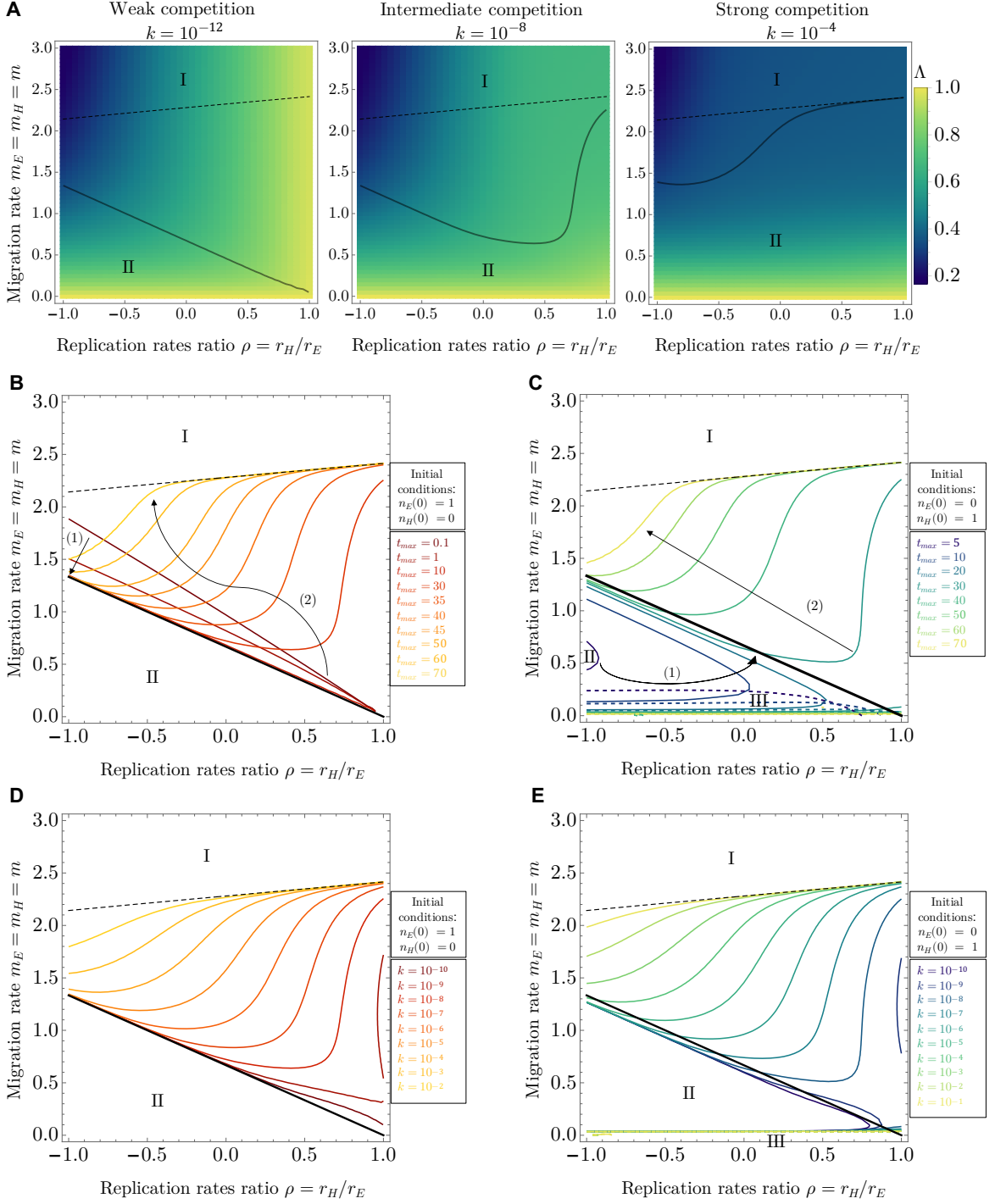


Figure 3: **Optimal strategies in the model with competition in the host only.** (A) Change in the fitness landscape with the within-host competition intensity  $k = k_{HH}$ . Black line: contour of equal sensitivities. Thin dashed line: contour of equal sensitivities of the equilibrium population sizes. Other parameters:  $t_{max} = 30$ ,  $n_E(0) = 1$ ,  $n_H(0) = 0$ . (B-E) Change in the contour lines delimiting the regions of optimality of the strategies with (B-C)  $t_{max}$  (probing time chosen to measure the population size) and (D-E)  $k$  (within-host competition intensity), with initial conditions where all the microbes are in the environment (B and D,  $n_E(0) = 1$ ,  $n_H(0) = 0$ ) or where all the microbes are initially in the host (C and E,  $n_E(0) = 0$ ,  $n_H(0) = 1$ ). Solid lines: limit between the regions of optimality of strategy I (increasing the replication rates ratio) and II (decreasing migration). Dashed lines: between strategies I and III (increasing migration). Other parameters:  $k = 10^{-8}$  (B-C),  $t_{max} = 30$  (D-E). In the case of competition limited to the host, whatever be the initial conditions, at sufficiently large  $t_{max}$ , or sufficiently large  $k$ , the region of optimality of strategy I (increasing the replication rates ratio) tends to narrow down and shift out of the  $m < 1$  region to reach the contour of equal sensitivities of the equilibrium population sizes (thin dashed line). The arrows in panels (B) and (C) indicate the two steps of contour shift with increasing  $t_{max}$ : in the first phase, when competition has a limited effect due to low abundances, the contours approach the limit of no competition (black line, as in Figure 2). In the second phase, competition in the host kicks in, and the contours move away from the baseline limit.

