Many pathogens have evolved the ability to induce latent infections of their hosts. The bacteriophage λ is a classical model for exploring the regulation and the evolution of latency. Here, I review recent experimental studies on phage λ that identify specific conditions promoting the evolution of lysogenic life cycles. In addition, I present specific adaptations of phage λ that allow this virus to react plastically to variations in the environment and to reactivate its lytic life cycle. All of these different examples are discussed in the light of evolutionary epidemiology theory to disentangle the different evolutionary forces acting on temperate phages. Understanding phage λ adaptations yield important insights into the evolution of latency in other microbes, including several life-threatening human pathogens.

Phage Life Cycles
Some pathogens exploit their host for a very limited amount of time. In humans, for instance, most influenza virus infections last for less than 10 days [1]. The duration of the infection is generally limited by host immunity but it can also be reduced by the death of the infected host. In contrast, other pathogens can remain in their host for a very long time in a latent state. Many human viruses have adopted this alternative life history strategy. For instance, infections by herpes simplex viruses lead to short acute and usually localized infections of the skin, yielding durable infection of nearby sensory neurons [2]. These latent infections can later reactivate to cause recurrent disease and transmission. Hence, latency can guarantee lifetime persistence of the infection and constitutes a major therapeutic challenge in several human pathogens [3–6]. Yet, the adoption of a latent life cycle is a dramatic feature that requires specific pathogen adaptations. When should pathogens adopt such latent life history strategies? When the pathogens are in a latent state, when should they reactivate and attempt to reach another host?

These fundamental questions are relevant for a broad range of pathogens but are often difficult to tackle experimentally. Bacteriophages, however, provide a powerful model system to explore these questions. Bacteriophages are viruses that infect bacteria and they are one of the most abundant organisms in the biosphere [7–9]. They can adopt very different exploitation strategies of their host, ranging from lytic to lysogenic life cycles (Figure 1, Key Figure). Lytic life cycles are characterized by bacterial adsorption, replication of the phage genome, synthesis of new viral particles, and bacterial lysis. Lysogeny, however, is characterized by the integration of the virus as a prophage in the bacterial genome and the indefinite persistence of the infection. Lysogeny was discovered in bacteriophages by Bail and Bordet 90 years ago [10–12]. The dichotomy between these two alternative lifestyles provides a fantastic opportunity to study the evolution of latency in microbes. Bacteriophage λ [13] is a temperate coliphage that has been involved in major molecular biology advances [14]. In particular, it has been used as a classical model system to unveil the regulation of lysogeny [15].
The regulation of lysogenisation is mainly under the control of the repressor gene cl. The repressor protein turns off the expression of other genes of the phage but activates its own transcription. This prevents the expression of the lytic life cycle and allows the lysogenic bacteria to divide and to transmit the prophage vertically. Other phage genes are also involved in this regulation. The gene cro is an antagonist of cl, and its expression is associated with the expression of other phage genes leading to the launch of a productive infection and the lysis of the host. The balance between the expression of cl and cro is at the heart of the genetic switch that allows λ to control the fate (lysogeny or lysis) of its host [15]. Interestingly, genetically identical cells infected with the same number of viruses can give rise to distinct outcomes (Figure 1). Detailed models of within-host dynamics of the accumulation of gene products of cl and cro have shown that this binomial variability can be explained by spontaneous biochemical noise during the development of the infection [16,17]. Yet, this fact does not imply that cell fate is exclusively stochastic. First, several mutations are known to alter the propensity for lysogenisation. For instance, the cl857 mutant has a cl repressor that decreases the probability of lysogeny, φ, and increases the induction rate, \( \alpha \). Second, several environmental factors are known to affect lysogenisation and induction.
Earlier studies of λ were mainly focused on the molecular details of phage infections. Here, I want to discuss the biology of λ with an evolutionary perspective to question the adaptive nature of specific features of its life cycle. First, I discuss the adaptive nature of fixed latency in different environmental conditions. I review two recent experimental studies that explore the effects of (i) epidemiological dynamics, and (ii) spatial structure on the evolution of viral latency. Second, I discuss the ability of λ to adopt plastic exploitation strategies in response to fluctuations of its environment. I use theoretical models to explore different drivers of the evolution of pathogen plasticity: (i) variations in the environment inside the host, and (ii) variations in the environment outside the host. These theoretical results are discussed in the light of plastic transmission strategies observed in λ. Finally, I discuss the relevance of these studies for the evolution of both fixed and plastic transmission strategies of other bacteriophages and other pathogens.

