



Bacteriophage evolution given spatial constraint

Stephen T. Abedon*, Rachel R. Culler

Department of Microbiology, The Ohio State University, Mansfield, OH 44906, USA

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Abstract

Spatial structure can impede mixing, diffusion, and motility. In microbiology laboratories, spatial structure is commonly achieved via formation of agar gels, within which bacteriophage (phage) replication results in localized clearings called plaques. Developing a better understanding of phage plaque formation is relevant because of the ubiquity of phage plaquing in the laboratory; because plaque size has been employed as a measure of phage fitness; because many bacteria exist within environments that display significant spatial structure (e.g., biofilms, soils, sediments, and in or on plant or animal tissues); and because spatial structure could impede phage exploitation of bacterial communities. There is, however, a relative dearth of experimentation and analysis considering phage plaque formation from the perspective of selection acting on individual phage growth parameters—latent period, burst size, and adsorption rate. Here we consider the impact of these parameters on rates of plaque wavefront velocity (rates of radial plaque enlargement), especially as functions of existing phage and environmental properties. We do so based on analyses of published equations which predict plaque enlargement rates. These indicate that greater wavefront velocities should be associated with (i) latent period reductions, (ii) larger burst sizes, or (iii) faster virion binding to bacteria. We suggest, however, that exceptions could occur, respectively, (i) if virion adsorption is unlikely or if burst sizes are large, (ii) if burst sizes are already large, or (iii) if virion binding rates are already fast, bacterial densities are especially high, or burst sizes are large. Higher initial lawn bacterial densities could also contribute to faster plaque expansion, but only if adsorption is otherwise slow or burst sizes are large. By contrast, faster virion diffusion is always expected to result in greater plaque wavefront velocities. Overall, we provide a snapshot of how phage populations may respond evolutionarily to selection for more-rapid propagation during spatially constrained growth.

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1. Introduction

Phage ecology is the study of the interactions between phage—viruses that productively infect bacteria—and their environments. Most phage ecology studies consider well-mixed environments (as reviewed by Abedon, 2006, and Weinbauer, 2004). An exception has been exploration of the physiochemical (e.g., Langmark et al., 2005), macromolecular (e.g., Sauer et al., 2004; Webb et al., 2004), or predatory (e.g., Resch et al., 2005; Webb et al., 2003; and “therapies”, below) interactions between phage and the bacteria that inhabit biofilms (Costerton et al., 1987; Cvikovitch, 2004; McLean et al., 2001; Sutherland et al.,

2004). Because a large fraction of environmental bacteria exist as biofilms, gaining a greater appreciation of the nature of phage population growth among bacteria that are not freely mixing, that is, which are spatially constrained, is important toward enhancing our understanding of phage ecology.

In the laboratory, spatial structure is more commonly encountered within agar gels rather than within biofilms. We believe, however, that agar gels can serve as simplified models of phage population growth within naturally occurring, spatially structured environments. Phage growth within this agar-based semi-solid medium occurs via a combination of virion diffusion, infection of bacteria, and phage-induced bacterial lysis. Since lysis results in precipitous declines in culture turbidity, phage population

*Corresponding author. Tel.: +1 419 755 4343; fax: +1 419 755 4327.

E-mail address: abedon.1@osu.edu (S.T. Abedon).

1 Table 1
Description of parameters employed in predicting plaque wavefront velocity^a 59

3 Abbr.	Parameter	Units	Description	61
5 B	Burst size (“yield”)	Phage	Number of phage produced upon lysis per infected bacterium	
6 c	Wavefront velocity	cm/min	Rate of outward expansion (increase in radius) of a plaque	63
7 f	Relative volume bacteria	None	Fraction of culture volume occupied by bacteria of a maximum that bacteria could occupy ($= N_o/N_{max}$)	
9 f_o	Critical f value	None	Employed by Ortega-Cejas et al. (2004), $f = f_o$ is the point at which c in Eqs. (10) and (11) are equal; $f_o = 1/[L(B-1)k_1N_{max}]$	65
11 D	Diffusivity	cm ² /min	Rate of diffusion of virion particles; a measure of rates of phage movement (absent environmental mixing) when not infecting bacteria	67
13 k	Adsorption constant ^b	ml/min	Rate of phage-to-bacterial attachment given phage suspension with bacteria (a diffusion-limited phage-infection rate)	69
15 k_1	Adsorption rate constant	ml/min	Rate of specific phage-to-bacterial attachment given phage encounter with bacterium (a not diffusion-limited phage attachment rate)	71
17 k_{-1}	Desorption rate constant	min ⁻¹	Rate of conversion of reversibly bacteria-adsorbed phage to free phage	
19 k_2	Infection lysis-rate constant	min ⁻¹	Rate of conversion of infected bacteria to lysed (progeny phage releasing) bacteria (which may be approximated as $1/L$)	73
21 K	Equilibrium adsorption constant	None	Rate of binding of phage to bacteria, given encounter, taking into account losses due to desorption ($= k_1/k_{-1}$)	75
23 K_{max}	Maximum K	None	Inherent binding affinity of a phage for a bacterium ($= k_1N_{max}/k_{-1}$)	
25 L	Latent period	min	Period during which a phage spends not diffusing as a virion because of infection of a bacterium	77
27 N_o	Bacterial density	ml ⁻¹	Bacterial lawn density, held as a constant (assumption of no division)	79
29 N_{max}	Maximum N	ml ⁻¹	Maximum number of bacteria that could be packed within a given volume of agar	81

^aSee Yin and McCaskill (1992) for further discussion.