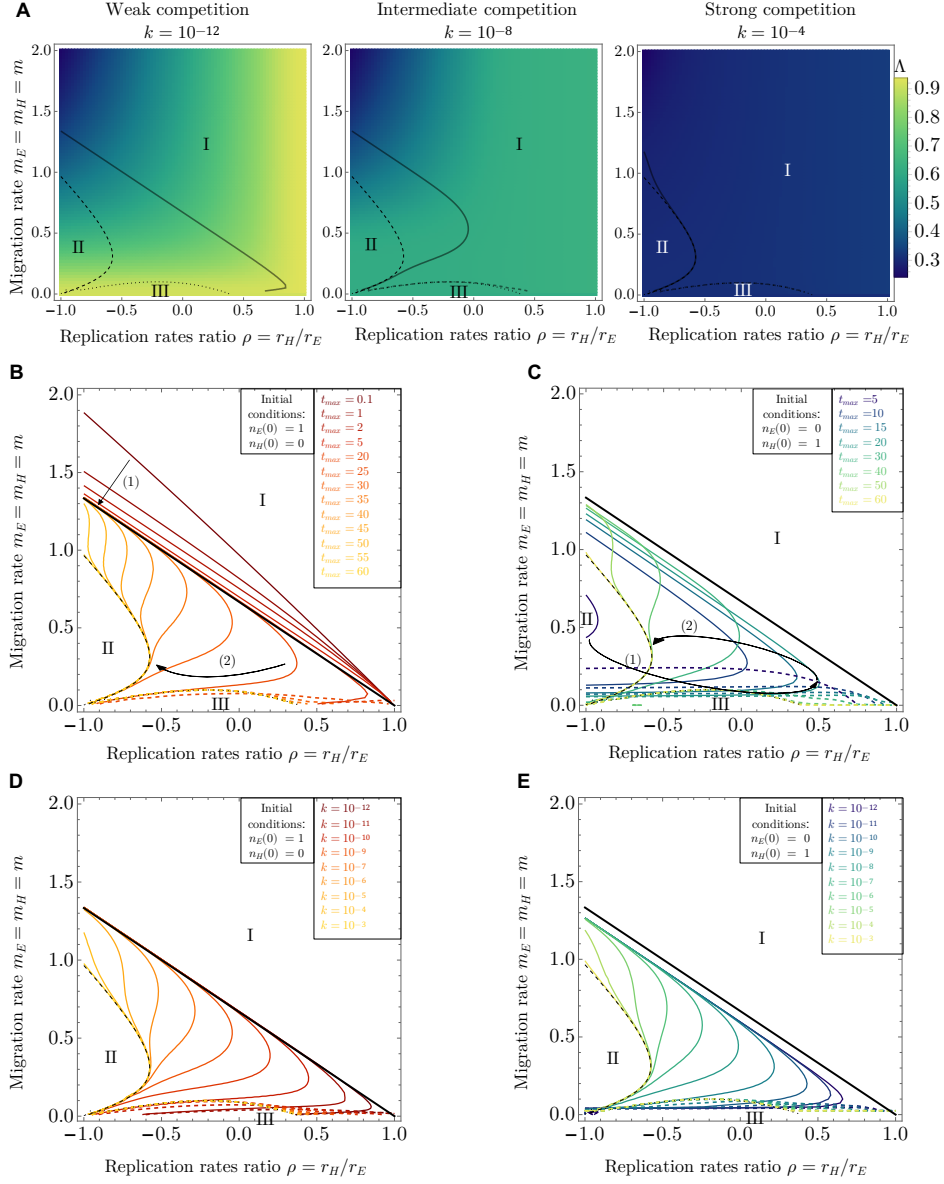


### 272 3.3.2 Competition in the environment only (fast-replicating compartment)

273 When there is competition in the environment only, there is no (positive) equilibrium for all  $m < \rho$ . In  
274 this region of the phenotypic space, the size of the environmental population becomes substantially smaller  
275 than that of the host-associated population after some time. As a consequence, strategy I (increasing the  
276 replication rate within the host) becomes more important, so that we see its area of optimality extend, see  
277 Figure A.2. For a fixed  $t_{max}$ , with a small value of  $k$  we recover the shape of the fitness landscape from the  
278 baseline model with no competition, but increasing  $k$  shifts the contour line to lower  $\rho$  until the strategy II  
279 (decreasing migration) disappears from the  $m < \rho$  region and the delimitation of the strategies approaches the  
280 contour of equal sensitivities of the equilibrium population sizes, calculated analytically. Like in the previous  
281 sections, we also observe the appearance of a third optimal strategy around  $m = 0$ , increasing migration.  
282 Unlike in the previous sections, this time the third strategy also appears when the microbes are all initially  
283 in the environment (Figure A.2B and D), and is also predicted by the sensitivity analysis of the equilibrium  
284 population sizes. Intuitively, having competition in the fast-replicating environment reduces the advantage of  