**Latency as a Fixed Strategy**

An understanding of the regulation of lysogeny in λ provides invaluable information on the evolution of latency in pathogens. When should pathogens adopt such latent life-history strategies? To answer this question, Berngruber *et al.* [18,19] studied the competition between two λ variants with distinct life history strategies. The wild-type temperate λ has a high lysogenisation rate ($φ \sim 0.5$) and a low induction rate ($κ \sim 10^{-6}$). In contrast, the virulent λcI857 is a thermosensitive mutant with a low lysogenisation rate ($φ < 0.1$) and a high induction rate ($κ > 10^{-2}$). Monitoring the frequency of these variants provides a measure of selection on those life-history traits. In other words, it provides a way to determine when latency is favored and when it is counterselected.

**Evolution during Epidemics**

During the early stage of a phage epidemic the density of susceptible bacteria is very large, and free viruses have many opportunities for horizontal transmission. This promotes the spread of phage variants with more aggressive host exploitation strategies that rapidly get access to a larger number of hosts through the production of free virus particles. In contrast, at the end of an epidemic, the availability of susceptible cells is reduced and the probability of finding a new host to infect is very low. In this case, more prudent host exploitation strategies become adaptive because they secure the durable exploitation of a host and invest in vertical transmission [20,21]. This argument is akin to the effect of host availability on optimal lysis time in lytic phages [22,23]. But the dynamic nature of the density of susceptible hosts requires models that keep track of epidemiology and selection to understand and predict the competition between different life-history strategies [24].

Berngruber *et al.* [20] formalized the effects of epidemiology on λ evolution throughout epidemics and tested these predictions with experimental epidemics in chemostats. Each chemostat was seeded with a 1:1 ratio of the temperate wild-type λ and the virulent λcI857. These experiments supported three theoretical predictions (Figure 2). First, the frequency of the virulent mutant increases rapidly during the early phase of the epidemics and decreases later on. Second, the evolutionary outcome is governed by the epidemiology. When the experiment is initiated with a small number of infected cells the availability of a large number of susceptible cells favors the virulent λcI857 in the early stage of the epidemic. In contrast, when the experiment starts with a larger fraction of infected cells, the initial benefit of the virulent λcI857 is reduced. Finally, the experiments confirm that the frequency of the virulence mutant is always higher in the free virus compartment. The validation of these three qualitative predictions demonstrates the predictive power of models integrating epidemiology and evolution, and accounting for the specificities of λ life cycle.

One key element of λ biology is the ability of lysogenic bacteria to prevent the exploitation of the host by superinfecting viruses. In other words, superinfection inhibition ensures that the
prophage controls the fate of its cell. Note, however, that lambdoid phages have evolved the ability to escape superinfection inhibition, and several immunity groups are circulating. A phage from a different immunity group is able to escape the repression induced by the resident phage. This dramatically alters the prediction regarding the evolution of lysogeny because the coexistence between different immunity groups selects for more virulent strategies [25].

Evolution in Spatially Structured Environments
The previous section focused on the transient evolutionary dynamics taking place in a well-mixed environment during an epidemic. Epidemics, however, often spread in spatially structured environments. Theory predicts that spatial structure generally selects for lower pathogen virulence [26–28]. Berngruber et al. [19] extended previous models to account for localized migration of the bacteria and localized transmission of the phage. In addition, this model allows us to track the contribution of horizontal and vertical transmission of the two phage genotypes. This helped to identify conditions promoting the evolution of the temperate phage. To test these theoretical predictions, Berngruber et al. [19] manipulated the amount of mixing among bacteria and phages and recovered two main theoretical predictions. First, low mixing promotes the spread of the temperate phage. Indeed, in a spatially structured environment the virulent phage rapidly runs out of susceptible cells (Figure 3). Second, the relative contributions of horizontal and vertical transmissions are modulated by spatial structure. When mixing is high, most of the fitness of the virulent mutant depends on horizontal transmission. When mixing is low, vertical transmission is key because it allows perpetuation of the infection. In other words, spatial structure changes the availability of susceptible cells and empty sites in the local environment of an infection. The magnitude of this effect varies with the genotype of the parasite and this is what selects for latent (i.e., more prudent) exploitation strategies [19].