^bThe adsorption constant, k , is not employed in this study, but is listed here to contrast its definition with that of the adsorption rate constant, k_1 .

29 growth within bacterial lawns growing in or on agar produces circular clearings called plaques. Simplifying their understanding of, for example, the phage ecology of

31 study, plaque radii typically increase at constant rates for a given combination of phage, bacterium, bacterial growth

33 phase, and plaquing conditions (Kaplan et al., 1981; Koch, 1964; Lee and Yin, 1996a; Mayr-Harting, 1958; Yin, 1991). Larger plaques have been presumed to indicate greater

35 phage fitness (Burch and Chao, 2004; Lee and Yin, 1996a, b) and we assume that individual phage that can more rapidly form into larger plaques can also acquire

37 more bacteria during competition at a plaque’s edge (Abedon, 2006; Bull, 2006; Lee and Yin, 1996a; Wei and Krone, 2005; Yin, 1993). What properties of a hypothetical phage mutant would supply it with a growth advantage

83 terrestrial environments (Gill and Abedon, 2003).

2. Methods 89

91 We consider, via algebraic manipulation, models of phage plaque enlargement provided by Koch (1964), Yin and McCaskill (1992), and Ortega-Cejas et al. (2004). We introduce the models, put them into a common form, address issues concerning their relative utility, and then discuss their differences, especially in terms of the impact of phage latent period (L), phage burst size (B), phage-binding rate given bacterial encounter (k_1), and bacterial density (N_o), on plaque wavefront velocity (c). We will assume in our analysis that each of these parameters may be varied independently, leaving for future analyses consideration of the impact of lack of independence, e.g., such as tradeoffs between parameter values (as discussed for phages more generally by Breitbart et al., 2005). See Table 1 for discussion of the parameters employed. 105

3. Results 107

3.1. Modeling plaque wavefront velocity 109

111 There exist seven published equations that purport to predict rates of plaque enlargement (c) during phage growth within semi-solid agar-based media (Koch, 1964; Ortega-Cejas et al., 2004; Yin and McCaskill, 1992). We 113

1 first introduce the oldest and simplest of these equations,
2 that of Koch (1964). The four Yin and McCaskill (1992)
3 equations are more complex, incorporating additional
4 parameters, and we introduce these by focusing especially
5 on their least-limiting form. See Appendix A for introduc-
6 tion to the two equations of Ortega-Cejas et al. (2004).

7 Koch (1964) estimated the rate of plaque enlargement in
8 radius, c , as

$$9 \quad c = 10 \left(\frac{D}{L} \right)^{1/2}, \quad (1)$$

11 where D is the virion diffusivity (i.e., the rate at which
12 phage diffuse) and L is the phage latent period (i.e., the
13 duration of the period during which phage are not
14 diffusing). In words, this equation indicates that the rate
15 of radial plaque enlargement is predicted as equal to ten
16 times the square root of the rate of phage–virion diffusion
17 divided by the square root of the phage latent period. Thus,
18 $c \propto D^{1/2}$ and $c \propto L^{-1/2}$. In the Section 4 we provide further
19 consideration of the proportionality of c to various
20 parameters.

21 By explicitly incorporating phage growth parameter
22 values in addition to diffusivity and latent period, Yin
23 and McCaskill (1992) provide more mechanistic models of
24 phage plaque enlargement. These differ as functions of
25 phage properties, as we will describe. For example, under
26 conditions of “equilibrated” adsorption between phage
27 and bacterium (i.e., explicitly somewhat reversible phage
28 adsorption), Yin and McCaskill provide this solution:

$$29 \quad c = 2 \left[\frac{Dk_2(B-1)fK_{max}}{(1+fK_{max})^2} \right]^{1/2}, \quad (2)$$

31 where B is the phage burst size and k_2 the rate of
32 conversion of infected bacteria to virions.

33 The parameters k_1 and k_{-1} are an association constant
34 between bacteria and phage virions (given encounter
35 between phage and bacterium) and a corresponding
36 dissociation constant (which provides explicit adsorption
37 reversibility in this equation). According to Yin and
38 McCaskill, $f = N_o/N_{max}$ and $K_{max} = k_1N_{max}/k_{-1}$ (respec-
39 tively, the fraction of culture volume occupied by bacteria
40 and the inherent binding affinity of a phage for a
41 bacterium), where N_o is a constant density of bacteria
42 found within the lawn surrounding a plaque, and N_{max} is
43 the “bacterial concentration required to completely occupy
44 the diffusional medium.” We make these substitutions into
45 Eq. (2) to produce

$$46 \quad c = 2 \left[\frac{Dk_2(B-1)N_o k_1/k_{-1}}{(1+N_o k_1/k_{-1})^2} \right]^{1/2}. \quad (3)$$

47 Since the above algebraic manipulation is easily repeated
48 for the three “limiting” cases provided by Yin and
49 McCaskill (1992)—when phage burst sizes are large, when
50 phage adsorption is “slow” (that is, k_1 is small), or when
51 phage adsorption is “fast” (k_1 is large)—we present these
52 equations only in a “finalized” form (as Eqs. (7)–(9),

below). See Appendix A for similar consideration of the
53 model of Ortega-Cejas et al. (2004). 59

