DOI: 10.1534/genetics.105.045922

Lysis Timing and Bacteriophage Fitness

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Manuscript received May 20, 2005
Accepted for publication September 29, 2005

ABSTRACT

The effect of lysis timing on bacteriophage (phage) fitness has received little theoretical or experimental attention. Previously, the impact of lysis timing on phage fitness was studied using a theoretical model based on the marginal value theorem from the optimal foraging theory. An implicit conclusion of the model is that, for any combination of host quantity and quality, an optimal time to lyse the host would exist so that the phage fitness would be the highest. To test the prediction, an array of isogenic λ-phages that differ only in their lysis times was constructed. For each phage strain, the lysis time, burst size, and fitness (growth rate) were determined. The result showed that there is a positive linear relationship between lysis time and burst size. Moreover, the strain with an intermediate lysis time has the highest fitness, indicating the existence of an optimal lysis time. A mathematical model is also constructed to describe the population dynamics of phage infection. Computer simulations using parameter values derived from phage λ-infection also showed an optimal lysis time. However, both the optimum and the fitness are different from the experimental result. The evolution of phage lysis timing is discussed from the perspectives of multiple infection and life-history trait evolution.

LYSIS of the infected bacterial host is the last event in the infection cycle of a lytic bacteriophage (phage). The molecular mechanisms of phage lysis have been studied quite extensively (Young 1992; Wang *et al.* 2000; Young *et al.* 2000). However, the potential impact of lysis timing, traditionally called the latent period (Ellis and Delbrück 1939), on phage fitness has received few theoretical or experimental examinations (Abedon 1989; Wang *et al.* 1996; Abedon *et al.* 2001, 2003). A lysis-defective phage can continue to accumulate progeny virions intracellularly long beyond the normal time of lysis (Hutchison and Sinsheimer 1966; Josslin 1970; Reader and Siminovitch 1971), resulting in up to a 10-fold increase in virions per cell (Wang *et al.* 1996). Thus, host lysis is not prompted by lack of exploitable resource inside the infected host, and, in principle, a delay in the timing of lysis can lead to an immediate benefit of increased fecundity by producing more progeny per infected host. To explain why lysis occurs long before the production capacity of the host is exhausted, Wang *et al.* (1996) adopted the marginal value theorem from the optimal foraging theory (Charnov 1976; Stephens and Krebs 1986) to explore ecological factors influencing the evolution of phage lysis timing. Two factors were examined: the host quantity (density), which dictates the average time for a phage to find and infect an uninfected host, and the host quality (physiological state), which influences the rate by which the phage progeny are assembled/matured. The analysis showed that for any given ecological setting, an optimal lysis time would exist that results in the highest phage fitness. Also, the optimal lysis time is shorter under the conditions of high host quantity or quality, and vice versa. From this study, the existence of an optimal lysis time can be intuitively understood as the result of the trade-off between a present immediate linear gain by extending the vegetative cycle of the phage and a future uncertain exponential gain derived from lysing the current host and releasing the progeny virions (Wang *et al.* 2000; Young 2002; Bull *et al.* 2004).

To release the accumulated progeny to the environment, a phage has to overcome the physical barrier of the host envelope, consisting of the cytoplasmic membrane, the cell wall, and, in the case of Gram-negative bacteria, the outer membrane. The molecular mechanisms for overcoming these barriers have been studied extensively (Young 1992; Wang *et al.* 2000; Young *et al.* 2000). For phages with complex, double-stranded nucleic acid genomes (generally 15 or more genes), a phage-encoded membrane protein (the holin) forms a lesion in the cell membrane at a programmed, allele-specific time. As a result of the disruption of the membrane, the phage-encoded endolysin (a muralytic enzyme) is able to attack the cell wall, leading to lysis within seconds (Gründling *et al.* 2001). Holins are extremely diverse, found in many unrelated sequence families with at least three membrane topologies, suggesting that they may have evolved from multiple distinct origins (Wang *et al.* 2000).

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The holin-endolysin system, although apparently universal among complex phages, is not used by the simpler lytic phages like the single-stranded, circular DNA phages (Microviridae) and the single-stranded RNA phages (Leviviridae). For these viruses, lysis is effected by a single gene without a phage-encoded muralytic activity. In two cases, the single lysis gene encodes an inhibitor of a specific step in cell wall biosynthesis, and lysis is thus formally analogous to that obtained by treating the host cells with a cell wall antibiotic (Bernhardt *et al.* 2000, 2001, 2002).

The most extensively studied system of phage lysis is that of λ (Young 1992; Wang *et al.* 2000). The genes involved in λ-lysis are clustered in a lysis cassette, consisting of four genes, *S*, *R*, *Rz*, and *Rz1*, encoding a total of five proteins. The *S* gene encodes two proteins: the holin S105, for which the initiation codon is Met3 and extends 105 residues, and S107, beginning at Met1 and differing from S105 by the amino-terminal sequence Met-Lys. Although the sequences are nearly identical, the two *S* gene products have opposing function, for S107 is the "antiholin," which binds to S105 and inhibits its lytic function (Gründling *et al.* 2000c). S105 consists mainly of three α-helical transmembrane domains with short intervening loop regions (Gründling *et al.* 2000a). S105 accumulates in the membrane without any detectable deleterious effect on the cell, until it suddenly triggers and disrupts the host membrane, after which lysis ensues within seconds (Gründling *et al.* 2001). Both S105 and S107 are expressed during the infection. The proportion of S105 and S107 is determined by RNA structures in the 5′-end of the *S* gene and is, for wild-type *S*, ~2:1. Altering these structures can lead to different proportions of S105 and S107, and, in general, the higher the amount of S107 is compared to S105, the slower the lysis time. In fact, when S107 is produced in excess of S105, spontaneous lysis triggering does not occur (Bläsi *et al.* 1989). In contrast, the absence of S107 has a relatively minor effect; the *S105* allele (in which the start codon for S107 is abolished) causes lysis 5–8 min earlier than S^+ (*S* wild type), which, under standard conditions, allows lysis at ~50–52 min after induction.