Figure 2. Virulence Evolution During an Epidemic in Bacteriophage λ. (A) Change in the virulent/avirulent ratio in the provirus stage. (B) Change in the virulent/avirulent ratio in the free virus stage. The initial value of the virulent/avirulent (λcI857/λ) ratio in the provirus was 1:1, and competition was started from two different initial prevalence values: 1% (red) and 100% (blue). The lines indicate the mean over four replicate chemostats, and the envelopes show the 95% confidence intervals of the log-transformed data. Modified from Berngruber et al. [19].
Latency as a Plastic Strategy

In the above section, latency is assumed to be a fixed strategy. Bacteriophage $\lambda$, however, is also known to exhibit plastic life-history strategies. For instance, the efficacy of lysogenisation and the induction rate of the prophage can vary with the environment. These conditional strategies are well studied on a molecular level but the adaptive nature of this plasticity is often overlooked.

Escaping a Stressful Environment

One of the best studied examples of plasticity is perhaps the effect of stress on the induction rate, $\alpha$, of bacteriophage $\lambda$. Whenever a lysogen undergoes DNA damage due to UV irradiation or other chemical stress, the protein RecA is activated. RecA is involved in the inactivation (cleavage) of the LexA repressor and in the launch of the SOS response, activating different mechanisms of DNA repair. Interestingly, RecA is also involved in the inactivation (cleavage) of the $cI$ repressor and thus in the launch of the lytic cycle of $\lambda$. Is this conditional induction rate adaptive? One way to answer this question is to show that such plasticity may be adaptive under some theoretical scenario. In Box 1 I developed a simple model where the infected host can be in two different states. The host can be in a ‘normal’ state in which host cells replicate and have low mortality, but with a rate $\sigma$ host cells may enter a ‘stressful’ state where both fecundity and survival are reduced. The model shows that the virus infecting a stressed cell should shift towards a more intensive exploitation strategy. In other words, this simple model provides an adaptive explanation for the conditional induction rate of $\lambda$ because the activation of RecA signals that the host is in a critical state.

Lysogenisation or Not in a Variable Environment

The probability to lysogenize, $\phi$, is another life history trait of $\lambda$ modulated by variations in the environment. In particular, this probability increases when the number of virus particles
Box 1. Leaving a Sinking Ship

Let us assume a simple epidemiological model where the host can fall into two different states: (i) a ’normal’ host with high fecundity, $λ$, and low mortality, $δ$, and (ii) a ’stressed’ host with low fecundity, $λ_*$, and high mortality, $δ_*$. We can model the dynamics of phage $λ$ under these assumptions which yields the following epidemiological model (the dot notation refers to differentiation with respect to time):

\[
\begin{align*}
L &= \lambda L + \phi a S V - (\lambda + \alpha + \varsigma) L \\
L_* &= \lambda_* L_* + \alpha_* L_* - (\lambda_* + \alpha_* + \varsigma_*) L_* \\
V &= B (\lambda L + \alpha L_* + \beta (1 - \phi) a S V - m V)
\end{align*}
\]