3.2. Presenting equations in a common form 61

62 The Koch equation is the oldest and simplest model of
63 phage plaque formation. To facilitate further comparison
64 among models, first we justify the inclusion of additional
65 parameter values employed by Yin and McCaskill (1992),
66 as seen in Eq. (3). We then show how the Yin and
67 McCaskill equations may be presented as modifications of
68 that of Koch. Subsequently, we provide additional
69 algebraic manipulation so as to allow more facile
70 comparison especially among the Yin and McCaskill
71 (1992) equations. In Appendix A, we repeat this process
72 for the equations of Ortega-Cejas et al. (2004). 73

74 Koch constrains the applicability of his model so as to
75 minimize the impact of burst size on bacterial density. The
76 suggestion, by Eqs. (2) and (3), that burst size would
77 contribute positively to plaque size (i.e., the presence of B
78 in the numerator) therefore seems reasonable despite the
79 seemingly contradictory modeling approach of Koch. 79
80 Similarly, though less intuitive, the negative contribution
81 of higher bacterial densities, suggested by Eqs. (2) and (3)
82 (i.e., when $N_o k_1/k_{-1} \gg 1$), is consistent with the experi-
83 mental results of Mayr-Harting (1958). 83

84 Koch provides evidence for the reasonableness of
85 ignoring rates of phage adsorption when predicting rates
86 of plaque expansion. Hershey et al. (1944), though,
87 describe experimental results indicating that very low
88 adsorption rates can result in small plaque sizes. Consistent
89 with Hershey et al., in Eq. (3) the k_1 term negatively
90 impacts c if it is very small, i.e., if $1 \gg N_o k_1/k_{-1}$ (in
91 which case Eq. (3) approaches $(4Dk_2(B-1)N_o k_1/k_{-1})^{1/2}$). Also
92 inconsistent with Koch, Eq. (3) predicts a negative impact
93 on c with a larger k_1 . This latter behavior, though, is lost
94 when the same model is numerically approximated (You
95 and Yin, 1999). 95

96 Given these considerations, we find the Yin and
97 McCaskill (1992) model (Eq. (3)) to be consistent with
98 expectation and experiment, though with caveats regarding
99 the impact of larger values for k_1 . To provide a more
100 intuitive view of differences, however, we present Eq. (3) as
101 a modification of Eq. (1), 101

$$102 \quad c = 10 \left(\frac{D}{L} \right)^{1/2} \cdot \left[\frac{BN_o k_1/k_{-1}}{25(1+N_o k_1/k_{-1})^2} \right]^{1/2}, \quad (4)$$

103 where $1/L$ replaces k_2 (as consistent with numerical
104 solutions by Yin and McCaskill, 1992) and B replaces
105 $(B-1)$. This “1” following B represents virion loss to
106 initiate a phage infection, which may be readily ignored for
107 all but the smallest of phage burst sizes. Thus, plaque
108 enlargement according to Eq. (4) occurs at a rate that is
109 equivalent to that indicated by Koch (1964) but multiplied
110 by an expression that, minimally, increases as a function of
111 one-fifth the square root of the phage burst size (B). 113

1 To facilitate further comparison, we restate, in a
 2 common form, Koch's (1964) equation as Eq. (5) and
 3 Yin and McCaskill's (1992) equations as Eqs. (6)–(9).
 4 These are a general Eq. (6) that explicitly considers a
 5 reversibility in phage adsorption (“equilibrated adsorp-
 6 tion”) and, as introduced in Section 3.1, three limiting
 7 cases: large burst size (Eq. (7); larger B), slow adsorption
 8 (Eq. (8); smaller k_1), and fast adsorption (Eq. (9); larger
 9 k_1):

$$11 \quad c = 10 \left(\frac{D}{L} \right)^{1/2} \cdot (BN_o k_1)^{1/2} \cdot 1.00 \left[\frac{1}{BN_o k_1} \right]^{1/2}, \quad (5)$$

$$15 \quad c = 10 \left(\frac{D}{L} \right)^{1/2} \cdot (BN_o k_1)^{1/2} \cdot 0.20$$

$$\times \left[\frac{1}{k_{-1}(1 + N_o k_1/k_{-1})^2} \right]^{1/2}, \quad (6) \quad 59$$

$$c = 10 \left(\frac{D}{L} \right)^{1/2} \cdot (BN_o k_1)^{1/2} \cdot 0.16 \quad 61$$

$$\times \left[\frac{L^{1/2}}{(BN_o k_1)^{1/2}} \right]^{1/2}, \quad (7) \quad 63$$

$$c = 10 \left(\frac{D}{L} \right)^{1/2} \cdot (BN_o k_1)^{1/2} \cdot 0.20 L^{1/2}, \quad (8) \quad 65$$

$$c = 10 \left(\frac{D}{L} \right)^{1/2} \cdot (BN_o k_1)^{1/2} \cdot 0.26 \left[\frac{B}{LN_o^2 k_1^2} \right]^{1/2}. \quad (9) \quad 67$$