As noted above, holin triggering, although lethal to the cell, does not *per se* cause rapid lysis. Lysis requires the endolysin R, a 158-residue lytic transglycosylase that accumulates fully folded and functional in the cytosol throughout the latent period and gains access to its substrate, the murein layer, when the holin disrupts the membrane. The lesion formed by the S protein has not been described; however, it is large enough to allow lysis by intact fusions of the R protein and full-length β-galactosidase (Wang *et al.* 2003). The functions of two other proteins, Rz and Rz1, although dispensable in standard conditions, are required for host lysis in the presence of high concentrations of divalent cations (*e.g.*, 20 mM Mg^{2+}) known to stabilize the outer membrane (Young *et al.* 1979; Zhang and Young 1999).

Not all holin-endolysin systems involve the release of cytoplasmic endolysin through holin-mediated lesions. Recently, endolysins with secretory signals have been described. In these cases, the endolysin is exported outside the cell membrane by the host *sec* system but is inactive until activated by holin-mediated membrane disruption (Sao-Jose *et al.* 2000; Xu *et al.* 2004, 2005). Such findings accentuate the general role of holins: to act as a molecular timer for lysis. By using a holin as a timing mechanism, the phage ensures that the membrane integrity, hence the metabolic capacity, of the host cell is maintained while the progeny virions are being synthesized and assembled, and at the same time an excess of endolysin is being accumulated. At a proper time, presumably dictated by past environmental conditions, the phage effects a prompt and efficient lysis to release its progeny.

To be able to evolve toward an optimal lysis time under any ecological setting, three conditions are necessary: (1) there is a genetic basis for lysis timing, (2) different alleles for the gene(s) responsible for lysis timing can result in different lysis times, and (3) differences in lysis timing can be translated into differences in fitness. The existence of holin-endolysin genes in large phages demonstrated that there is a dedicated system for host lysis. In this study, the existence of an optimal lysis time predicted by a previous theoretical model is tested with isogenic λ-strains that differ only in their *S* genes.

MATERIALS AND METHODS

Bacterial and phage strains: All the bacterial and phage strains used in this study are listed in Table 1.

Construction of isogenic λ-phages with different lysis times: Plasmids carrying different λ *S* alleles were originally constructed for the purpose of mapping the topology of λ S protein, using the cysteine-scanning method (Gründling *et al.* 2000a,b). All are derived from the plasmid pS105, which is itself a derivative of pBR322 containing the λ-lysis cassette (Smith *et al.* 1998). The phage strain, λ-*cI*857 *Sam*7, was used as the parental strain for isogenic strain construction. First, the lysogen IN158 (MC4100 [λ-*cI*857 *Sam*7]) was transformed with each *S* plasmid. A culture of the transformant was grown to an $A_{550} \approx 0.2$ in 25 ml LB plus 100 μg ml^{-1} ampicillin at 30° in a shaking water bath. To induce the excision of the prophage from the host chromosome, the culture was then transferred to 42° for 15 min and then 37° until lysis (Chang *et al.* 1995). The lysate was then plated for plaque-forming phages, which are the results of either reversion of the parental *Sam*7 allele or recombination between the prophage-borne and the plasmid-borne *S* alleles (Figure 1). To confirm the sequence identity, four single plaques were picked from each lysate and individually mixed with 10 μl MC4100 overnight culture in 1 ml LB and let grow at 37° for several hours. Supernatant of the lysate was used directly as the template for PCR DNA sequencing to screen for the phage strain carrying the desired *S* allele. Once the correct allele was identified, the same supernatant was then used to lysogenize MC4100. The resulting lysogen was again PCR DNA sequenced to confirm the identity of the *S* allele. Each isogenic λ-strain was then heat induced from the lysogen as described above. The lysate was cleared by centrifugation and stored at 4° after mixing with 1%

TABLE 1

List of the bacterial and phage strains

Strains	Genotype[a]	Source
Bacterial strains		
MC4100	*lac*Δ_{U169} *araD139 relA rpsL thi*	Silhavy *et al.* (1984)
IN158	MC4100 (λ *cI*857 *Sam7*)	This study
IN63	MC4100 (λ *cI*857 $S105_{C51S/S76C}$)	This study
IN61	MC4100 (λ *cI*857 $S105_{C51S}$)	This study
IN67	MC4100 (λ *cI*857 $S105_{C51S/I13C}$)	This study
IN68	MC4100 (λ *cI*857 $S105_{C51S/L14C}$)	This study
IN69	MC4100 (λ *cI*857 $S_{C51S/L14C}$)	This study
IN62	MC4100 (λ *cI*857 *S105*)	This study
IN64	MC4100 (λ *cI*857 $S_{C51S/F94C}$)	This study
IN56	MC4100 (λ *cI*857 *S*)	This study
IN76	MC4100 (λ *cI*857 S_{HK97})	This study
IN77	MC4100 (λ *cI*857 $S_{H7N/L10/F78}$)	This study
IN66	MC4100 (λ *cI*857 S_{S68C})	This study
Phage strains		
All phage strains listed in Table 2 were thermally induced from the above lysogens.		

[a] The genotype *S105* indicates the presence of an *M1L* mutation that abolishes the expression of the S107 protein.