where $L$ and $L_*$ are the densities of normal and stressed hosts respectively, $V$ is the density of free viruses, and $S$ is the density of susceptible hosts. The parameters $\lambda$, $\phi$, $\delta$, $a$, $m$, $\sigma$, $\delta_*$, and $B$ refer to the fecundity of lysogens, the lysoypisation rate, the natural death rate of lysogens, the adsorption rate of the virus, the death rate of free virus, efficiency of the induction rate of lysogens, the rate at which a bacterium enters the stressed state, and the burst size of a productive infection (see Figure 1 in main text). This simple model can be used to explore the effect of host stress on the evolution of the parasite exploitation strategy. To study the evolution of conditional lysis strategies, we focus on the ability of a mutant pathogen (with a strategy $\alpha^M$ and $\alpha^C_1$) to invade a resident pathogen population (with a strategy $\alpha$ and $\alpha_1$) at equilibrium. In other words, we assume that the density $S$ of susceptible hosts is fixed by the resident strategy of the pathogen. The dynamics of the mutant can be fully described by the matrix $F^M$ which accounts for how many mutants are created in the two different types of host and in the environment (as free-living viruses) and the matrix $V^M$ which refers to transition between these three compartments:

\[
F^M = \begin{pmatrix}
\lambda & 0 & a S \phi \\
0 & \lambda_* & 0 \\
0 & 0 & a B S (1 - \phi)
\end{pmatrix} \\
V^M = \begin{pmatrix}
\delta + a^M + \sigma & 0 & 0 \\
-\sigma & \delta_* + a_*^M & 0 \\
-a^M B & -a_*^M B & m
\end{pmatrix}
\]

The per-generation growth rate of the mutant is given by the dominant eigenvalue $\lambda_M$ of the next generation matrix $F^M$:

\[
F^M \cdot V^{M-1} = \begin{pmatrix}
\lambda^M_{1,1} & \lambda^M_{1,2} & \lambda^M_{1,3} \\
\lambda^M_{2,1} & \lambda^M_{2,2} & \lambda^M_{2,3} \\
\lambda^M_{3,1} & \lambda^M_{3,2} & \lambda^M_{3,3}
\end{pmatrix}
\]

where $\lambda^M_{i,j}$ is the per-generation transition between compartment $X$ and $Y$. For the sake of simplicity, we focus on the special case where $\lambda = 0$. We derive the gradient of selection on conditional lysis:

\[
\frac{\partial \lambda_M}{\partial \alpha} = \begin{pmatrix}
\frac{\partial \lambda_M}{\partial \alpha} \\
\frac{\partial \lambda_M}{\partial \alpha*}
\end{pmatrix} = \begin{pmatrix}
\beta S (\delta_* + \alpha_*) - \beta (\delta_* + \alpha_* + \varsigma_* + \alpha_1) L \\
\alpha B \phi S (\delta_* + \alpha_*) - \alpha B (\delta_* + \alpha_* - \varsigma_* + \alpha_1) L
\end{pmatrix}
\]

In other words, lysis can evolve towards intermediate rates in normal hosts to balance the benefit associated with access to new hosts and the cost of losing the current host. In stressed hosts, however, lysis is maximized because there is no benefit associated with delaying lysis in a sterilized and moribund host.

attempting to infect a host cell is high [29,30]. Some of the molecular details governing this regulation have been largely uncovered and modeled [16,17,31]. Yet, the adaptive nature of this regulation remains unclear. Why should pathogens coinfecting the same host adopt a latent strategy? Classical virulence evolution theory, in contrast, often predicts that coinfections should yield more aggressive exploitation strategies [21]. Such conditional lysoypisation strategy, however, may be adaptive in some environments. Avlund et al. [32] argue that it may evolve as a response to the stochasticity of the environment. Indeed, conditional dormancy in plants and other organisms has often been viewed as a bet-hedging strategy that evolved in random environments [33,34]. Yet, I want to argue here that conditional latency may also be adaptive in fully deterministic but temporally variable environments. Indeed, the fact that several viruses are attempting to enter the same cell indicates that very few hosts are available and, in this case, it may be advantageous to take care of the host. Lysogenisation may thus be a way to stay in the host and avoid the perils of the free-living stage of the virus life cycle. Box 2 is an attempt to formalize the evolution of plastic transmission strategies in a variable environment. Theory shows that plasticity may evolve when it allows transmission to coincide with a transitory increase in the availability of susceptible cells. But the model does not specify which cues can be used by the virus to trigger a life-history switch. The difficulty for the virus is to use local information obtained
Box 2. Plastic Transmission Strategies in Variable Environments