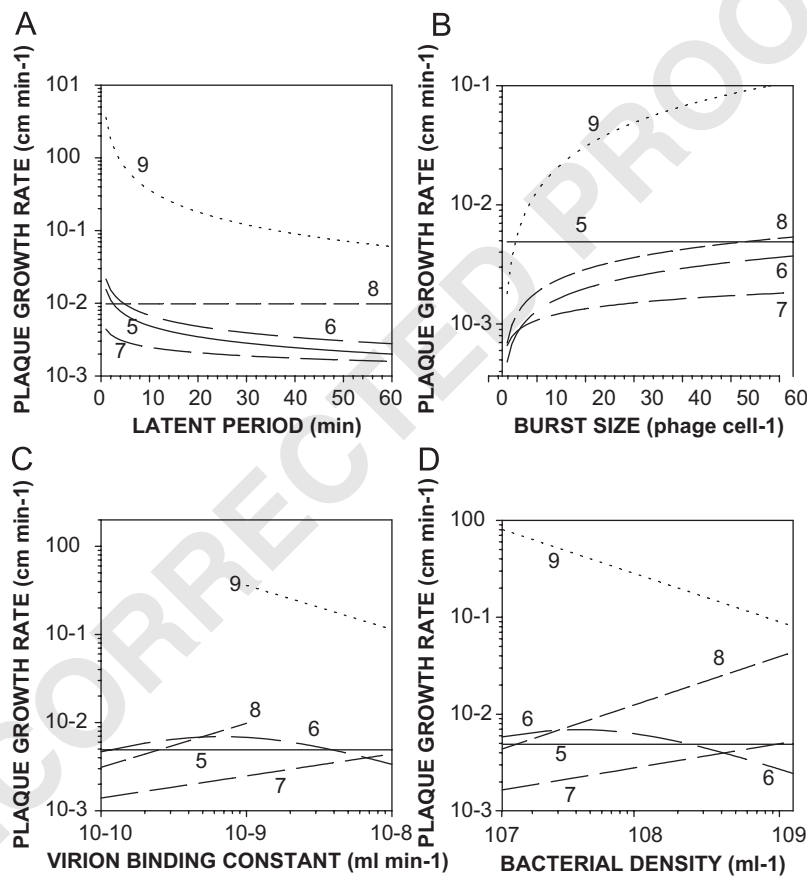


Fig. 1. (A–D) Plaque growth rate (wavefront velocity) as functions of various parameters and variables. Shown are model solutions based upon the following parameter values (unless otherwise varied as indicated on the x axes of each panel): D = diffusivity = 2.4×10^{-6} cm²/min (Yin and McCaskill, 1992), L = latent period = 10 min (Yin and McCaskill, 1992), B = burst size = 200 phage/bacterium (Yin and McCaskill, 1992), N_o = cell density = 5×10^7 bacteria/ml (arbitrary value), $k_1 = 10^{-9}$ ml/min (Garen, 1954; Ortega-Cejas et al., 2004; You and Yin, 1999), and $k_1/k_{-1} = 3 \times 10^{-8}$ ml (Garen, 1954; Yin and McCaskill, 1992). The latter is also as employed by Yin and McCaskill (1992). Numbers refer to equation numbers corresponding to the different models discussed. Note (i) that the values in panel B to the extreme left are distorted by our equating B with $B-1$, (ii) that the curve associated with Eq. (7), also in panel B, is somewhat invalid over the burst size range indicated (i.e., see Fig. 3 of You and Yin, 1999) since this model's validity is limited to “large” burst sizes, (iii) that we have adjusted in panel C the k_1/k_{-1} term of Eq. (6) as a function of k_1 , and (iv) that we truncate the curves corresponding to Eqs. (8) and (9), also in panel C, to illustrate that these curves are limiting cases corresponding to low and high k_1 , respectively. Upon inspection of these graphs, we note that Eq. (9) predicts plaque wavefront velocities somewhat in excess of the rest of the curves presented, e.g., such as relative to that of Eq. (6) in panel C. We speculate that this difference could reflect the discrepancy seen between analytic and numeric solutions as observed by You and Yin (1999); (their Fig. 4) at higher values of k_1 .

Table 2
Impact of changes in latent period, burst size, virion binding constant, and cell density on rates of plaque enlargement

Description	Eq.	L	B	k_1	N_o	Predictions (c = wavefront velocity)
Koch (1964)	5	$c \propto L^{-1/2}$	$c \propto B^0$	$c \propto k_1^0$	$c \propto N_o^0$	Dependence of c on latent period but independence of c of burst size, adsorption rate, and cell density
Yin and McCaskill (1992) "equilibrated adsorption"	6	$c \propto L^{-1/2a}$	$c \propto B^{1/2}$	$c \propto k_1^{-1/2} \dots k_1^{1/2}$ ($c \propto k_1^0 \dots k_1^{1/2}$) ^b	$c \propto N_o^{-1/2} \dots N_o^{1/2}$	Dependence of c on burst size, virion adsorption rate, and cell density
Yin and McCaskill (1992) "large burst size"	7	$c \propto L^{-1/4}$	$c \propto B^{1/4}$	$c \propto k_1^{1/4}$	$c \propto N_o^{1/4}$	Only moderate dependence of c on latent period, burst size, rate of adsorption, and cell density
Yin and McCaskill (1992) "slow adsorption"	8	$c \propto L^0$	$c \propto B^{1/2}$	$c \propto k_1^{-1/2}$	$c \propto N_o^{-1/2}$	Independence of c of latent period
Yin and McCaskill (1992) "fast adsorption"	9	$c \propto L^{-1}$	$c \propto B^1$	$c \propto k_1^{-1/2}$ ($c \propto k_1^0$) ^b	$c \propto N_o^{-1/2}$	Maximal dependence of c on latent period and burst size. Negative impact of higher cell densities

^aAccording to You and Yin (1999), given very short phage latent periods, e.g., less than ~ 10 min, then one might replace $k_2^{1/2}$, in $c \propto k_2^{1/2}$, with k_2 raised to a power that is less than $1/2$ but greater than or equal to zero (in the table this would be seen as replacing $L^{-1/2}$ in $c \propto L^{-1/2}$ with L raised to a power that is greater than $-1/2$ but less than or equal to zero), which is to say that the relative impact of latent period on c is lower when L is small.