$CHCl_3$. Note that, as a result of intragenic recombination, several *S* alleles listed in Table 1 (*e.g.*, in lysogens IN64, IN66, and IN69) showed recombinant genotypes.

Phage plating: To obtain a consistent plaque size to facilitate plaque counting, the plating conditions were rigorously standardized as follows. The bacterial strain MC4100 was grown in 3 ml TB broth plus 0.2% maltose (TBM) at 37° overnight. After addition of 3 ml TBM the culture was grown at 37° for another 1.5 hr. For plating, 100 μl of these cells was mixed with 100 μl of phage suspension appropriately diluted with λ-dil (Davis *et al.* 1980). After incubation at 37° for 20 min, 2.5 ml molten H-top agar (Miller 1992) was added. The mixture was lightly vortexed, poured on a freshly prepared plate (1–2 days old) containing 35 ml LB agar, and incubated at 37° for 18 hr before plaque counting.

Lysis time determination: A standardized method similar to the previously described procedure (Chang *et al.* 1995) was used to determine the lysis time for each λ *S* allele. Briefly, a 25-ml culture of λ-lysogen was grown to $A_{550} \approx 0.2$ with constant aeration in a 30° water bath. The culture was then aerated at 42° for 15 min for thermal induction of the prophage, followed by aeration at 37° until lysis. Beginning ~5–6 min before the onset of lysis, the A_{550} of the induced culture was recorded every 2 min using a spectrophotometer equipped with a fast-acting sipper device.

Burst size determination: A standard procedure for determination of phage burst size (number of phage progeny produced per infected bacterial cell), as developed by Delbrück (Ellis and Delbrück 1939; Delbrück 1940), was used for each phage construct. Phage at a concentration of $2–3 \times 10^7$ PFU ml^{-1} was mixed with MC4100 at 2×10^8 cells ml^{-1} ($A_{550} \approx 0.6$ in TBM) and 5 mM $MgSO_4$ for 15 min at 37° and then diluted 10^4-fold in LB broth, prewarmed to 37°, to a final culture volume of 10 ml. A 1-ml volume was withdrawn to determine the concentration of total phage [*T, i.e.*, unadsorbed phages (free-floating phages) plus infective centers (bacterial cells infected with phages)], while another 1-ml aliquot, after vortexing with 2% $CHCl_3$, was used to determine the concentration of the unadsorbed phage (*U*). The remaining culture was shaken at 37° for 75 min. After adding $CHCl_3$ to a final concentration of 2% and shaking for another 5 min, the culture was appropriately diluted with λ-dil, and the final phage concentration (*F*) was determined. The burst size is calculated as $b = F/(T - U)$.

Fitness determination: The phage growth was conducted by adding phages (~2000 PFU ml^{-1}) and exponentially grown MC4100 cells (~3×10^5 cells ml^{-1}) to 3 ml LB broth plus 5 mM $MgSO_4$. The mixture was incubated at 37° and rolled in the tissue culture roller drum (New Brunswick Scientific, Edison, NJ) at setting 7. The fitness is calculated as $w = \ln(P_5/P_0)/5$, where P_5 and P_0 are the free phage concentrations at times 0 and 5 hr, respectively.

RESULTS

Isogenic λ-strains: Figure 2 shows the mutations of the 11 *S* alleles used in this study mapped onto the membrane topology of the λ S protein (Gründling *et al.*

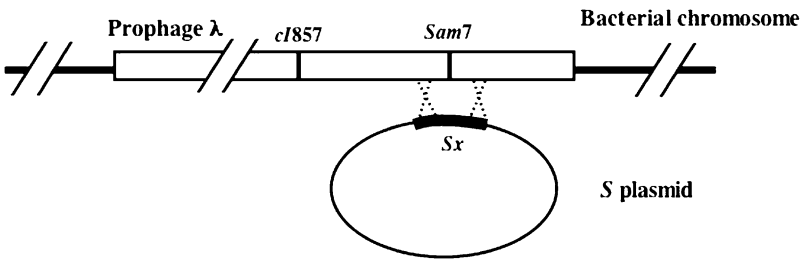

Figure 1.—Construction of isogenic λ-strains. Various plasmids carrying different λ *S* alleles (*Sx*) were each transformed into the *E. coli* lysogen carrying a prophage λ with mutations in *cI* (*cI*857) and *S* (*Sam7*) genes. After thermal induction of the lysogen, a mixture of phage progeny is produced, but only ones carrying the *Sx* allele (as a result of homologous recombination between prophage genome and the plasmid) can form plaques when plated on the bacterial host.

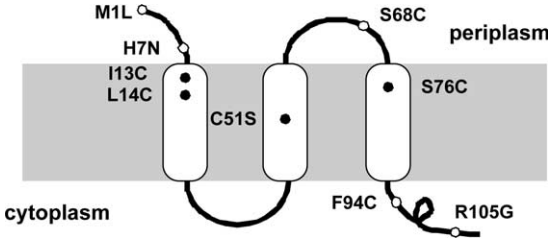

FIGURE 2.—Topology of the λ-S105 holin protein. The S105 protein has three α-helical transmembrane domains (rounded rectangles) with an N-out, C-in configuration. For various *S* alleles used in this study, mutations are also mapped onto the secondary structure. See Table 2 for the *S* allele genotypes.

2000a). Alleles with the M1L mutation produce only the S105 protein, while wild type produces both S105 and S107. *S* alleles carrying the C51S mutation are the ones previously used for the purpose of cysteine scanning (GRÜNDLING *et al.* 2000a). Also, the *S* allele from the lambdoid phage, HK97 (JUHALA *et al.* 2000), was used in this study.

