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Coinfection and superinfection in RNA virus populations: a selection–mutation model

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Abstract

In this paper, we present a general selection–mutation model of evolution on a one-dimensional continuous fitness space. The formulation of our model includes both the classical diffusion approach to mutation process as well as an alternative approach based on an integral operator with a mutation kernel. We show that both approaches produce fundamentally equivalent results. To illustrate the suitability of our model, we focus its analytical study into its application to recent experimental studies of in vitro viral evolution. More specifically, these experiments were designed to test previous theoretical predictions regarding the effects of multiple infection dynamics (i.e., coinfection and superinfection) on the virulence of evolving viral populations. The results of these experiments, however, did not match with previous theory. By contrast, the model we present here helps to understand the underlying viral dynamics on these experiments and makes new testable predictions about the role of parameters such the time between successive infections and the growth rates of resident and invading populations.
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1. Introduction

RNA viruses, due to their intrinsic simplicity and adaptability, offer a unique opportunity for exploring long-term evolutionary dynamics [1]. It is possible that RNA genomes were the first life forms and constituted the first biosphere, later supplanted by the more stable DNA genomes. But RNA genomes have persisted as molecular parasites in cells with DNA genomes and they are nature's closest counterparts of early molecular replicators [2,3]. It is perhaps not surprising that, due to their simplicity, they have been amenable to useful theoretical modeling [4,5]. Their success can be understood in terms of both their simplicity and their enormous plasticity and adaptability to changing environments.

Such plasticity stems, to a large extent, from the high mutation rates operating during RNA genome synthesis and rapid replication. These two influences lead to an extremely heterogeneous population structure termed *molecular quasispecies* [2,6–12]. The quasispecies structure has numerous implications for the biology of RNA viruses. The most obvious one for viral pathogenesis is that mutant swarms contain rervoirs of variants with potentially useful phenotypes in the face of environmental change [13].

The dynamics of the viral population can be visualized as a dynamical process of growth, competition and selection which takes place in the sequence space. The fitness landscape (an idea first introduced by Sewall Wright and later extended by several authors, see [14,15]) is defined in terms of some particular traits which are implicit in the virus particle phenotype and are usually described in terms of replication rate or infectivity.

One of the most interesting aspects of virus evolution is related to the different pathways of host-pathogen interactions that can occur. One specially important aspect is the outcome of multiple infections, in terms of the final fitness displayed by selected viral populations. In this context, mathematical and computer models can be extremely helpful in providing reasonable forecasts on how viral infections unfold, in particular when several strains of a given virus compete for a given population of hosts. In this context, recent experimental [16,17] and theoretical [18] studies have shown that long-term competition among different RNA strains of the vesicular stomatitis virus (VSV) can promote red Queen dynamics (RQD, see [19]). Under RQD both strains improve their fitness relative to their ancestors but with no relative gain between them. They thus coexist along their evolution in cell cultures but this coexistence ends eventually as a consequence of competitive exclusion. These and other experiments have been successfully interpreted by using one-dimensional fitness landscapes in which the only variable under consideration was replication rate [18,20]. In spite of the oversimplification implicit in such choice and the known fact that RNA viruses have multipeaked, rugged fitness landscapes [14,21,22] the success of these models suggests that an important part of the whole picture can be compressed into selection and mutation dynamics on a replication landscape.

Although most of the studies on the evolution of virulence are restricted to scenarios in which a host or populations of hosts are infected by a single strain of the virus, multiple infections are more likely to occur under a wide range of circumstances. The simplest case (hereafter simple infection) has a predictable outcome: if each host is infected by a single strain, the one with highest reproductive rate will eventually take over through competitive exclusion. But if multiple rounds of infection are allowed, then the final outcome is far less simple.

Two pathways of multiple infection can be defined: *coinfection* and *superinfection*. In the first case, several strains invade the host simultaneously. In the second, different strains infect at different times. During natural virus outbreaks, the infection of available hosts can occur in three ways. At the beginning of the epidemic, when the density of available hosts is high but viral density is still low, a host would usually be infected by a single variant at a time. However, as the epidemic progresses, more and more hosts become infected and the density of virus in the population increases as well. Consequently, the probability of a newly released virus infecting an empty host would decrease as a function of time, and hosts would be simultaneously infected by more than one viral strain. Depending on the exact moment at with different strains meet inside the host, coinfection [23] or superinfection [24] will take place.

Since the two possibilities might lead to different final virulences, it is important to know which of the three possible infection scenarios might generate the more virulent strains. In this context, theoretical models can help to provide some clues. The evolutionary dynamics of coinfection and superinfection have been theoretically explored under a number of assumptions [23–26]. Using a weak form of interaction within hosts, May and Nowak [23] found that coinfection can support a highly diverse population of different strains closely clustered around the maximum virulence level. For superinfection, Nowak and May [24] assumed a competitive hierarchy among parasites. Under this assumption a new, more virulent strain can take over a host that is already infected by a less virulent strain. As a consequence, multiply-infected hosts would transmit only the most virulent of the strains they harbour. These models of superinfection have been used as a powerful framework to explore other related problems, such as the evolution of diversity in the context of metapopulation dynamics [23].

These models also allow to provide some predictions concerning the evolution of diversity (measured in terms of the number of strains present). But on the other hand the superinfection model does not strictly include the time separation between different infecting strains. It is actually a meta-model of the real process, in which infection occurs when the resident population has already exploited some amount of resources. Using ideas borrowed from community assembly theory [27,28] in which infection by a virus strain would map into invasion by a new species from a regional pool, it can be shown that competitive displacement might fail to occur depending upon the current species composition. In this context, as far as the ecological mapping is valid, the success of a new strain might be jeopardized by the structure of the resident community, independently of the virulence of the invader [29]. In this paper we further explore a recent set of experiments involving the three types of infection dynamics [30] in which some of these phenomena seem to be at play.

Precisely, we present a class of general mutation-selection models useful to describe the interaction between viral populations. In these models, mutation can be incorporated in two different ways: as a diffusion process in fitness space, by means of a Laplacian operator, or by means of an integral operator with a dispersal (or mutation) kernel. We will demonstrate that these two modeling alternatives, although having different assumptions on the mutation process, drive to almost identical conclusions. The mathematical description of coinfection and superinfection dynamics are treated as particular cases of these general models.

2. Multiple infection dynamics in VSV populations

In a recent paper [30], Miralles et al. presented a detailed analysis of an in vitro evolution experiment with vesicular stomatitis virus (VSV) in which the three different types of infection were used. Experimental populations were allowed to grow and compete (Fig. 1) under conditions that simulated single infections, coinfections and superinfections. After 100 generations of evolution, the average fitness of each population was measured by means of standard competition assays [31] against a reference strain. This reference strains and the clone used to initiate the evolution lines only differed in a single neutral amino acid replacement at the surface glycoprotein, hence, they had a relative fitness, expressed as a ratio between growth rate parameters, of 1.