^bAlternative possibility as suggested by You and Yin (1999).

Going from left to right, as separated by the larger multiplication dots ("•"), these equations present the Koch equation (left), a region that is constant between the seven equations (middle), and a region that represents the sole discrepancies between the different equations (right). Based on these equations, in Section 4 we consider the relative impacts of modifying phage latent period, burst size, or rate of binding to bacteria, as well as bacterial density, on rates of plaque-radius increase (as summarized in Fig. 1 and Table 2). See Eqs. (18) and (19) (Appendix A) for presentation of the Ortega-Cejas et al. (2004) equations in similar form.

4. Discussion

Equations predicting rates of plaque radial enlargement (c) are provided by Koch (1964), Yin and McCaskill (1992), and Ortega-Cejas et al. (2004). We have restated those of Koch and Yin and McCaskill, Eqs. (5) through (9), such that they may be easily compared by visual inspection (see Appendix A for those of Ortega-Cejas et al.). From these equations, we now determine the degree to which selection for faster plaque enlargement (greater c) may impact the phage latent period, burst size, or adsorption rate. We employ three criteria to discern this impact: (i) Does a shortening of latent period (L), an increase in burst size (B), or an increase in rates of virion binding (k_1) result in an increase (or decrease) in c ? (ii) What is the relative magnitude of the change in c as described by the different models given equivalent changes in L , B , or k_1 ? (iii) Can we infer trends in the impact of changes in L , B , or k_1 based on differences in assumptions underlying the various models? See Table 2 and Fig. 1 for illustration.

4.1. Relative impact of changes in latent period on rates of plaque enlargement

We can rank Eqs. (5)–(9) in terms of the size of the impact of equivalent changes in latent period (L) on c , from highest impact to lowest. That is, from a larger impact for a given degree of change in L to a smaller impact: (i) Eq. (9) (fast adsorption; $c \propto L^{-1} = 1/L$, which in words means that the rate of plaque enlargement increases proportionally as latent period decreases); (ii) Eqs. (5) and (6) (Koch's equation and Yin and McCaskill's equilibrated adsorption equation, respectively, where $c \propto L^{-1/2} = 1/\sqrt{L}$); (iii) Eq. (7) (large burst size; $c \propto L^{-1/4} = 1/\sqrt[4]{L}$); and finally (iv) Eq. (8) (slow phage adsorption; $c \propto L^0 = 1$, i.e., c is independent of L). We infer from these observations a reduced impact of latent period on rates of plaque growth when the influence of virion diffusion is enhanced, either due to more phage diffusing (Eq. (7), larger burst size) or lower phage affinity for bacteria (Eq. (8), slower adsorption). Consistently, a greater impact by latent period is seen when diffusion is relatively de-emphasized (Eq. (9), faster adsorption). The above-noted trends can be seen in Fig. 1A, where curve steepness corresponds to relative impact of changes in latent period on wavefront velocity. Note, however, that the modeling results of You and Yin (1999) call into question the increasing curve steepness seen with very short latent periods (extreme left, Fig. 1A).

4.2. Relative impact of changes in burst size on rates of plaque enlargement

We can rank Eqs. (5) through (9) in terms of the impact of changes in burst size (B) on rates of plaque enlargement (c). The greatest impact is seen (i) with Eq. (9) (fast adsorption; $c \propto B^1$). (ii) Eqs. (6) and (8) (equilibrated adsorption and slow adsorption, respectively) are indis-

1 tinguishable in terms of the impact of burst size on rates of
 2 plaque enlargement ($c \propto B^{1/2}$). (iii) Eq. (7), large burst size
 3 (e.g., ~ 200 and larger; You and Yin, 1999), actually
 4 displays the lowest impact of burst size on rates of plaque
 5 enlargement of the four cases presented by Yin and
 6 McCaskill (1992) ($c \propto B^{1/4}$). The relatively greater impact
 7 of burst size with fast adsorption (Eq. (9)) is suggestive, as
 8 in Section 4.1, that a reduced per-virion potential to diffuse
 9 (in this case because phage are more likely to adsorb
 10 bacteria) can be compensated for by supplying more phage
 11 via larger burst sizes. There apparently are limits to the
 12 impact of ever larger burst sizes, however, as indicated by
 13 the reduced influence of burst size in Eq. (7), which implies
 14 that similar percent changes in burst size are not as relevant
 15 to plaque wavefront velocities when burst sizes are very
 16 large versus when burst sizes are small. See Fig. 1B for
 17 graphical representation of these various trends; note that
 18 we truncate this graph at a burst size of 60 to better
 19 highlight the more-dramatic trends seen especially at lower
 20 burst sizes. We also note, for Eq. (1), that c does not vary
 21 as a function of burst size ($c \propto B^0$).