As shown in Figure 2, these mutations are relatively evenly distributed throughout the entire secondary structure, including the N terminus, each of the three transmembrane domains, one intervening loop region, and the C terminus. As has been known for a while, there does not seem to be a readily discernible pattern that correlates length of lysis time with type of the mutation and/or position of the mutation (see Table 2) (RAAB *et al.* 1988; GRÜNDLING *et al.* 2000a).

Lysis time determination: Figure 3 shows examples of typical lysis curves from thermally induced λ-lysogens. At a particular time after induction, the turbidity of the culture, as measured by A_{550}, declines precipitously. The onset of the decline is a characteristic of each λ *S* allele. In this study, the lysis time is defined as the time point where the thermally induced lysogen culture first shows a consistent decline in its turbidity. Since this is a population phenomenon, other criteria, such as the midpoint of the declining part of the lysis curve, may also be defined as the lysis time. However, since all the lysis curves have a similar slope in their declining part (data not shown), a midpoint definition will change only the numerical value of each lysis time, but not its ranking.

Table 2 lists the measured lysis times and the associated standard errors of the 11 *S* alleles. As shown in Table 2, the lysis time for each *S* allele, as determined under the carefully standardized laboratory conditions, is quite reproducible, with differences of 1–2 min between replicates. Within the collection of *S* alleles, the lysis time ranges from 28.3 to 63.0 min.

Lysis times and phage fitness: Depending on the purpose, several operational definitions of phage fitness have been used in the literature (BULL *et al.* 1997; CHAO and TRAN 1997). In this study, the phage fitness is defined as $w = [\ln(P_t/P_0)]/t$, where P_0 and P_t are the phage concentrations at hours 0 and t after infection, respectively. Preliminary studies were conducted first to assess the proper assay period t so that it would allow several cycles of infection without depleting too many host cells such that it becomes a limiting factor for phage growth. As shown in Figure 4, after 5 hr of coculturing of bacterial host and phages, the apparent bacteria-to-phage ratio is ~1.0, indicating that host availability would not be a limiting factor before the end of the 5-hr assay period.

The fitness of each isogenic λ-strain is listed in Table 2 and its relationship with lysis time is shown in Figure 5.

TABLE 2

Lysis times, burst sizes, and fitness of isogenic λ-strains

	Measurements ± SE[c] (n[d])		
Phage name	Lysis time (min)	Burst size (PFU cell^{-1})	Fitness (hr^{-1})
λ($S105_{C51S/S76C}$)	28.3 ± 0.75 (3)	9.7 ± 1.04 (3)	1.89 ± 0.039 (3)
λ($S105_{C51S}$)	35.0 ± 0.65 (3)	62.3 ± 4.95 (3)	2.71 ± 0.025 (5)
λ($S105_{I13C/C51S}$)	37.5 ± 0.54 (4)	58.2 ± 3.22 (4)	2.69 ± 0.022 (3)
λ($S105_{L14C/C51S}$)	38.5 ± 1.04 (4)	85.3 ± 6.69 (4)	2.70 ± 0.020 (3)
λ($S_{L14C/C51S}$)	38.5 ± 1.04 (4)	62.5 ± 4.08 (3)	2.71 ± 0.039 (3)
λ(S105)	42.7 ± 0.38 (3)	88.5 ± 4.08 (3)	2.72 ± 0.037 (5)
λ($S_{C51S/F94C}$)	42.7 ± 0.38 (3)	79.7 ± 7.36 (3)	2.60 ± 0.027 (3)
λ(*S*)[a]	*51.0 ± 0.65 (3)*	*170.0 ± 10.46 (4)*	*2.83 ± 0.024 (4)*
λ(S_{HK97})[b]	53.7 ± 0.75 (3)	215.9 ± 13.07 (3)	2.19 ± 0.061 (3)
λ($S_{H7N/L10/F78}$)[b]	55.3 ± 0.65 (6)	213.47 ± 5.17 (3)	2.11 ± 0.064 (3)
λ(S_{S68C})	63.0 ± 1.13 (3)	255.0 ± 22.19 (5)	2.47 ± 0.030 (3)

[a] Italics indicate the wild-type *S* allele.

[b] The λ($S_{H7N/L10/F78}$) *S* allele has one nonsynonymous mutation at H7N and two synonymous mutations at codon positions 10 and 78. The genotype of λ(S_{HK97}) is like that of λ($S_{H7N/L10/F78}$), except that it also carries an additional R105G mutation.

[c] The standard errors have been corrected for small sample sizes according to SOKAL and ROHLF (1995), p.53.

[d] Number of replicates.

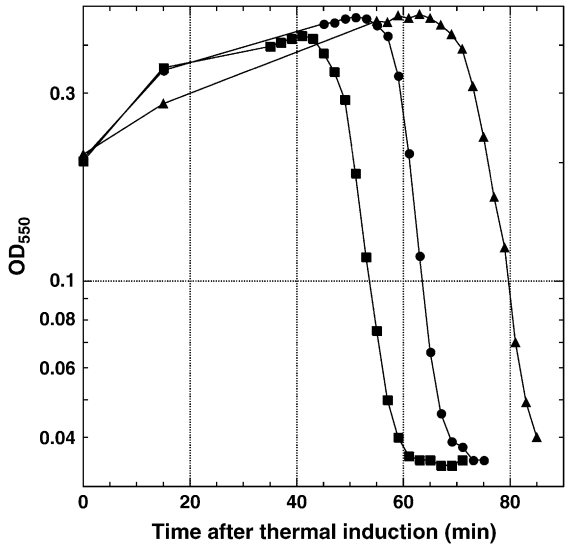

FIGURE 3.—The lysis curves of three λ *S* alleles. The culture turbidity (in A_{550}) was sampled every 2 min using a sipper-equipped spectrophotometer. In this particular example, the lysis times for λ($S105_{L14C/C51S}$) (■), λ(Swt) (●), and λ(S_{S68C}) (▲) are 41, 51, and 63 min, respectively.