Miralles et al. simulated different types of multiple infections in the laboratory. Multicellular host were simulated by a cell culture flask containing several million mammalian cells. The experiment was initiated by infecting the first set of flasks with a certain amount of a VSV MARM clone. After 24 h, cells were completely killed and viral progeny harvested. Approximately 1.5×10^5 viruses from the progeny were used to infect next-day hosts. For the case of single infection, next-day hosts were infected with virus from a single host (Fig. 1(A)). In the case of multiple infections, next-day hosts were infected with virus from two different sources at different frequencies (Fig. 1(B)). For coinfection both viruses were added at the same moment, whereas for superinfection, next-day flasks were infected with virus from only one flask and after several hours virus from a different flask was added at the convinient proportion (Fig. 1(B)). This daily protocol was repeated during 25 days. Two different frequencies of multiple infection were simulated, 5% and 50% (for details on the design, see [30]).

Experimental results in [30] show that infection passages with coinfection increased fitness compared with the single infection case (Fig. 2). The magnitude of the increase was proportional to the frequency of the added virus. In contrast, passages with superinfection did not generate increases in fitness larger than those obtained under single-infection regimes (Fig. 2). Similarly, an increased virulence during coinfections, and a lack of significant effect for superinfection, have been observed with other viruses. Alonso et al. [32] observed that when hematopoietic necrosis (IHNV) and pancreatic necrosis (IPNV) viruses coinfected a cell culture, the former had a lower growth rate and was systematically displaced by IPNV. By contrast, if cells were infected with IHNV and later
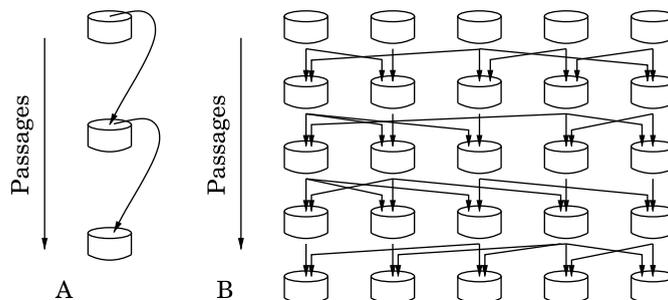

Fig. 1. Schematic representation of the experimental protocol designed in [30] to test for the effect of multiple infections. Panel A represents the daily transfers done according with a single-infection case. Panel B represents the multiple-infection case (coinfection or superinfection). For details, see main text and [30].
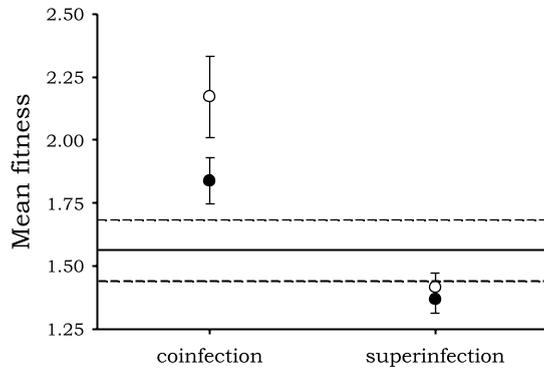
Fig. 2. Overview of the experimental results reported in [30]. The horizontal bands represent the mean ($\pm$ standard error) for the fitness estimates obtained for the case of single infection ($n = 20$ replicates). Open circles correspond with a 50% frequency of multiple infection, whereas solid circles correspond with a 5% frequency. Significant differences were obtained for coinfection but not for superinfections.

superinfected with IPNV, IHNV productivity was not affected by IPNV. Another example can be found in nuclear polyhedrosis viruses (NPV) [33]. When cells were coinfected with two different variants of NPV, the replication of one of them was completely inhibited by the other. In contrast, if the cells were already infected with the first, superinfection with the second did not produce inhibition, regardless of the exact moment in which superinfection occurred. Finally, it has been recently reported that coinfection with two different groups of the human immunodeficiency virus type 1 (HIV-1) subtype B resulted in an increased virulence of both of them, characterized by more production of viral particles and by the induction of massive apoptosis/cell killing in PBMC cultures that were not affected in such way when infected individually with either group [34]. This synergism arose as a consequence of using different co-receptors for cell entry.

Using simple arguments from a Lotka–Volterra model of two-species competition, it was conjectured that a plausible explanation for these results (in particular for the unexpected low fitness observed in the superinfection case) was the numerical superiority of the resident population. Once the resident takes some advantage, it might be difficult for the invader to be able to change the course of events. This is specially clear if the rate of generation of new beneficial mutants is not fast enough so that actually the ecological dynamics of competition play a leading role within passages. In theoretical terms, once the population is deep enough into one of the basins of attraction the entrance of a new small population will not modify the final outcome. In this way, simple infection and superinfection are not so different under the present conditions.

A diffusion approximation derived from Lotka–Volterra competition model was proposed in order to explain the reported experimental results. This model is an extension of a fitness-space model presented in [20] for RNA virus evolution. In this sort of model, each viral strain is characterized by a value in a finite one-dimensional discretized fitness space. The population of RNA viruses, indicated by $P_n^f(r, t)$, evolves on a one-dimensional discretized fitness space where the fitness increases linearly, i.e. $r_i = \alpha i$, $(i = 1, \ldots, N_c)$. Here r_i indicates replication rate (here discretized in N_c classes), $n = 1, \ldots, n_p$ is the passage number and 'f' is the flask.

The basic dynamics (assuming that the carrying capacity is normalized) was described by the following set of equations:

$$\frac{\mathrm{d}P_n^{\mathrm{f}}(r_i,t)}{\mathrm{d}t} = r_i P_n^{\mathrm{f}}(r_i,t)\left(1 - P_n^{\mathrm{f}}(r_i,t) - \sum_{j \neq i}^{N_{\mathrm{c}}} \beta_{ij} P_n^{\mathrm{f}}(r_j,t)\right) + \mu\left[P_n^{\mathrm{f}}(r_{i+1},t) + P_n^{\mathrm{f}}(r_{i-1},t) - 2P_n^{\mathrm{f}}(r_i,t)\right]$$

which are a discretized version of Fisher's diffusion model [35]. Here the competition coefficients [18] are $\beta_{ij} = 1/\beta_{ji} = r_j/r_i$. The last term is nothing but the diffusion in fitness space due to by mutation (at a rate μ). Although an additional drift term would be added to the model, it has been shown that is relatively unimportant for the qualitative features of the fitness dynamics for the VSV experiments [20]. All flasks started with an initial population $P_0^{\mathrm{f}}(r_i,0) = P_0$. The same size P_0 is used at the beginning of each passage.