22 4.3. Relative impact of adsorption rapidity on rates of 23 plaque enlargement

24 The term k_1 describes the intrinsic rate of binding of a
 25 phage to a bacterium, given physical virion-bacterium
 26 encounter. The greatest positive impact on c of an increase
 27 in k_1 (i) is seen with Eq. (8) (slow adsorption; $c \propto k_1^{1/2}$).
 28 Depending on circumstances, this impact may also be
 29 approached by Eq. (6) (equilibrated adsorption) when
 30 bacterial densities are low (such that $1 \gg N_o k_1 / k_{-1}$). Next in
 31 terms of positive impact (ii) is Eq. (7) (large burst size;
 32 $c \propto k_1^{1/4}$). No impact of change in k_1 on c (iii) is indicated by
 33 Eq. (1) (Koch's equation). A negative impact of faster
 34 phage adsorption (iv) can be seen with Eq. (9) (fast
 35 adsorption) and may be approximated by Eq. (6)
 36 (equilibrated adsorption) when bacterial densities are high
 37 (such that $N_o k_1 / k_{-1} \gg 1$). This interpretation is another way
 38 of saying that if fast adsorption can inhibit rates of plaque
 39 growth by too-efficiently converting diffusing virions into
 40 infecting virions, then even faster adsorption should be
 41 more inhibiting. However, the results of the plaque
 42 formation simulation of You and Yin (1999) contradict
 43 the existence of such a negative impact, instead suggesting
 44 that with faster adsorption c proportionally approaches k_1^0
 45 rather than the $k_1^{-1/2}$ value indicated by Eq. (9) (or by Eq.
 46 (6) at higher lawn bacterial densities). See Fig. 1C for a
 47 graphical summary of these predictions.

48 The trends are thus: A sufficiently low rate either of
 49 phage encounter with bacteria, or low probability of
 50 successful phage adsorption per encounter, should select
 51 for higher rates of phage adsorption in a given encounter.
 52 This is observed with Eq. (8) (slow adsorption) or is seen in
 53 Eq. (6) under conditions where bacterial densities are low.
 54 In other words, reduced opportunities for adsorption by

55 various means could result in selection for increases in rates
 56 of phage adsorption, given encounter with a bacterium. 59

60 4.4. Relative impact of changes in bacterial density on rates 61 of plaque enlargement

62 In addition to phage properties—latent period, burst
 63 size, and adsorption rate—we can also consider the impact
 64 of lawn bacterial density (N_o) on rates of plaque enlarge-
 65 ment. Note that there exists a resemblance between the
 66 individual equations when graphed varying the phage
 67 adsorption intrinsic binding rate (Fig. 1C) versus varying
 68 cell density (Fig. 1D; comparing especially the right side of
 69 panel C with the left side of panel D). This is because rates
 70 of plaque-size increase are similarly dependent on these two
 71 parameters (that is, see the consistent pairing of $N_o k_1$ in
 72 Eqs. (6)–(9)), just as are rates of phage adsorption in broth
 73 (Stent, 1963). Consistently, if phage are less effective (i.e.,
 74 slower) adsorbers, as seen with Eq. (8), then they can
 75 benefit from greater cell density (thus the upward slope of
 76 curve 8 in Fig. 1D). However, because a given virion
 77 cannot simultaneously diffuse and infect, there are limits,
 78 in terms of plaque growth rates, to the benefits of faster
 79 adsorption. Thus, for phage which are already fast
 80 adsorbers (Eq. (9)), an increase in bacterial density can
 81 bias phage even further toward infecting bacteria rather
 82 than diffusing outward within plaques, resulting in a
 83 predicted negative impact of increasing bacterial density on
 84 rates of plaque enlargement (the downward slope of curve
 85 9 in Fig. 1D). This prediction of slower plaque growth with
 86 greater bacterial density is consistent with the results of
 87 Mayr-Harting (1958), who measured plaque growth rates
 88 as a function of seeded bacterial density.

89 With Eq. (6) (reversible adsorption), the smaller the
 90 value of the expression, $N_o k_1 / k_{-1}$, the more similar a
 91 change in c as a function of N_o is to that seen with Eq. (8)
 92 (slow adsorption; $c \propto N_o^{1/2}$; Fig. 1D). As $N_o k_1 / k_{-1}$ becomes
 93 large relative to 1, then $(1 + N_o k_1 / k_{-1})$ approaches $N_o k_1 /$
 94 k_{-1} and Eq. (6) instead comes to resemble Eq. (9) (fast
 95 adsorption; $c \propto N^{-1/2}$; Fig. 1D) with regard to the impact of
 96 changes in N_o on c . Indeed, we expect from Eq. (6) that
 97 increasing lawn bacterial densities will result in increasing
 98 rates of plaque enlargement until N_o approaches k_{-1} / k_1 .
 99 Rates of plaque enlargement as a function of lawn bacterial
 100 density will then decline as N_o becomes larger. It is
 101 important to realize, though, that no consideration in these
 102 analyses has been made toward the impact of bacterial
 103 densities on phage growth parameters, e.g., such as can
 104 occur as bacteria enter stationary phase.