Since there is no *a priori* quantitative model describing the relationship between lysis time and phage fitness, a quadratic function was used to fit the entire data set. With the current data set, the estimated maximum fitness is 2.71 hr^{-1} and the optimal lysis time is 44.62 min [$y = 2.486 + 0.005x - 0.002(x - 43.816)^2$, $P = 0.0006$, using the statistical package of JMP v.5.0.1a for the Macintosh computer]. In contrast, linear regression analysis of the same data set is not significant ($P = 0.6615$). This result supports the general expectation

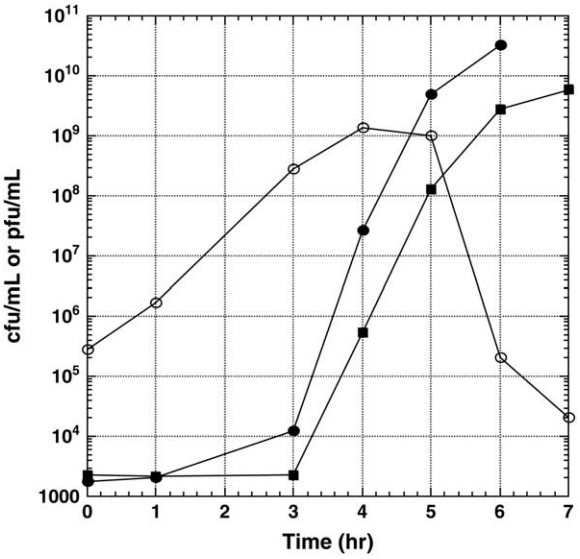

FIGURE 4.—An example of the population dynamics during fitness assay. The concentrations of *E. coli* (○) infected with λ(S105) (●) were plotted against time. Another phage strain, λ($S105_{C51S/S76C}$) (■), was also plotted (the corresponding host concentration is not shown).

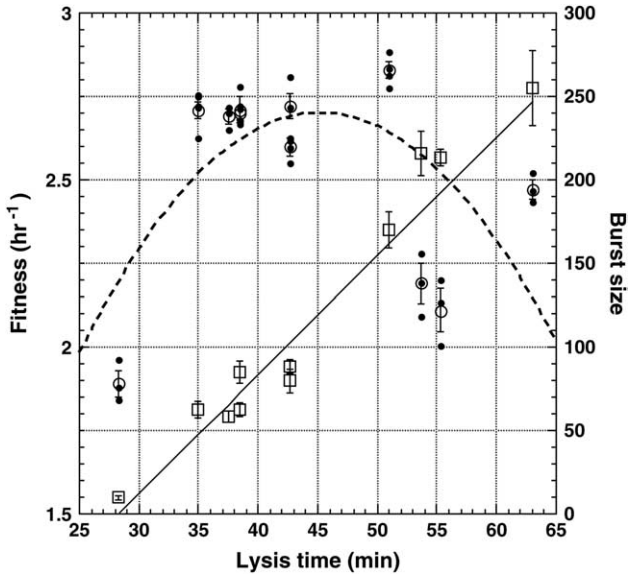

FIGURE 5.—The effect of lysis time on burst size and fitness. For each of the 11 *S* alleles, burst size (□), individual fitness measurements (●), and average fitness (○) are plotted against their corresponding lysis times. Error bars indicate one standard error. The dashed curve shows the quadratic fit of the individual fitness measurements, and the solid linear line represents the regression of burst size on lysis time.

that, at least under the experimental condition, an optimal lysis time exists for phage fitness. Too long or too short a lysis time is detrimental to phage fitness.

Lysis times and burst sizes: Theoretical analysis of the optimal lysis time assumed a positive relationship between lysis time and burst size; *i.e.*, the longer the lysis time is, the larger the burst size. It is the trade-off between the current, guaranteed linear gain and a future, potential exponential gain that a phage will evolve toward an optimal lysis time. Therefore, it is important to determine that under the experimental condition there is a positive linear relationship between lysis time and burst size. This also ensures that mutations at each *S* allele do not have a negative pleiotropic effect on production of phage progeny. A plot of the burst size *vs.* the lysis time (Figure 5) clearly shows a linear relationship. Linear regression analysis shows that phage progeny matures at a rate of 7.7 phages min^{-1}, and the eclipse period (the time elapsed between phage infection and appearance of the first mature phage progeny) is ~28 min. This result also suggests that there is no obvious pleiotropic effect of the *S* alleles on phage progeny production.

Simulation of the infection dynamics: The previous optimality model (WANG *et al.* 1996) assumed an environment with constant host density and physiological state, a condition clearly violated by the current fitness assay design, namely phage growth in a tube culture. To understand the relationship between lysis timing and phage fitness under the current experimental condition, a model of phage population dynamics, modified from LEVIN *et al.* (1977), was constructed.