These equations describe the intra-passage dynamics. At the end of each passage (i.e. at a given step T once $\sum_j P_n^{\mathrm{f}}(r_j,T) = 1$, time in arbitrary units) a stochastic sampling of the final distribution is performed. The number of flasks and replicas is the same as in the experimental setup. The new initial condition (with different frequencies of multiple infection) applied to the $n+1$ passage is defined differently under each scenario [30]. The results of these numerical experiments supported the idea that the basic mechanism at work is the competition of strains in fitness space plus the founder effects introduced by initial conditions in the superinfection case. The relative differences between the three types of infection mechanisms are not affected by changes of the parameters used (as far as they remain biologically sensible).

These results were based on computer simulations of the diffusion model, but a stronger argument for this interpretation would be obtained by considering a more general mathematical analysis. Independently to this viral context, a finite one-dimensional continuous fitness space has been considered in papers as [36,37] to derive what is called a selection–mutation model. Mathematically, these models are analogous to non-local reaction–diffusion models with reaction and diffusion terms representing selection and mutation processes, respectively. Mutation is modeled as a diffusion process in a fitness-space by means of the Laplacian operator and the non-local dependence appears in the selection term to take into account the resource competition among all types of individuals in the population, which are characterized by a value of an evolutionary variable assumed to be related to a fitness measure. Recently, mutation has been also modeled using an integral operator with a continuous kernel $\gamma(x,y)$ that represents the probability of mutation from the type y to a type in the interval $(x, x+dx)$ [38]. Finally, in [39] it was presented a selection–mutation–recombination model for bacteria adding a third term for the recombination process and using the Laplacian for the mutation process. A similar model is also considered in [40] where an integral operator with a piece-wise constant kernel is used to model mutation.

3. Selection–mutation models

Let $u(x,t) \geqslant 0$ be a density with respect to some fitness measure x (infectivity, virulence, a function of both of them, etc.) describing a viral population at time t. Here $x \in \Omega := [a,b]$, $a \geqslant 0$, $b < \infty$, where Ω stands for the *fitness space* which is assumed to be finite since any of the previous fitness measures cannot attain infinite values due to existence of metabolic limits. From a biological point of view, $u(x,t)$ is required to be non-negative and integrable with respect to x for any fixed $t \geqslant 0$. Under this formulation, the viral population consists of a continuum of viral strains characterized by a value of x.

Let $P(t) := \int_a^b u(x,t)\,dx$ denote the *total viral population* at time t and let $B(t) := \int_a^b r(x)u(x,t)\,dx$, the *total production rate of virus* at time t, be a measure of the experienced competition by any viral strain. Therefore, $B(t)$ affects the growth or replication rate of the x-viral strain by reducing it as $r(x) - B(t)$. Since $u(x,t)$ is assumed to be integrable and Ω is finite, $P(t)$ and $B(t)$ are finite as far as $u(x,t)$ exists and $r(x)$ is bounded in Ω (see below). These are two quantities that describe the viral population as a whole and that will define the non-linearities appearing in the models. As we have mentioned before, the usual form of these models contain two terms. The first one is the selection term which is taken to be the following:

$$r(x)\left(1 - \int_a^b \beta(x,y)u(y,t)\,dy\right)u(x,t) = \left(r(x) - \int_a^b r(y)u(y,t)\,dy\right)u(x,t),$$

where, as in the previous discretized version of the Fisher model, the carrying capacity of the cell culture where the virus is replicating is assumed to be normalized to 1, $r(x)$ is the replication rate which is a function of the fitness x and $\beta(x,y)$ are the competition coefficients which verify $\beta(x,y) = 1/\beta(y,x) = r(y)/r(x)$. It is worth noting that for the simulations done in [30], $r(x)$ was assumed to be a linear form. However, here we only assume it to be a positive and continuous function.

The second ingredient of the model, the mutation term, has been usually modeled in terms of a classical diffusion process in the fitness space Ω by means of the Laplacian operator, which is the more frequent approach. Precisely, the mutation term is given by $\mu(x)\Delta u$ with μ being the mutation rate of the strain labeled by x (see, for instance, [20,36,37,39,41]).

According to the previous assumptions, namely, a logistic-like selection term and the mutation process described by the Laplacian operator, and taking a constant mutation rate μ for all viral strains, the equation governing the selection–mutation process is

$$\frac{\partial u(x,t)}{\partial t} = \left(r(x) - \int_a^b r(y)u(y,t)\,dy\right)u(x,t) + \mu\frac{\partial^2 u(x,t)}{\partial t^2}, \quad a < x < b, \tag{1}$$

which has to be endowed with an *initial condition* $u(x,0) = u_0(x)$, $0 \leqslant a \leqslant x \leqslant b$, and, moreover, with *boundary conditions* in order to have a well-posed initial value problem.

Here, we will assume the so-called homogeneous Neumann boundary conditions, $\partial_x u(a,t) = \partial_x u(b,t) = 0$, which implies that no individual leaves the fitness space Ω. In other words, we assume *non-flux boundary conditions*. Although such boundary conditions as well as the zero boundary conditions ($u(a,t) = u(b,t) = 0$) can be found in the literature in the context of selection–mutation models (see, for instance [36,37,39]), our claim is that only the non-flux conditions are suitable when Ω is a fitness space.

If mutation only implies a redistribution of the potential offspring of each viral strain into different strains, then it has no sense to leave the fitness space by mutation. First, because we are assuming a maximum value of the fitness measure which is imposed by, let us say, metabolic limits. So, to leave the fitness space through this boundary ($x = b$) would mean that some members of the population achieve even higher values of fitness by mutation, which contradicts our initial assumption. Second, because, from a demographical point of view, lethal mutations produce viral particles which are not able to self-replicate. This fact means that we can label these particles with the minimum value of the fitness measure we admit ($x = a \geqslant 0$) as long as the replication rate at this value is equal to zero ($r(a) = 0$). In other words, since there is nothing

worst than a lethal mutation, it has no sense to assume that mutation process provokes a flow of individuals leaving Ω through the boundary $x = a$. Moreover, taking into account that the competition term in the model is given by $B(t)$, by assuming $r(a) = 0$ it follows that there is no contribution to competition effects from particles affected by lethal mutations (since $r(a)u(a, t) = 0$), as it is expected from a biological point of view.

Note that the previous description is not the case when Ω is a physical domain and one considers a spatial spread by diffusion in it. In this case, individuals themselves can move at random along Ω and leave it across its boundaries (when these are not isolated). Such a spatial spread is, therefore, completely different from spread in fitness space.