105 A negative consequence of increasing lawn bacterial
 106 densities is not apparent if phage display larger burst sizes,
 107 as modeled using Eq. (7) (Fig. 1D). We speculate that
 108 greater burst sizes can supply sufficient numbers of
 109 outward-diffusing phages that rates of plaque formation
 110 are less affected by losses due to phage adsorption than
 111 they would be with smaller burst sizes. Consistently,
 112 increasing burst size is predicted to be more important
 113

when adsorption is fast, as seen with Eq. (9) (Fig. 1B). Thus, excessive biases toward infection, whether via longer latent periods (Fig. 1A), greater rates of adsorption (Fig. 1C), or more bacteria (Fig. 1D), all tend to lead to reductions in rates of plaque-size increase, though these biases to a degree may be overcome by supplying more phage to the pool of diffusing virions (Fig. 1B).

4.5. Conclusions

Phage population growth within well-mixed broth culture—an environment that is not spatially structured—has been considered by a number of authors, especially in terms of tradeoffs and the evolution of phage latent period (e.g., Abedon, 1989, 1994, 2006; Abedon et al., 2001, 2003; Breitbart et al., 2005; Bull et al., 2004; Bull, 2006; Levin and Lenski, 1983; Wang et al., 1996; Wang, 2006). However, for the most part the resulting findings are not presented in a form that allows comparison to the predictions presented in here. Furthermore, comparison of the impact of parameter values on rates of phage population growth within broth and rates of phage spread within plaques is difficult because the former is a description of phage increase in number whereas the latter is a description of phage movement through space. Nevertheless, there should be little contention that phage broth growth would be enhanced given shorter phage latent periods, faster phage diffusion, larger phage burst sizes, or, to a point, greater bacterial densities (unpublished observations based on simulations). That is, those things which allow phage to find new bacteria to infect faster (i.e., shorter generation times) and produce more phage progeny upon doing so (greater per-infection phage fecundity) should lead to faster phage population growth within well-mixed broth. However, consideration of just how such rates of phage growth within broth culture should vary as a function of changes in these values, under different conditions, is beyond the scope of this study.

Here, instead, we have focused on consideration of the impact of phage growth parameters (and bacterial density) on rates of phage spread during growth within plaques. This is distinct from overall plaque fecundity, which could serve as an alternative measure of phage growth within plaques. In the absence of tradeoffs—such that modification of parameter values toward greater plaque wavefront velocity does not have the effect of changing other parameter values toward reduced wavefront velocity—we have an expectation that faster diffusion, shorter latent periods, and larger burst sizes all will give rise to faster rates of plaque-size increase. Seemingly, however, only faster diffusion would contribute to greater wavefront velocities under all circumstances of spatially constrained phage population growth, and there may be less of an impact of rates of bacterial acquisition on rates of plaque expansion than on rates of phage population growth within broth. Testing these hypotheses, especially quantitatively, will require well-behaved phage variants which differ in

terms of individual growth parameters, such as burst size but not latent period nor adsorption rate. The goal, ultimately, should be the development of robust, quantitatively predictive models of phage population expansion within spatially structured environments, including such environments as they exist outside the laboratory.

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Appendix A. Consideration of Ortega-Cejas et al. (2004) model

The Ortega-Cejas et al. (2004) equations represent a refinement on the efforts of Yin and McCaskill (1992). For completeness we consider in this appendix the Ortega-Cejas et al. model, indicating as we do why we feel that this model and that of Koch (1964) are algebraically equivalent under typical laboratory plating conditions.

A.1. Two equations valid under different conditions

The Ortega-Cejas et al. (2004) model consists of two equations. The first consists of

$$c = 2 \left[\frac{D}{L} \frac{(1-f)}{(1+(f/x))} \left(\frac{L(B-1)N_o k_1}{(1+L(B-1)N_o k_1)} \right) \right]^{1/2}, \quad (10)$$

where $f = N_o/N_{max}$ (Section 3.1) and x describes a bacterium's shape (Fort and Méndez, 2002). Note that Eq. (10) includes the substitution of $(L(B-1)k_1 N_{max})^{-1}$ for " f_o " as indicated by Ortega-Cejas et al., plus has been subject to subsequent algebraic manipulation. The second equation consists of

$$c = \left[\frac{2D}{L} \frac{(1-f)}{(1+(f/x))} \right]^{1/2} \quad (11)$$

Eqs. (10) and (11) may be presented in a form equivalent to those of Eqs. (5) through (9):

$$c = 10 \left(\frac{D}{L} \right)^{1/2} \cdot (BN_o k_1)^{1/2} \cdot 0.20 \times \left[\frac{L(1-f)}{(1+LBN_o k_1)(1+(f/x))} \right]^{1/2}, \quad (12)$$

$$c = 10 \left(\frac{D}{L} \right)^{1/2} \cdot (BN_o k_1)^{1/2} \cdot 0.14 \times \left[\frac{(1-f)}{BN_o k_1(1+(f/x))} \right]^{1/2}. \quad (13)$$

1 These two equations are valid under different conditions
 2 (Section A.2).

3
 4
 5 *A.2. Relative importance of Eq. (11) under laboratory
 6 conditions*

7 Of interest are the conditions under which Eqs. (10) and
 8 (11) of Ortega-Cejas et al. (2004) differentially hold, which
 9 is when $0 \leq f \leq f_o$ versus $f_o \leq f \leq 1$, respectively. For f
 10 equivalent to f_o we can factor out extraneous variables:

$$11 \quad f \equiv f_o \rightarrow \frac{N_o}{N_{max}} \equiv \frac{1}{L(B-1)k_1 N_{max}},$$

$$12 \quad \rightarrow N_o \equiv \frac{1}{L(B-1)k_1}. \quad (14)$$

13 Thus, for Eqs. (10) and (11), respectively,

$$14 \quad N_o \leq \frac{1}{L(B-1)k_1} \quad \text{or} \quad N_o \geq \frac{1}{L(B-1)k_1}. \quad (15)$$

15 From Eq. (14) we can consider the utility of Eqs. (10) and
 16 (11) in terms of bacterial densities, phage latent periods,
 17 phage burst size, and rates of phage adsorption.