Since the purpose of this model is to simulate the phage population dynamics during the fitness assay in a tube culture, the growth rates of the susceptible (N) and infected (M) hosts, μ_N and μ_M, respectively, are assumed to be modulated in a density-dependent fashion, with the carrying capacity of the tube culture to be K and the total cell density to be N_T ($= N + M$). The adsorption of the phage particle (P) onto the cell surface is assumed to be a mass-action reaction, with r (the adsorption coefficient) as the rate constant. For the infected host, the phage progeny will be matured/assembled after a certain eclipse period e. After a certain lysis time l, the infected cell will be lysed and releases b amount of phage progeny (burst size). The actual burst size depends on the maturation rate v and the lysis time l in a linear fashion; *i.e.*, $b = v(l - e)$. However, the maximal burst size of an infected host is assumed to be b_{MAX}, no matter how long the lysis time is. The host-independent removal rate (phage mortality) for the phage is δ.

The model describing the infection process is shown,

$$\frac{dN}{dt} = \mu_N N\left(1 - \frac{N_T}{K}\right) - rNP$$
$$\frac{dM}{dt} = \mu_M M\left(1 - \frac{N_T}{K}\right) + rNP - M_{(t-l)}$$
$$\frac{dP}{dt} = bM_{(t-l)} - rNP - \delta P,$$

where $M_{(t-l)}$ is the infected host concentration at l time ago.

There are several things to be noted in this model. First, both the uninfected and infected host densities are assumed to contribute to the modulation of the host growth rate. This is a reasonable assumption for it has been shown that materials forming phage progeny are derived from the growth medium of the bacterial host (Adams 1959; Wang *et al.* 1996), indicating that the infected host is still metabolically active and therefore would deplete limiting nutrient in the culture, thus contributing to the regulation of bacteria growth rate. Although the infected host is metabolically active, it is in fact genetically dead; therefore the growth rate of the infected host is set to zero ($\mu_M = 0$) during simulations. Second, it is assumed that only the uninfected host can be infected by phages. Obviously, this assumption can be violated easily if the phage density is high. However, to modify this assumption, the consequence of superinfection needs to be specified. Unfortunately, this information is currently unavailable. Third, there is a built-in time delay in the model in the form of $M_{(t-l)}$, *i.e.*, the infected host at l lysis time ago. However, unlike Levin's (1977) model in which the lysis time is a model parameter with a fixed value, the length of the time delay in this study is a variable. Consequently, the burst size will differ depending on the lysis time.

To conduct the simulation, the kinetics simulation package Berkeley Madonna version 8.0.1 (Macey and Oster 1996) was used. The parameter values used in the simulation were derived from *Escherichia coli* K-12 growing in LB broth and phage λ infecting the *E. coli*. The intrinsic growth rate of the host (μ_N) is 2.04 hr^{-1} and the carrying capacity (K) of the aerated LB culture at 37° is 7.35×10^9 cells ml^{-1} (both are estimated by fitting data points with a logistic equation using the statistical package of JMP v.5.0.1a for the Macintosh computer). The adsorption coefficient (r) of laboratory phage λ is 10^{-8} $cell^{-1}$ $phage^{-1}$ ml^{-1} hr^{-1} (estimated from Hendrix and Duda 1992). The eclipse period (e) is 28 min (from this study, Figure 5). The maturation rate (v) is 7.7 phages min^{-1} (from this study, Figure 5). The maximum burst size (b_{MAX}) is 1000 (estimated from Reader and Siminovitch 1971, Figure 1). However, for the parameter values used here, the lysis time is never long enough to reach b_{MAX}. The phage mortality rate (δ) in LB at 37° is -0.163 hr^{-1} (unpublished data). To initialize the simulations, the host density of 3×10^5 cells ml^{-1} and phage concentration of 2×10^3 phages ml^{-1} were used (see materials and methods).

Figure 6 shows an example of the simulated infection dynamics, using λ(S105)'s parameter values (lysis time $l = 42.7$ min, see Table 2). Clearly, the model is able to capture the infection dynamics qualitatively. The concentration of the uninfected host first increases and then declines after being converted into the infected host. The infected host also shows a similar boom-and-bust pattern at a somewhat delayed time. The phage concentration increases steadily with time. However, by comparing it to the experimental result from λ(S105) infection (from Figure 4), it is clear that this model did not capture the quantitative aspect of the dynamics. For the first three hours of simulation, the model can roughly describe the outcome of bacteria–phage coculturing. After that, the experimental and simulation results diverge significantly. The bacterial population crashes 2 hr earlier than the simulation. This is accompanied by a much faster rise of phage population size than that of the simulation. Such a discrepancy may explain why the phage fitness estimated using the simulation data is much lower than the experimental result (Figure 7). Clearly, this simple population dynamics model could not fully describe the quantitative aspect of phage infection in a test tube environment.

DISCUSSION

Lysis time and phage fitness: The existence of a "correct" lysis time is somewhat anticipated from genetic studies of the λ-lysis system. It is known that some mutations in the λ *S* gene would result in no phage production, but for different reasons. The most dramatic example is at the amino acid sequence position 52, an alanine residue in the wild-type sequence. Neither the alanine-to-glycine (A52G) nor the alanine-to-valine (A52V) mutation can produce phage progeny.