Under the previous interpretation of the mutation process, selection–mutation models have to be equivalent to the pure selection models when we look at the total population level, i.e., we consider the dynamics of $P(t)$. And this is actually the case when the non-flux boundary conditions are assumed since, then, it follows that the dynamics of $P(t)$ are given by the following non-autonomous ordinary differential equation:

$$\frac{\mathrm{d}P(t)}{\mathrm{d}t} = B(t)(1 - P(t)), \quad t > 0, \tag{2}$$

which is the same equation that the one obtained from our model when mutation does not occur. Note that, if $r(x) > 0 \ \forall x \in \Omega \setminus \{a\}$, then $B(t) > 0$ if $P(t) > 0$. Therefore, $P'(t) > 0 \ \forall t \geqslant 0$ for any $0 < P(0) < 1$. That is, the total population $P(t)$ has logistic-like dynamics with $P^* = 1$ (the normalized carrying capacity) being a globally asymptotically stable equilibrium for any $P(0) > 0$.

The use of the Laplacian operator means that mutation process is modelled by Brownian motion in fitness space. Such an approach has been object of many criticisms [20] due to some well-known deficiencies of both the approach itself and the corresponding solutions, criticisms that, in fact, have been also formulated in all the applications of the Laplacian in physics, chemistry and biology: only local interactions are taken into account, stochastic processes different from Brownian motion (as correlated random walks) are not included, the phenomenon of a infinite propagation in Ω of the solutions of the corresponding models, etc. (see [42,44]). Most of these criticisms are related to the fact that the use of the Laplacian implies a limit modeling approximation of a discrete (stochastic) process and, under such a perspective, one has to look at diffusion models. However, there is new a criticism that can be formulated when such an approach is applied to the mutation process, namely, diffusion is not a proper way of modeling propagation in a fitness space Ω. The reason is very simple: individuals in Ω are fixed since they have a particular fitness value x during all their lives; they are not particles having a random motion according to a given stochastic process. The apparent motions in Ω are, in fact, the dispersal in Ω of new individuals, dispersal that is provoked by mutations. Therefore, although diffusion models can reproduce more or less correctly some features of our system at a population level, the underlying mechanism is not the correct one.

On the other hand, there is another factor that affects these motions in Ω: the existence of the so-called *fitness-dependent mutational bias*. This bias is due to the fact that, when Ω is finite, the "overall probabilities of going up and down in fitness are not the same" [45]. In other words, there is a bias in the appearance of mutations which is related to the location itself in the fitness space Ω. Such a bias reflects the fact that deleterious mutations are more frequent than beneficial mutations, specially when locations in Ω corresponding to high levels of fitness are achieved. Indeed,

experimental estimates of the deleterious and beneficial mutation rates obtained for VSV suggest that the former is very high whereas the latter is very low. For instance, averaging across different genotypes of VSV, the estimated genomic deleterious mutation rate is approximately 1.2 per generation [46]. At the other side, the genomic beneficial mutation rate has been estimated to be as low as 6.4×10^{-8} per generation [47]. In other words, the vast majority of mutations produced during RNA replication must be considered as deleterious and only a tiny fraction as beneficial.

With regard to this bias, it is clear that the description of mutations using the Laplacian implies no bias in the appearance of such mutations since, as we have said, the use of this operator means that we are modeling the mutation process by a Brownian motion in fitness space. To overcome such a difficulty, one has to consider a generalization of this diffusion process, the so-called *biased diffusion* (see [48] and references in for details). However, in the context of this paper and as far as we know, no approach to mutation process has been done using biased diffusion.

3.1. An alternative selection–mutation model

A different approach to model mutation comes also from population dynamics and it is based on the so-called transport models for spatial spread (see [35,42]). Transport models have been developed in ecology, for instance, to model non-local effects and long range diffusion. In particular, they have been applied to the describe seed dispersal in plant populations, the evolution of adaptive traits, and chemotaxis [35,43,44,48]. The basic idea underlying these transport models is to replace the Laplacian operator by an integral term with a dispersal kernel $k(x, y)$ representing the density of new positions/types, given the (parental) position/type y. Since this term describes pure motion, the dispersal kernel must verify $k(x, y) \geqslant 0$ and $\int_{\Omega} k(x, y)\,\mathrm{d}x = 1$. (See [35] for a relationship among short range diffusion, long range diffusion and integral operators.)

In the context of selection–mutation models, some authors have adopted a similar approach to model mutation. Under this approach, an integral operator adds all the contributions to a given type x of viral strain coming from all types by means of a mutation kernel $\gamma(x, y)$. For instance, in [38] it is proposed and analyzed an evolutionary model of a population divided amongst two groups, young individuals and adults, both of them distributed with respect to the evolutionary trait $x = 1/T$ with T being the maturation age. However, this model, as well as the one we present below, is not a transport model with respect to the mutation process since it does not describe pure motion of the population members in Ω. Instead of this, it describes the motion in Ω of the new members of the population.

According to our description of dispersal in fitness space and using this second approach, we will assume the following *integral mutation operator*:

$$\int_a^b \gamma(x, y) r(y) u(y, t)\,\mathrm{d}y,$$

which gives the production rate of x-viral strain when the viral density is $u(y, t)$. Here $\gamma(x, y)$ is the *mutation kernel*: it gives the density of new types x of viral strains (locations in the fitness space Ω), given the parental type y. This means that, as the dispersal kernel above, γ satisfies $\gamma(x, y) \geqslant 0$ and $\int_{\Omega} \gamma(x, y)\,\mathrm{d}x = 1$, i.e., $\gamma(\cdot, y)$ is a probability density. The size of the support of $\gamma(\cdot, y)$ is, of course, related to the maximum size of a mutation (the range of mutation for fixed type y). In particular, a desirable feature of the mutation kernel is that, when the range of mutation tends to zero for all

types, the selection–mutation model has to tend to the selection term. This implies that $\gamma(x, y) \to \delta(y)$, a Dirac's delta concentrated at y, as the size of the support of $\gamma(\cdot, y)$ tends to zero.

Moreover, also from a modeling point of view, we want the mutation kernel $\gamma(x, y)$ to be *non-symmetric* (i.e., $\gamma(x, y) \neq \gamma(y, x)$ if $x \neq y$). Such an asymmetry permits a fitness-dependent mutational bias, i.e., it allows us to model a more realistic situation in which, as we have previously commented, beneficial mutation rates are lower than deleterious mutation rates.

Finally, since small mutations have higher probabilities to happen than those that are very big, it seems quite suitable for modeling purposes that $\gamma(x, y)$ is concentrated along both sides of the line $x = y$. This assumption is fully realistic for RNA viruses; it has been shown that the observed distribution of deleterious fitness effects fits very well to a compound probability model with most of the effects explained by an asymmetric gamma probability distribution and a small portion of effects drawn from a $(0, 1)$ uniform probability distribution [46]. This compound model had the advantage of explaining those mutants with larger deleterious effects.