285 starting with a microbial population exclusively located there, and in this case too migration towards the host  
286 becomes initially more important than limiting migration out of the environment. For a fixed value of  $k$ , with  
287 increasing  $t_{max}$  the contour line starts by getting closer to the baseline model contour, before diverging from this  
288 limit and approaching the contour of equal sensitivities of the equilibrium population sizes. This finally leaves  
289 strategy I as the only optimal strategy on almost all the phenotypic space at sufficiently long times (Figure  
290 A.2B-C), except for a region of small  $\rho$  and intermediate  $m$ . Increasing  $k$  for a fixed value of  $t_{max}$  (Figure  
291 A.2D-E) has a very similar effect on the contour, except for the initial dynamics towards the baseline model.

### 292 3.4 Competition of same intensity in each compartment

293 When there is competition of equal intensity in the host and the environment (*i.e.*  $k_{EH} = k_{HE} = 0$  and  
294  $k_{EE} = k_{HH} = k$ ), we observe very similar results to the previous section, with competition in the environment  
295 only (see Figure 4): increasing  $k$  or increasing  $t_{max}$  leads to the disappearance, at long times, of the area of  
296 optimality of strategy II (decreasing migration), except for a distinct region of small  $\rho$  and intermediate  $m$ ,  
297 predicted by the contour of equal sensitivities of the equilibrium population sizes. This implies that the effect of  
298 competition in the fast-replicating compartment has a dominating effect on the global population growth rate.