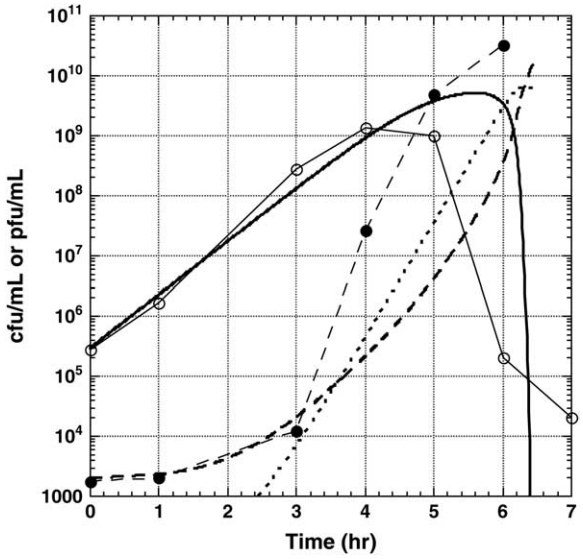

FIGURE 6.—Simulated infection dynamics in a tube culture. The uninfected host (thick solid line), infected host (short dotted line), and phage (dashed line) concentrations were plotted against time after infection. The parameter values used in the simulation are derived from phage λ(S105) with lysis time of 42.7 min and from its bacterial host *E. coli* K12 (see text for more details). The experimental data of uninfected host (thin solid line with ○) and phage (thin dashed line with ●) (from Figure 4) were superimposed on the simulation result.

The A52G mutant lyses too early, well before phage progeny can be assembled, while the A52V mutant never triggers the formation of membrane lesion to cause lysis (WANG *et al.* 2000). Naturally, between these two extremes, there should lie a lysis time that would result in progeny production and release. The question is: Would there be an optimal lysis time so that the phage fitness is the highest? By using an array of isogenic λ-strains that differ only in their lysis timing, this study has demonstrated the existence of an optimal lysis time, a conclusion from a previous theoretical model (WANG *et al.* 1996). Under the current experimental condition, the fittest phage strain is the one that lyses its host at an intermediate time. Unfortunately, it is somewhat difficult to determine exactly what that intermediate time is with the current results. This is in part due to the fact that no *S* allele that would result in lysis times between 29 and 35 min and between 43 and 51 min was used. These time gaps make the optimal lysis time difficult to pinpoint. Since we do not yet understand how different mutations in the *S* gene would result in different lysis times, these time gaps can be filled only by systematic site-directed mutagenesis and screening, a potentially laborious undertaking. Even with the current result, the existence of an optimal lysis time is contingent upon the mutant λ($S105_{C51S/S76C}$) being included in the fitness assay. In this regard, the case for the existence of an optimal lysis time will be greatly strengthened if *S* alleles lysing between 29 and 35 min can be obtained.

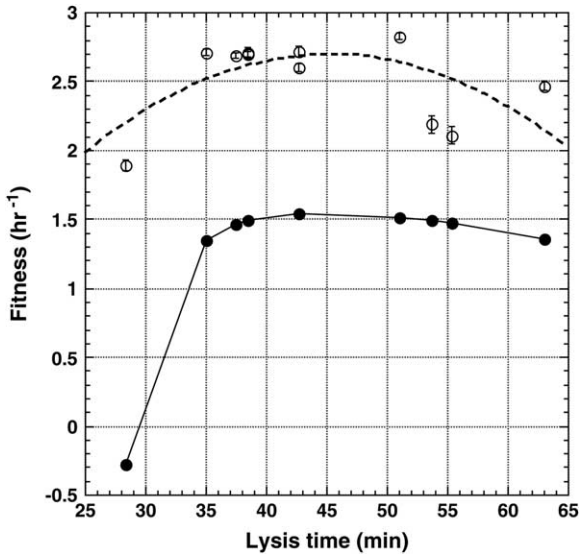

FIGURE 7.—Phage fitness calculated from simulated infection dynamics. The calculated phage fitness [●, using $w = (P_5/P_0)/5$], the experimental results with error bars (○, from Figure 5), and the quadratic fit (dashed curve, from Figure 5) are shown.

Simulation of phage infection dynamics: Interestingly, simulated population dynamics using parameter values derived from phage λ-infection have also shown the existence of an optimum. However, this model can describe only the qualitative aspect of the infection dynamics and the resulting phage fitness. Quantitatively, the experimental results showed a faster pace of bacteria and phage growth and an overall higher phage fitness. One likely factor contributing to this discrepancy is how lysis time is defined. In this study, the lysis time is defined as the time point when the culture turbidity first shows a consistent decline. This definition is consistent with that of the latent period, which is defined as the time period between initial phage infection and the first appearance of phage progeny in the culture (FRAENKEL-CONRAT and KIMBALL 1982). The latent period is usually determined using the one-step growth curve (DELBRÜCK 1940; DOERMANN 1952). Although it takes some time to have the entire lysogen culture completely lysed or to have all the phage progeny fully released, both definitions use the shortest time period possible. However, it is conceivable that lysis time can also be defined as the time point when the culture has declined to half of its maximum value, roughly the median lysis time. The lysis time thus defined is quite different from what is actually used in this study. A casual inspection of Figure 3 shows that the median lysis time would be ~10 min longer. Adopting the median lysis time as the "real" lysis time will also change the estimate of eclipse period in Figure 5, although the maturation/assembly rate will not be affected. Instead of the estimated 28 min, the new median eclipse period will be ~38 min. The overall impact of using the median lysis

time is that the phage will produce less progeny per unit of time. If these redefined estimates are used in the simulation, the calculated fitness would be even lower than the current ones shown in Figure 7. Clearly, the discrepancy between the observed and the simulated fitness is not due to the definition of lysis time.

Also, in this study, only two time points, 0 and 5 hr, were used to determine phage fitness. Although this definition has the advantage of incorporating more than one infection cycle into the fitness determination, it nevertheless underestimates the maximally attainable growth rate. However, even if the fitness was determined using data between 4 and 5 hr after infection, the resulting observed fitness value would still be larger than the simulated result. For example, in Figure 6, if determined between hours 4 and 5, the observed fitness of λ(S105) will be 5.2 hr^{-1} and the simulated fitness 3.0 hr^{-1}, again a gross deviation. Therefore, even though the current fitness definition underestimates the intrinsic phage growth rate, the discrepancy between the observed and the simulated fitness shown in Figure 7 is not due to the definition of phage fitness.