Note that, since for each viral strain the mutation kernel is multiplied by the offspring production rate at time t, dispersal in the fitness space only takes place at the moment of reproduction. In fact, under the hypotheses on γ and assuming enough regularity in all the ingredients of the model, we obtain the total production rate of virus in the population by integrating with respect to all produced types x, that is,

$$\int_a^b \int_a^b \gamma(x, y) r(y) u(y, t) \, dy \, dx = \int_a^b r(y) u(y, t) \, dy = B(t).$$

Therefore, the previous modelling assumptions leads to the following integro-differential equation for the proposed selection–mutation model:

$$\frac{\partial u(x, t)}{\partial t} = \int_a^b \gamma(x, y) r(y) u(y, t) \, dy - \left(\int_a^b r(y) u(y, t) \, dy \right) u(x, t), \quad a \leqslant x \leqslant b, \tag{3}$$

which has to be endowed by an initial condition $u(x, 0) = u_0(x)$, $a \leqslant x \leqslant b$. Note that this formulation does not require boundary conditions, in contrast to the diffusion approach. Moreover, under this formulation there is no decomposition of the population growth in two separate parts, namely, selection (reaction) and mutation (diffusion), since mutation is already included in the selection term of the equation.

As we have already mentioned, the hypotheses on γ guarantee the convergence of the model equation to the pure selection one as the range of mutations tends to zero. This implies, in particular, that the equation for the total viral population $P(t)$ is also given by (2) since mutation is assumed to involve only random redistributions of the offspring over the fitness space. Therefore, the dynamics of the total viral population are governed by the same equation under both approaches when non-flux boundary conditions are assumed in the diffusion case, an equation that is also equal to the one for the model without mutation (the selection model). However, it is important to realize that (2) is a non-autonomous differential equation because $B(t)$ itself depends on the distribution of the viral population with respect to fitness. Hence, populations with the same size $P(t)$ but with different composition, i.e., with different viral densities $u(x, t)$, will have different values of $B(t)$ and, so, different growth rates (see Section 4 for details for the simpler case in which mutations are not considered).

4. Selection dynamics

When no mutation is present in a population with dynamics described by one of the previous equations, the resulting model, usually called a generalized logistic model [49], is given by

$$\frac{\partial u(x,t)}{\partial t} = \left(r(x) - \int_a^b r(y)u(y,t)\,\mathrm{d}y \right) u(x,t), \tag{4}$$

with $u(x,0) = u_0(x)$ for $x \in [a,b]$. The equation says that, when the total viral population approaches the carrying capacity (i.e., $\int_a^b u(x,t)\,\mathrm{d}x \approx 1$), the per capita growth rate at time t of a x-viral strain is equal to the difference between its replication rate $r(x)$ and the *mean replication rate* of the population at this time t, $\bar{r}(t) := \int_a^b r(y)u(y,t)\,\mathrm{d}y$. In fact, since $r(x)$ can be considered itself as a measure of the fitness of the x-viral strain when is an increasing function of x, $\bar{r}(t)$ is a measure of the fitness of the population as a whole at time t.

It is not difficult to verify that the results of [49] about the asymptotic behaviour of the solutions to a quite similar generalized logistic model (in some sense, even more general than one we are dealing with) hold also true for our model. The first difference between both models is the fact that, in [49], it is $P(t)$ instead of $B(t)$ the term that represents the competition among strains. The second main difference is that the authors consider growth rate and a competition coefficient (or mortality parameter in their words) as the two variables defining a two-dimensional space of evolutionary characteristics (or traits) and with respect to which a population is distributed. Roughly speaking, the main result in [49] is that the asymptotic behaviour of a solution u with a positive initial condition u_0 having as a support the interval $[a_0, b_0] \subset [a, b]$, is given by the convergence of the solution $u(x,t)$ to $b_0 \delta_{b_0}$, the maximum point of the support of u_0 times a Dirac's delta concentrated at this point, as $t \to \infty$.

Indeed, it is easy to obtain an explicit expression of the solution by looking for u with the form

$$u(x,t) = T(t)\exp(r(x)t)u_0(x)$$

with $T(0) = 1$ and, then, take the limit as $t \to \infty$. The solution is given by

$$u(x,t) = \frac{\mathrm{e}^{r(x)t}u_0(x)}{\int_{a_0}^{b_0} \mathrm{e}^{r(y)t}u_0(y)\,\mathrm{d}y - P_0 + 1}$$

with $P_0 = \int_{a_0}^{b_0} u_0(x)\,\mathrm{d}x$ is the total initial viral population.

From this expression of u and also assuming that $r(x)$ is a strictly increasing function (as in [49]), it formally follows that

$$\lim_{t\to\infty} u(x,t) = \begin{cases} 0, & x \in \Omega \setminus \{b_0\}, \\ \infty, & x = b_0 \end{cases}$$

with $\int_{a_0}^{b_0} u(x,t)\,\mathrm{d}x \to 1$ as $t \to \infty$ (see Section 3). Therefore, this (formal) limit can be thought as δ_{b_0}, a Dirac's delta concentrated at the maximum point (b_0) of the support of the initial condition u_0. Note that in case that the maximum of $r(x)$ in Ω was in $x^* \in (a_0, b_0)$, then this limit would be formally equal to δ_{x^*}.

In words, if mutation is not present, selection dynamics drive the viral population to eventually adopt the maximum fitness value of any viral strain initially present.

We would like to emphasize that, although the previous limit is a formal computation, the method we have used to obtain $u(x,t)$ illustrates a procedure that will be used in the next sections

for the full model. Moreover, it has to be pointed out that such a method has been already used in [36] for the diffusion case with zero boundary conditions.

On the other hand, as we said in the previous section, differences in the initial composition of the viral populations affect their growth. To illustrate this fact, let us consider two initial viral populations $u_1(x,0)$ and $u_2(x,0)$ both of them having $\Omega = [a,b]$ as support and competing under pure selection dynamics, i.e., no mutations occur. Finally, let us assume $r(x)$ to be strictly increasing with $r(a) = 0$ and, moreover, that $u_1(x,0)$ is biased to high values of fitness while $u_2(x,0)$ is biased to low values of fitness. Under these hypotheses and if $\int_a^b [u_1(x,0) + u_2(x,0)]\mathrm{d}x < 1$, there exists a value $x^* \in (a,b)$ such that

$$r(x^*) = \int_a^b r(x)[u_1(x,0) + u_2(x,0)]\,\mathrm{d}x.$$

Hence, from (4) it follows that all the viral strains of both populations with $x < x^*$ will have a negative growth (i.e., $\partial_t u_1(x,t)|_{t=0} < 0$, $\partial_t u_2(x,t)|_{t=0} < 0 \; \forall x < x^*$) while those strains with $x > x^*$ will have a positive growth. However, due to the bias of the initial compositions, the negative growth of $u_2(x,0)$ will be more negative than that of $u_1(x,0)$ and, in its turn, the positive growth of the latter will be higher than that of $u_2(x,0)$. Therefore, if biases of the initial compositions are strong enough, it follows an initial decline of the population size of $u_2(x,t)$ since, at $t = 0$,

$$\frac{\mathrm{d}}{\mathrm{d}t}\left(\int_a^b u_2(x,t)\,\mathrm{d}x\right) < 0$$

and, at the same time, an increase of the size of $u_1(x,t)$ because, at $t = 0$,

$$\frac{\mathrm{d}}{\mathrm{d}t}\left(\int_a^b u_1(x,t)\,\mathrm{d}x\right) > 0,$$

although the whole population $P(t) = \int_a^b [u_1(x,t) + u_2(x,t)]\,\mathrm{d}x$ always has a positive growth as long as the total initial population is lower than the carrying capacity (see (2)).