Fluctuation in the quality of the environment is modeled with $\lambda(t)$ which describes the periodic influx of susceptible cells in the population. The epidemiological model introduced in Box 1 becomes:

$$S = \lambda(t) - aSV - kS$$
$$L = mL + \phi aSV - (\delta + \alpha)L$$
$$V = BeL + B(1 - \phi) aSV - mV$$

Under the assumption that the dynamics of the free virus compartment is much faster than the other processes, we can assume that the density of free viruses is always at equilibrium:

$$V = \frac{BeL}{m - B(1 - \phi) aS}$$

For the sake of simplicity, we focus on the case where $\phi = 1$. The dynamics of a mutant virus may thus be described by:

$$L_m = \frac{\lambda + \phi aBS_0}{m} - (\delta + \alpha)L$$

If the system oscillates periodically, the mutant will invade if its average growth rate over one period $T$ (noted with a tilde) is positive:

$$\tilde{r}_m = \frac{1}{T} \int_0^T r_m dt = \lambda + \phi aBS_0 - (\delta + \alpha) + \frac{\phi aB}{m} \text{cov}(S, S_0) > 0$$

The covariance in the above condition captures what may select for plasticity [43]. A strategy building up a covariance between the timing of lysis and the high density of susceptible hosts is going to be selected because it synchronizes the release of free virus with the availability of susceptible hosts. Multiplicity of infection may be used by the virus to build up this covariance because it carries valuable information on the epidemiological state of the population. Note that, in contrast to Avlund et al. [32], phases do not ‘play dice’ in the above model because the dynamics is fully deterministic. They do not evolve plastic transmission strategies because of stochasticity but because of the periodic fluctuation of the environment.

Outstanding Questions

- The details of the genetic switch in $\lambda$ have been unveiled. Is it also possible to disentangle the molecular mechanisms underlying the regulation of latency in other pathogens?
- What is the amount of natural variation in the propensity to develop fixed or plastic latency among pathogens? Is it possible to relate this variation to specific features of their local environment?
- How do stochastic and deterministic variations in the environment interact with the evolution of latency?
- Is it possible to demonstrate experimentally the adaptive nature of plastic latency in the laboratory?
- Do different pathogen species share similar environmental cues that trigger relapses?
- Is it possible to develop new drugs that prevent latency and facilitate within-host eradication?

Concluding Remarks

The discovery and the characterization of bacteriophage $\lambda$ has led to major discoveries in molecular biology [14]. In particular, the regulation of the lysis–lysogeny decision remains a very fruitful topic that stimulated a broad range of research studies [26,37]. The molecular details of the genetic switch of $\lambda$ have been scrutinized for decades and led to a deep understanding of gene regulation that shed light on many other biological regulatory processes. The lysis–lysogeny decision has clear-cut implications on the virus life cycle and offers unique opportunities to study the ecology and evolution of virus latency. Here, I reviewed a few recent studies and discussed the adaptive nature of both constitutive and conditional latency. Competition between different $\lambda$ strains (with different life-history strategies) has allowed the study of which environmental factors promote the evolution of fixed lysogenisation within an infected host to infer the epidemiological state of the population (i.e., outside the infected host). The number of coinfected viruses carries valuable information on the epidemiological state of the population, and this is why it may be a good cue to evolve conditional lysogenisation. But other cues may also be used to modulate lysogenisation. In particular, host starvation is known to increase the probability of lysogenisation [29]. Starvation is a very good indicator of the environmental state of the population because it signals that very little bacterial growth is feasible. In a variable environment, where starvation oscillates, it is adaptive for the virus to hide in infected cells (lysogenisation) during a famine to avoid the risks associated with the free-living stage. The above argument does not account for the potential benefits on host fitness associated with the integration of the phage in the host genome. For instance, Chen et al. [35] showed that lysogenisation with $\lambda$ may carry benefits for the Escherichia coli bacteria because it lowers the growth rate of lysogens in energy-poor environments. The potential symbiotic nature of lysogenisation in some environments clearly deserves further investigation [36].
strategies. To explore the adaptive nature of conditional lysogenisation I developed new models of life-history evolution in fixed and variable environments. Evolutionary epidemiology theory provides a way to illustrate how λ developed various adaptations to cope with variable environmental conditions. I do not claim that every feature of the biology of λ is well adapted. As any organism, λ evolves under a set of evolutionary constraints. Evolutionary epidemiology, however, provides a fruitful framework for trying to make sense of some peculiar features of the λ life cycle. As such, it may also help to understand the evolution of latency in other phages and other pathogens.