One possible reason for the discrepancy is the stochastic nature of the adsorption process during infection. For the current fitness assay condition, ~2000 phages ml^{-1} were used to infect 3×10^5 cells ml^{-1}. Using the estimated adsorption coefficient of 10^{-8} $cell^{-1}$ $phage^{-1}$ ml^{-1} hr^{-1}, the calculated production rate for the infected host would be on average 6 ml^{-1} hr^{-1}, a very low number. However, if the adsorption process is stochastic, then the time it takes to have a successful infection event would be distributed randomly; that is, a fraction of these initial 2000 phages would have successfully infected the host at a much shorter time span than the average. Once an infection has commenced, the resulting progeny would increase the overall phage concentration, thus further increasing the production rate of the infected host. That is, there would be a positive feedback cycle from those early, although small, successful infections. This interpretation is reflected in Figure 6, where the observed phage population level rises faster than that from the simulation data. This early rise in phage concentration in turn contributed to the earlier decline of the host population. It would be interesting to see if the simulated infection process can mimic the experimental data if the population dynamics model is recast in a stochastic form.

Testing the marginal value theorem: Although the main purpose of this study is to demonstrate the existence of an optimal lysis time for phage infection, the current experimental setup can be easily adapted to independently manipulate and maintain the host density and/or physiological state to test the model predictions on lysis time evolution (Wang *et al.* 1996). The growth rate (and thus the physiological states) can be experimentally manipulated by growing cells with different substrates (Bremer and Dennis 1987), while the cell density can be approximately maintained in a constant density by serial twofold dilution of the culture at a predetermined time interval. In fact, this simple technique has been successfully used to isolate a short lysis time mutant of phage RB69, a phage T4 relative (Abedon *et al.* 2003). By subjecting the array of isogenic λ-phages used in this study to various combinations of host density and host physiological state, it would be possible to assess whether the peak of the fitness curve would also be shifted according to the model's prediction.

Effect of multiple infection on the evolution of lysis time: All the theoretical and experimental studies so far implicitly assumed single-infection dynamics. No interference competition, mediated via various molecular mechanisms, is considered during the evolutionary process. However, if multiple infection is the norm of the infection dynamics, it is not clear if the evolution of lysis timing would be as straightforward as the theoretical model would have predicted. A few studies revealed a glimpse of potential underlying complexity if multiple infection is the norm. Two studies have shown a very interesting, but also complex, pattern of holin–holin interactions (Raab *et al.* 1988; Ramanculov and Young 2001). By simultaneously coexpressing two λ *S* alleles in the same host, one with a long (t_L) and the other with a short (t_S) lysis time, it was found that the actual time of host lysis (t_C) can be: (a) $t_C = t_S$, where the host lysis time is dictated by the shorter time of the two; (b) $t_C > t_L$, where the lysis time is delayed beyond the longer time of the two; or (c) $t_C < t_S$, where the lysis time is accelerated to be shorter than the short lysis time (Raab *et al.* 1988). The exact outcome of t_C depends on the identity of the two coexpressing alleles. It is also found that coexpression of two entirely unrelated holin genes, the λ *S* and T4 *t*, also resulted in an accelerated lysis, with the actual lysis time being shorter than that of either of the two when expressed alone (Ramanculov and Young 2001). It is not clear how representative these observations are. Nevertheless, it is clear that to understand or even be able to predict the outcome of coinfection/expression would require the understanding of how the protein–protein interaction plays a role in the timing and formation of the holin lesion. In this respect, a more detailed understanding of molecular mechanism(s) would greatly inform and facilitate our understanding of the relationship between genotypic (holin sequence) and phenotypic (lysis timing) evolution. Also, given the observed patterns of holin–holin interaction, it remains to be seen whether the relentless march toward optimality would actually be thwarted by intrahost interaction, if the infection dynamics are shifted from single to multiple infection.

Effect of other life-history traits on the evolution of lysis timing: The understanding of lysis time evolution would be further complicated by seeing lysis timing in a larger context of life-history trait evolution. Undoubtedly, the evolutionary trajectory of lysis time will depend not only on the ecological conditions but also on the character states of other life-history traits, such as adsorption

rate (dictating the probability of finding a host per unit of time) and maturation rate (determining the rate by which the progeny is assembled). That is, the selective value of a holin gene allele would be context dependent. In fact, in the original model of lysis time evolution (Wang *et al.* 1996), the ecological condition of high host density can be seen as an equivalent of a phage population with high adsorption rate, for both will have the same effect of reducing the average time to finding a host. As the analysis predicts, the optimal lysis time would be shorter in the background of high adsorption rate. It is interesting to note that all laboratory λ-strains carry a frameshift mutation at the *stf* (side tail fiber) gene that renders them morphologically side tail fiberless. The impact of this mutation on the life-history trait is that the laboratory λ has a greatly reduced adsorption coefficient when compared to Ur-λ (Hendrix and Duda 1992), the original λ-strain isolated by Esther Lederberg (Lederberg 1951). To what extent other life-history traits may influence the evolutionary trajectory of lysis timing is currently under investigation.

The author thanks Ry Young for his enthusiasm, encouragement, and invaluable assistance and support during the course of this study; Angelika Grüendling for providing various λ *S* plasmids; and anonymous reviewers for constructive suggestions. This study was supported by Public Health Service grant GM27099 to R. Young and by a Basic Bioscience minigrant to I.-N. Wang.

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Communicating editor: N. Takahata