Notice that this scenario is compatible with the asymptotic behaviour we have obtained for the solutions of the pure dynamics, namely, that $u(x,t) = u_1(x,t) + u_2(x,t) \to \delta_b$ as $t \to \infty$. Nevertheless, it is not possible to predict the fraction of the total eventual population $P^*(= 1)$ that will correspond to $u_1(x,t)$ and to $u_2(x,t)$, respectively, since this fraction will depend on the transient behaviour which, in its turn, depends on the initial conditions as we have already seen. Only when initial populations have different supports, will the whole final population correspond to the eventual population of one of them. However, this situation is no longer valid when mutations are present since they provoke that, sooner or later, both populations have the same support.

In summary, differences in fitness of initial viral distributions imply different transient behaviours of populations, behaviours that are responsible for the success or the failure of invasion events (superinfections). However, it is not possible to have a more realistic analysis of the outcome of these invasion events without including mutations. At this point, we can only say that since mutations influence the motions in the fitness space Ω, differences in the way of modeling them can also have consequences on the outcomes of these invasions. For this reason, in the following sections we will carry out a detailed analysis about the dynamics of the viral density $u(x,t)$ under the occurrence of mutations (Section 5) and try to find a relationship between the time of the invasion (superinfection) and the invader and resident viral densities at this moment under an abstract approach which includes different approaches to the mutation process (see Section 6).

5. Selection–mutation dynamics

5.1. The model in an abstract form

Let us write, in an abstract form, an equation that includes both ways of modeling mutation. We can do that since the required properties of the solutions to the equations that we need will not depend on the precise approach used to represent the mutation process.

To see that let us rewrite the selection–mutation model given by (1) as follows:

$$\frac{\partial u}{\partial t} = (r(x)I + \mu\Delta)u - \mathcal{N}(u)u =: A_\mu u - \mathcal{N}(u)u,$$

where $\mathcal{N}$ is the integral operator given by $\mathcal{N}(u) = \int_a^b ru$. For the integro-differential equation model, given by (3), we write

$$\frac{\partial u}{\partial t} = \int_a^b \gamma(x,y)r(y)u(y,t)\mathrm{d}y - \mathcal{N}(u)u =: A_\gamma u - \mathcal{N}(u)u.$$

Although A_μ and A_γ are different linear operators, they share properties that imply the same asymptotic behaviour of the solutions to the corresponding model equations when the following hypotheses hold:

(H1) The replication rate $r(x)$ is a continuous function which is strictly positive for all $x \in \Omega \setminus \{a\}$ with $r(a) \geqslant 0$.

(H2) The mutation kernel $\gamma(x,y)$ is a positive and continuous function in $\Omega \times \Omega$. Moreover, we assume that there exists $\epsilon > 0$ such that the support of $\gamma(\cdot, y)$ contains the interval $I_\epsilon = (\max\{a, y - \epsilon\}, \min\{y + \epsilon, b\})$ for each $y \in \Omega$.

Hypothesis (H1) is a sufficient condition to guarantee that A_μ verifies the next properties (in fact, the weaker condition $\int_a^b r(x)\,\mathrm{d}x > 0$ is also a sufficient condition, see [39]). Hypotheses (H1) and (H2) guarantee the same for A_γ (see Remark 2). Indeed, both hypotheses can be relaxed but they are natural from a modeling point of view. First, because viral replication rates for $a < x \leqslant b$ are always strictly positive without competition among strains. This implies that (H1) is satisfied if one assumes that these rates change smoothly with respect to the fitness. In particular, the replication rate assumed in [30], namely, $r(x) = \alpha x$, $\alpha > 0$, $x \in [0,1]$, satisfies (H1) for both settings with $a = 0$ and $r(0) = 0$. Second because, as a mutation kernel, $\gamma(x,y)$ has to be concentrated along both sides of the line $x = y$ in the sense given by (H2). Of course, all the assumptions on γ in Section 3 are compatible with (H2), in particular, that $\gamma(x,y) \neq \gamma(y,x)$ and that $\int_a^b \gamma(x,y)\,\mathrm{d}x = 1 \ \forall y \in \Omega$.

According to the previous models, a general abstract form of selection–mutation models is

$$\frac{\partial u}{\partial t} = Au - \mathcal{N}(u)u$$

with A being a linear operator satisfying the following properties (see [50] for definitions):

(i) A is the infinitesimal generator of a C_0-semigroup, denoted by e^{At}, in a Banach space X (in our case, $X = L^1(a,b)$).

(ii) A has a positive and strictly dominant eigenvalue λ^* with algebraic multiplicity 1, i.e., its spectrum $\sigma(A)$ is entirely to the left of the line $Re\lambda^* = \lambda^* > 0$ of the complex plane $\mathbb{C}$ (there exists $\delta > 0$ such that $Re\lambda < \lambda^* - \delta$ for any $\lambda \in \sigma(A), \lambda \neq \lambda^*$).

(iii) The eigenfunction corresponding to λ^*, φ^*, has constant sign and, since $u_0 > 0$, it is chosen to be positive.

Under these conditions the generated semigroup e^{At} has the property of *asynchronous exponential growth* (see Theorem 1).

From a biological point of view, (ii) implies that the maximum growth rate of the viral population without competition effects, which is given by λ^*, has to be strictly positive. Note that this property is not always satisfied, even with positive intrinsic growth rates $r(x)$. For instance, in the diffusion case with zero boundary conditions, the existence of a positive strictly dominant eigenvalue λ^* is not guaranteed unless a small enough mutation coefficient μ is assumed (see [36,37]), which implies that not many individuals leave the fitness space at the boundaries. In other words, the potential growth of a viral population decreases with high mutation rates because a part of the population 'leaves' the fitness space through the boundaries $x = a$ and $x = b$. In contrast, imposing conditions of non-flux at the boundaries ($\partial_x u(a,t) = \partial_x u(b,t) = 0$), the population, as a whole, does not lose individuals by mutation at the boundaries and, so, there is no need to assume low mutation rates in order to have $\lambda^* > 0$. In particular, under these non-flux boundary conditions and for any value of the mutation coefficient μ, a positive lower bound of λ^* is obtained from Rayleigh quotient, namely,

$$\lambda^* \geqslant \bar{r} := \frac{1}{b-a} \int_a^b r(x)\,\mathrm{d}x,$$

that is, the maximum growth rate λ^* of the whole viral population when competition effects are not present is, at least, equal to the mean replication rate. Similarly, for the transport-like approach, the positivity of $r(x)$ and $\gamma(x,y)$ guarantees $\lambda^* > 0$. In the next section, we will see that, under any of both approaches, λ^* is given by the mean replication rate of a viral population whose density u is proportional to φ^*.