18 Garen (1954) reported a phage T1 binding constant to
 19 bacteria (k_1) that was in the range of 10^{-9} ml/min (see also
 20 Ortega-Cejas et al., 2004; You and Yin, 1999), burst sizes
 21 tend to range from about 25 to 200 phage per bacterium,
 22 and latent periods range from about 10 to 30 min and
 23 upward. Assuming 10^{-9} ml/min, 11 phage/bacterium, and
 24 10 min as the respective parameter values, then
 25 $(L(B-1)k_1)^{-1} \approx 1 \times 10^7$, or lower with greater rates of
 26 phage adsorption, larger phage bursts, or longer phage
 27 latent periods, e.g., such as those measured or employed by
 28 Garen (1954) or, subsequently, by Yin and McCaskill
 29 (1992). During plaque development within the laboratory,
 30 $\sim 10^7$ bacteria/ml would be at best a minimum value for N_o .
 31 Therefore, under most circumstances that we can imagine,
 32 at least in the laboratory or except for extremely slowly
 33 adsorbing phage (e.g., Kasman et al., 2002), f will exceed f_o ,
 34 and therefore Eq. (11) rather than Eq. (9) will describe
 35 plaque wavefront velocity. The result is a prediction of a
 36 reduced or modified impact of various parameters on
 37 plaque wavefront velocity, as Eq. (11) replaces Eq. (10),
 38 that can result from higher bacterial densities (N_o), longer
 39 latent periods (L), larger burst sizes (B), or faster
 40 adsorption (k_1).

41 Eqs. (10) and (11) are identical for
 42 $N_o = (L(B-1)k_1)^{-1} \approx (LBk_1)^{-1}$. While the bacterial den-
 43 sities at this break point are low compared with typical
 44 bacterial densities during plaque formation, in fact during
 45 phage propagation outside of the laboratory there is
 46 greater potential for these conditions to be met since
 47 bacterial densities may be low, phage adsorption slower,
 48 and burst sizes small (i.e., such that $N_o < (LBk_1)^{-1}$).
 49 Though not numerically identical, nevertheless with
 50 increasing bacterial densities the behavior of Eq. (10) is
 51 equivalent to that of Eq. (11) in that both define wavefront

velocities that do not vary except as a function of latent
 period and diffusivity.

A.3. Equivalence of Eq. (11) to Koch's equation

Ortega-Cejas et al. (2004) take into account, in their
 equations, suggestions that D can decline as a function of
 N_o . That is, Yin and McCaskill (1992) propose that

$$D > D_{effective} = D2(1-f)/(2+f), \quad (16)$$

while Ortega-Cejas et al. (2004) supply a similar

$$D > D_{effective} = D(1-f)/(1+f/x). \quad (17)$$

Though potentially important toward understanding phage
 plaque formation, for a number of reasons we suggest that
 this phage “effective diffusion” may be ignored when
 comparing the model of Ortega-Cejas et al. model with
 those of Koch (1964) or Yin and McCaskill (1992). For
 instance, such declines (i) may be important only at
 relatively high bacterial densities, (ii) have been inferred
 (Yin and McCaskill, 1992) but not robustly experimentally
 demonstrated during plaque formation (indeed, in liquid
 culture as many as 2.5×10^{10} resistant bacteria/ml has been
 found to be insufficient to impact phage adsorption to
 6×10^7 sensitive bacteria/ml; Stent and Wollman, 1952),
 (iii) may be validly included in any of the presented models
 (i.e., by replacing D with $D_{effective}$), and (iv) would have
 exactly the same effect on wavefront velocity in all seven
 Eqs. ((5) through (9) plus (10) and (11), since D is modeled
 in the same manner in each). For these reasons, for the
 comparison of the models of Ortega-Cejas et al. model with
 those of Koch (1964) or Yin and McCaskill (1992) we
 replace the effective diffusion expression found in Eqs. (12)
 and (13) with simply D , as indicated in Eq. (17). Thus, Eqs.
 (12) and (13) may be simplified to

$$c = 10 \left(\frac{D}{L} \right)^{1/2} \cdot (BN_o k_1)^{1/2} \cdot 0.20 \left[\frac{L}{(1 + LBN_o k_1)} \right]^{1/2}. \quad (18)$$

$$c = 10 \left(\frac{D}{L} \right)^{1/2} \cdot (BN_o k_1)^{1/2} \cdot 0.14 \left[\frac{1}{BN_o k_1} \right]^{1/2}. \quad (19)$$

We note, with this manipulation, that Eq. (19) is equal to
 Eq. (5), i.e., that of Koch, multiplied by 0.14 (which is a
 rounded square root of 2 that has been divided by 10).
 Since Eq. (18) should be less valid under typical laboratory
 plaquing conditions (Section A.2), we therefore consider,
 for the purposes of this study, an algebraic equivalence of
 the Koch and Ortega-Cejas et al. models. Additional
 considerations of the latter model are presented elsewhere
 (Fort et al., 2006; Fort and Méndez, 2002).

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