From λ to Other Bacteriophages
It is important to remember that while lysogenisation is the classical way to generate latent infections, alternative strategies are used by lytic phages. For instance, lysis inhibition is the ability to delay the timing of lysis in T4 phages, and this inhibition is triggered by multiplicity of infection [38]. It is tempting to think that the same forces have led to the evolution of the regulation of lysogenisation in λ and lysis inhibition in T4. In both cases, multiplicity of infection carries information about the epidemiological state of the population that may be used by the virus to better time the induction of a productive infection. Similarly, pseudolysogeny provides an alternative life-history strategy that enables the virus to delay lysis in starved hosts [39]. Pseudolysogeny is distinct from lysogeny because the phage genome does not integrate into the host chromosome. The effect of starvation, however, recalls the increased lysogenisation of λ in starved cells. In both cases, conditional latency is likely to be adaptive because it allows the virus to avoid the risks associated with the free-living stage of the life cycle.

From Bacteriophages to Other Pathogens
It is also tempting to use our understanding of the evolution of latency in λ to study the evolution of both constitutive and plastic latency in other pathogens. We explored the effect of epidemiology and spatial structure on λ evolution, but other environmental factors may trigger higher investment in latency. In malaria, for instance, it is clear that the seasonality of the environment has dramatic effects on the availability of mosquito vectors and thus on epidemiological dynamics. Plasmodium vivax is one of the main causative agents of malaria in humans, and this pathogen has the ability to remain dormant for several months before relapsing and causing new infections. The rate at which P. vivax relapses is variable among different pathogen isolates and has been found to depend on latitude [40, 41]. In particular, the relapsing rate is much lower (i.e., latency is more pronounced) at high latitude where seasonality implies that transmission is hindered by the lack of mosquito vectors in winter. This pattern is consistent with classical theoretical predictions on the evolution of latency [33, 34, 42–44].

The analysis of the λ life cycle indicates that different types of cue may trigger the relapse of latent infections. First, relapses may be induced by cues indicating that the environment inside the host has changed and is unlikely to sustain long-lasting infections (Box 1). Many infections by herpes simplex viruses and varicella zoster virus have been shown to reactivate when the host is immune-depressed or physiologically stressed [2]. For instance, asymptomatic reactivation of varicella zoster virus has been observed in astronauts after a mission in space [45]. Of course, a drop in the efficacy of host immunity may provide a proximal cause for the replication of the virus leading to a relapse. Yet, the ultimate cause of the relapse may be related to the evolved ability of the virus to react actively to variations in the physiological state of its host. Second, relapses may also be induced by cues indicating that the environment outside the host has changed and is becoming suitable (Box 2). Higher multiplicity of infection carries information regarding the epidemiological state of the population. Interestingly, malaria parasites, similar to bacteriophages, have plastic transmission strategies with regard to the multiplicity of infection [46]. Again, it is unclear whether this pattern is adaptive or not, but given the
tremendous amount of plasticity in *Plasmodium* parasites [43,47,48] this evolutionary perspective is worth investigating.

Bacteriophages live in complex environments and they have evolved elaborate strategies to cope with the variability of their habitat. In particular, bacteriophage λ has the ability to adopt constitutive and conditional latency. The study of evolutionary forces leading to these fascinating adaptations give insight into the diversity of other pathogens’ life-history strategies (see Outstanding Questions). Because the dormant stages of many human pathogens is challenging the efficacy of classical therapeutic interventions [3–6], a better understanding of the ultimate causes of these pathogen life histories may thus inspire new strategies to control and eradicate those infectious diseases.

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