Remarks

(1) In [36] it is given a suitable framework to include not only densities but also Dirac's deltas as initial conditions in the diffusion case with $u(a,t) = u(b,t) = 0$. This means that monomorphic populations with respect to the fitness value can be considered as initial populations (i.e., $u_0(x) = \delta_{x_0}$, $x_0 \in [a,b]$). However, due to the high mutation rates of the RNA viral populations, it is suitable to admit as initial conditions densities $u_0 \in D(A_\mu) = \{\phi \in L^1(a,b): \phi'$ is absolutely continuous and $\phi'(a) = \phi'(b) = 0\}$ as in [39]. In the transport-like case, the domain of A_γ is $D(A_\gamma) = L^1(a,b)$ and, so, any (positive) density on $[a,b]$ can be considered as initial condition.

(2) Under (H1) and (H2) A_γ is a positive integral operator of Fredholm type which is compact from $L^1(a,b)$ to $L^1(a,b)$. Therefore, (i) is fulfilled since A_γ is the infinitesimal generator of a uniformly continuous semigroup and, so, of a C_0-semigroup in $L^1(a,b)$. On the other hand, the semigroup $e^{A_\gamma t}$ is irreducible since A_γ itself is an irreducible operator when γ is concentrated

along both sides of the line $x = y$ (see [51] for definitions). Moreover, since A_γ is a compact generator of a positive semigroup, the spectral bound of A_γ, $s(A_\gamma)$, is an element of $\sigma(A)$ whose elements are poles of the resolvent (i.e., eigenvalues) [50,51]. Therefore, since $e^{A_\gamma t}$ is an irreducible, positive semigroup in $L^1(a,b)$ and $s(A_\gamma) \in \sigma(A)$, $s(A_\gamma)$ is a dominant eigenvalue with algebraic multiplicity 1 and the only eigenvalue admitting a positive eigenfunction φ^* (see Theorem 1.3 in [52]). Hence (iii) follows. Finally, since A_γ is compact, its spectrum is a countable set with no accumulation point different from zero [50] and, so, $s(A_\gamma)$ is isolated. Therefore, $s(A_\gamma)$ is a strictly dominant eigenvalue and, hence, (ii) is fulfilled with $\lambda^* = s(A_\gamma)$.

5.2. The solution and its asymptotic behaviour

Similarly to what we have done for the selection dynamics, we could look for a solution of the abstract equation $\partial_t u = Au - \mathcal{N}(u)u$ in the form $u(x,t) = T(t)e^{At}u_0(x)$ with $T(t)$ a function of time such that $T(0) = 1$ and with $e^{At}u_0$ being the solution to the associated linear problem $\partial_t w = Aw$ with initial condition $w(x,0) = u_0(x)$, i.e., the semigroup generated by A. In our case and according to the definition of $\mathcal{N}$, we would obtain that

$$u(x,t) = \frac{e^{At}u_0}{\int_0^t \mathcal{N}(e^{As}u_0)\,ds + 1} = \frac{e^{At}u_0}{\int_0^t \left(\int_a^b r(x)e^{As}u_0(x)\,dx \right) ds + 1}.$$

This procedure works as long as A is the infinitesimal generator of a C_0-semigroup and $\mathcal{N} : X \to \mathbb{R}$ is an integral operator (see [38]). However, it can also work even if the semigroup generated by A is non-linear (see, for instance, the models in [39,40]).

To determine the asymptotic behaviour of the solution to the non-linear problem, it is more convenient to rewrite $u(x,t)$ in terms of the rescaled semigroup e^{A^*t} generated by the linear operator $A^* := A - \lambda^*I$, i.e., $u(x,t) = T(t)e^{A^*t}u_0(x)$ with $T(t)$ a function of time such that $T(0) = 1$ and $e^{A^*t} = e^{-\lambda^*t}e^{At}$.

Note that, since A satisfies properties (i)–(iii), A^* has 0 as its strictly dominant eigenvalue with φ^* being its corresponding eigenfunction. Moreover, the asymptotic behaviour of the rescaled semigroup e^{A^*t} is given by the next theorem.

Theorem 1. *Let A be an operator satisfying hypotheses* (i)–(iii) *and let e^{A^*t} denote the C_0-semigroup generated by $A^* := A - \lambda^*I$. Then there exist constants $a > 0$ and $M > 1$ and a continuous linear form $\alpha : X \to \mathbb{R}$ such that, for all $u \in X$ and $t \geqslant 0$,*

$$\|e^{A^*t}u - \alpha(u)\varphi^*\|_X \leqslant Me^{-at}\|u\|_X.$$

Moreover, $\alpha(u) > 0$ whenever u belongs to $X^+ \setminus \{0\}$.

For the case $A = A_\gamma$, this is a well-known result since, as we have seen, under (H1) and (H2) A_γ is a bounded operator having a positive, strictly dominant eigenvalue λ^* with geometrical multiplicity 1 (see Remark 2 below). For the case $A = A_\mu$, see [36,39].

Roughly speaking, this result implies that, since $u(x,t) = T(t)e^{A^*t}u_0(x)$, the shape of the viral density tends (in the X-norm) to that of φ^* exponentially while the behaviour of the total viral population is given by $T(t)$. In demographical terms, we would say that the viral population tends to a *stable distribution* given by φ^*. It is worth noticing that the shape of φ^* depends on the choice of the operator used to model mutations: A_μ, A_γ or any other suitable linear operator satisfying properties (i)–(iii).

To know more precisely the behaviour of $u(x, t)$, let us write the abstract problem in terms of the auxiliar operator A^*, namely,

$$\partial_t u = A^* u + \lambda^* u - \mathcal{N}(u)u.$$

Replacing u by its expression in this equation, it follows that $T(t)$ satisfies the following asymptotically autonomous ODE:

$$T'(t) = \lambda^* T(t) - T^2(t) \int_a^b r(x) e^{A^* t} u_0(x) \, dx,$$

which has as a limit equation the following ODE:

$$T'_\infty(t) = \lambda^* T_\infty(t) - \alpha(u_0) T^2_\infty(t) \int_a^b r(x) \varphi^*(x) \, dx$$

since $e^{A^* t} u_0 \to \alpha(u_0) \varphi^*$ as $t \to \infty$. The solution to the latter is

$$T_\infty(t) = \frac{\lambda^*}{\lambda^* e^{-\lambda^* t} + [1 - e^{-\lambda^* t}] \alpha(u_0) \int_a^b r(x) \varphi^*(x) \, dx} \to T^*_\infty = \frac{\lambda^*}{\alpha(u_0) \int_a^b r(x) \varphi^*(x) \, dx}$$

as $t \to \infty$. Therefore, since $T(t)$ is bounded for all positive time, and T^*_∞ is the unique equilibrium of $T_\infty(t)$ and it is globally asymptotically stable for positive initial conditions, it follows that $T(t)$ also tends to T^*_∞ as $t \to \infty$ (see [53]).