**Figure 4: Optimal strategies in the model with limited growth in the host and the environment.** (A) Change in the fitness landscape with the competition intensity  $k_{HH} = k_{EE} = k$ . Black thick lines: contours of equal sensitivities (solid line: between strategy I and II, dashed line: between I and III). Black thin lines: contours of equal sensitivities of the equilibrium population sizes (dashed: between I and II and dotted: between I and III). Other parameters:  $t_{max} = 30$ ,  $n_E(0) = 1, n_H(0) = 0$ . (B-E) Change in the contour lines delimiting the regions of optimality of the strategies with (B-C)  $t_{max}$  (time chosen to measure the population size) and (D-E)  $k$  (competition intensity), in the case of initial conditions where all the microbes are in the environment (B and D,  $n_E(0) = 1, n_H(0) = 0$ ) or in the case where all the microbes are initially in the host (C and E,  $n_E(0) = 0, n_H(0) = 1$ ). Solid lines: limit between the regions of optimality of strategies I (increasing the replication rates ratio) and II (decreasing migration), and dashed lines: between I and III (increasing migration). Other parameters:  $k = 10^{-8}$  (B-C) and  $t_{max} = 30$  (D-E). In the case of competition in the host and in the environment, the effect of the competition in the environment (the fast-replicating compartment) seems to dominate, so that at sufficiently large times, the region of optimality of strategy II (decreasing migration) is reduced, and its limit approaches the contour of equal sensitivities of the equilibrium population sizes (black dashed line: between I and II). The black dotted line shows the contour of equal sensitivities of the equilibrium population sizes delimiting strategies I and III. The arrows in panels (B) and (C) indicate the two steps of contour disappearance with increasing  $t_{max}$ : in the first phase, when competition has a limited effect due to low abundances, the contours approach the limit of no competition (black solid line, as in Figure 2). In the second phase, competition  $k$  kicks in and the contours move away from the baseline limit.

## 299 4 Discussion

300 Out in the wild, microbes experience complex life cycles. Each of their steps can contribute to the overall  
301 reproductive success. In general, microbial fitness is thus more complex than the common approximation of  
302 growth yield used in the lab. This is particularly true for microbes experiencing life cycles that involve only  
303 a limited phase of host association, which translates as a selection pressure on phenotypic traits associated to  
304 migrating from an external environment to the host and vice-versa. A framework to study fitness in all its  
305 complexity is needed in the field of microbiome studies, which could benefit from approaches first introduced  
306 in demography. Here, we investigate a model of a microbial population living, replicating, migrating, and  
307 competing in and between two compartments: a host – assumed to be, throughout the paper, a compartment  
308 where replication is slower – and its environment. To analyze the selection gradient experienced by the microbial  
309 population going through this biphasic life cycle – with phases in the environment and phases in the host – we  
310 perform sensitivity analysis. We focus on the leading direction of the selection gradient at each point of the  
311 phenotypic space, thereby defining an optimal strategy for the microbial population to maximize its fitness.

312 We show that in the case of unconstrained exponential growth in both the compartments, there are two  
313 optimal strategies: increasing the replication rate in the host compared to the environment (strategy I), and  
314 decreasing the migration rates (strategy II) to maximize the time spent in the fast-replicating compartment.  
315 The first strategy is optimal at initially high ratios of replication rates and high migration rates, while the  
316 second strategy is optimal at initially small migration rates and small ratio of replication rates.

317 Next, we extend the model to a scenario where microbial growth is limited by competition. We start with  
318 global competition, a case which could describe competition for a resource homogeneously shared between  
319 the host and the environment. Biologically, this corresponds to communities of microbes that are associated  
320 with hosts, i.e. microbiotas, but have extensive contact with the environment, as the skin or other epithelial  
321 microbiotas for example [20, 21]. In this case, we show that apart from a transient effect, the optimality of  
322 the strategies is conserved from the case without competition. With competition in the host only (the slow-  
323 replicating compartment), at longer probing times, or at higher competition intensities, the strategy I (increasing  
324 the ratio of replication rates) is disfavored when migration out of the environment is slower than replication in  
325 the environment, *i.e.* where there is no equilibrium. Strategy II (decreasing the migration rates) thus increases  
326 its area of optimality. Inversely, with competition in the environment only (the fast-replicating compartment),  
327 or with competition of same intensity within the host and within the environment, the strategy II is selected  
328 against when migration out of the host is slower than replication in the host, leaving strategy I as the only  
329 optimal strategy on this region of the parameter space. Unsurprisingly, this suggests that competition within  
330 the fast-replicating compartment dominates the effect on the selection gradient.

331 While this analysis provides crucial information on the selection gradient that shapes microbial adaptation  
332 to life cycles involving host association, it does not take into account the evolvability of the traits themselves.  
333 Although the selection gradient is a good indicator of the expected evolutionary path in the phenotypic space,  
334 the underlying genotype/phenotype mapping does not always allow for this path to be taken [22, 23, 24, 25],

335 and the outcome of evolution may thus be different. The discrete nature, the non-additivity and non-linearity  
336 of genetic information, as well as the existence of costs, trade-offs and evolutionary constraints may prevent the  
337 predicted continuous change on the phenotypic trait. In addition, using sensitivities is built on the assumption  
338 that adaptation generates additive changes in life history traits. Although this is a common assumption,  
339 different choices are sometimes made. For example, multiplicative changes of the traits are assumed in elasticity  
340 analysis [3, 18, 24, 26], which presents the advantage of manipulating only proportional changes and thus  
341 non-dimensional quantities, but deals poorly with traits that can take the value of zero. These fundamental  
342 assumptions can sometimes result in different inferred selection gradients, as was shown for example in the  
343 context of age-classified populations [27]. However, although the exact shapes of the contours are modified, we  
344 have checked that our qualitative results remain robust when applying elasticity instead of sensitivity analysis.

345 Stepping back, we can evaluate the predictions of our model in the light of biological observations. Evo-  
346 lution experiments where microbial populations are serially passaged through a host and an environment are  
347 of particular interest here, to assess the response to selection resulting from biphasic life cycles. The key role  
348 of microbial immigration during the initial adaptation to their zebrafish host has for example been highlighted  
349 in [28]. In *Drosophila* [29] and in *C. elegans* [30], experimental selection towards host association resulted in  
350 adaptive changes in microbial life history with a direct impact on host fitness. In detail, in the first case, there is  
351 evolution towards by-product mutualism, and in the second, which concerns an initially pathogenic population,  
352 evolution towards less virulence and an increased carrying capacity.

353 Conceptually, using the effective population growth rate as a measure of fitness provides a complementary  
354 insight to invasion fitness approaches [31, 32] developed to analyze such evolution experiments, for example in  
355 [33, 34]. While invasion fitness analysis relies on assessing the long term chances of successful invasion of an  
356 established population at equilibrium by a new mutant strain of defined traits values, sensitivity analysis of  
357 the effective population growth rate provides a systematic framework that can be applied to out-of-equilibrium  
358 systems, and provides information on shorter time scales. Both frameworks rely on different proxies to assess a  
359 fitness capturing its different components - in one case, the frequency of patches where the microbe is present,  
360 and in the other, the microbial population growth, but both frameworks converge on the key role of migration  
361 between compartments. In fact, in many common cases like global competition, the long-term predictions of  
362 invasion fitness are recovered with the sensitivity analysis of the effective growth rate by setting  $t_{max}$  sufficiently  
363 large [18].

364 In future work, our framework could be extended in different directions to capture additional characteristics  
365 of microbial life cycles in host association. The first extension could be to increase the number of compartments.  
366 While the question of fluctuating environments has been studied before, in discrete times or in a different context  
367 [7, 18], in our context it may be profitable to consider and include host population dynamics. This would  
368 notably allow us to include microbial traits that affect host fitness in our analysis. A second direction could be  
369 to include stochasticity and non-homogeneities. Indeed, our deterministic description is valid only if the size of  
370 the microbial population is sufficiently large at all times and can only describe the average selection gradient  
371 experienced by the population. Introducing stochasticity would allow the study of differentiation, which may

372 play a role in the response to complex life cycles. Differentiation, in the form of speciation, phenotypic plasticity,  
373 or bet-hedging is indeed observed in evolution experiments and natural microbial populations [35, 36, 37, 38, 39,  
374 40]. It is also observed in host-associated populations [41] and may thus be expected in evolution experiments  
375 that include a host-association phase. Finally, a key aspect that we have so far excluded is spatiality. Effects  
376 of spatiality on the selection gradient are known for example in a simple Petri dish system, where the existence  
377 of an optimal expansion speed for a given habitat size is shown [42, 43]. Generally, hosts are highly structured  
378 habitats with variation in nutrients and chemical and physical gradients shaping for example the gut [44, 45, 46],  
379 which may also favor differentiation. The introduction of several compartments or sub-compartments within  
380 the hosts could represent a first step in this direction.

381 Thus, our model provides the key ingredients to study the consequences of host association for a microbe.  
382 It meets the need to conceptualize fitness as a holistic measure that captures all the aspects of microbial life  
383 cycles. With the development of this framework, we aim to contribute to a better understanding of the mutual  
384 benefits that microbes and hosts can retrieve from such associations.

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