Finally, the asymptotic behaviour of the solution to the selection–mutation model in any of the two proposed forms is

$$u(x, t) = T(t) e^{A^* t} u_0(x) \to u^*(x) = \frac{\lambda^*}{\int_a^b r\varphi^*} \varphi^*(x) \quad \text{as } t \to \infty.$$

That is, there exists a unique non-trivial equilibrium for any non-zero initial condition u_0 which is globally asymptotically stable. Moreover, this equilibrium is a positive multiple of φ^*, the eigenfunction associated to the dominant eigenvalue λ^*. (Under the diffusion approach, this result has been obtained in [36,39] for similar models.)

In particular, since the carrying capacity is normalized to 1 in the original models (Section 3), by imposing that $\int_a^b u^* = 1$ it follows that the dominant eigenvalue of A is given

$$\lambda^* = \frac{\int_a^b r\varphi^*}{\int_a^b \varphi^*}.$$

In other words, the maximum growth rate of the viral population under mutation when competition effects are not present is achieved when the viral density $u(x)$ is proportional to φ^*, and this maximum is equal to the corresponding *mean replication rate* of the population.

It is illustrative to relate this result to the corresponding to the pure selection dynamics. Precisely, to relate the shape of φ^* with the Dirac's delta δ_{b_0} as mutation size tends to zero (i.e., when $\mu \to 0$ or the support of $\gamma(\cdot, y)$ becomes very small). This property has been already established for similar models using the diffusion approach (see [36,37] for the case with zero boundary conditions), and for models using the probabilistic or transport-like approach [38]. In terms of our model, the result would say that, when mutation is small and $r(x)$ is strictly increasing, φ^* tends to be concentrated at the maximum point of the one-dimensional fitness space ($x = b$), and so does

$u(x,t)$ as $t \to \infty$. In this case, moreover, from the previous expression of λ^* it follows that $\lambda^* \to \max_\Omega\{r(x)\}$. Of course, this result is the one we expect from a biological point of view since, now, all types of viral strains will appear sooner or later by mutation and, as before, selection will drive viral strains to adopt the maximum fitness value that is possible. However, a rigorous proof of such a convergence of solutions of general selection–mutation models to solutions of pure selection models lies outside of the scope of the present paper.

This knowledge of the asymptotic behaviour of solutions to our initial value problem will be used in the next section for a qualitative analysis of competitive displacements in superinfection processes.

Remarks
(1) Since the domain of A_γ, $D(A_\gamma)$, is $X = L^1(a,b)$, the solution $u(x,t)$ of the transport-like case is classical for any initial condition $u_0 \in X$. However, in the diffusion case, since $D(A_\mu) \subset X$, $u(x,t)$ is a mild solution of the selection–mutation model unless $u_0 \in D(A_\mu)$. (See [54] for definitions.)
(2) Since A_γ is compact and has a strictly dominant eigenvalue, it follows that the spectrum of the rescaled operator is the disjoint union of two non-empty compact subsets $\sigma(A^*) = \{0\} \cup \sigma_2(A^*)$, and that the spectrum of the rescaled semigroup is $\sigma(e^{A^*t}) = e^{t\sigma(A^*)} = \{1\} \cup e^{t\sigma_2(A^*)}$. Hence, there exist a spectral projection P_{φ^*} of rank one on the eigenspace $X_0 = P_{\varphi^*}X = \langle\varphi^*\rangle$ along $X_1 = (I - P_{\varphi^*})X = \ker(P_{\varphi^*})$, and a unique spectral decomposition $X = X_0 \oplus X_1$ corresponding to $\{0\} \cup \sigma_2(A^*)$ such that the restrictions of e^{A^*t} to X_0 and X_1, denoted by $e^{A^*t}|_{X_0}$ and $e^{A^*t}|_{X_1}$, respectively, have bounded generators A_i^* $(i = 1, 2)$ with $\sigma(A_1^*) = \{0\}$ and $\sigma(A_2^*) = \sigma_2(A^*)$. More precisely, for all $\phi \in X$, $\phi = \phi_0 + \phi_1$ with $\phi_i \in X_i$, and $e^{A^*t}\phi = e^{A^*t}|_{X_0}\phi_0 + e^{A^*t}|_{X_1}\phi_1$ with $\sigma(e^{A^*t}|_{X_0}) = \{1\}$ and $\sigma(e^{A^*t}|_{X_1}) = e^{t\sigma(A_2^*)}$. Therefore, $e^{A^*t}|_{X_1}$ satisfies that $\|e^{A^*t}|_{X_1}\|_X \to 0$ exponentially as $t \to \infty$ because the spectral bound of A_2^*, $s(A_2^*)$, is strictly negative and, hence, the statement of the theorem follows. For definitions and results on spectral theory, see [50,51].

6. Superinfection vs coinfection

Although the asymptotic behaviour of solutions to the previous selection–mutation models is well established, we need to be able to say something about the transient behaviour of the competing viral strains in order to explain the reported experimental results on viral evolution in [30]. Note that, under the framework given by the selection–mutation model of the previous section, the equations governing the dynamics of resident strains and of invader strains in superinfection, and the dynamics of co-invaders in coinfection, are actually the same. The only difference is the distribution itself of the viral population in the fitness space: a distribution of fitter strains is more to the right in the fitness space with respect to a distribution of less fitted strains. This is in contrast with respect to the usual models in evolutionary dynamics where differences in the parameters of the equations distinguish between resident and mutant (or invader) types.

Coinfection is characterized by an initial condition given by a simultaneous infection of a new host with viral strains from two different sick hosts. That is, in coinfection, two different distributions of viral strains, $u_1^0(x)$ and $u_2^0(x)$, determine the initial condition. In contrast, superinfection

implies a two-step process: an initial infection of a new host with viruses from a sick host and, after a time τ, an addition of new viruses (second infection) coming from a different sick host.

Therefore, to study the transient behaviour of the solutions corresponding to each infectious process, we have to establish the corresponding initial conditions. Such conditions will be based on the reported experiments in [30] where two different frequencies of per host multiple infection were employed for both multiple infectious processes in the in vitro experiments, namely, 5% and 50%.

According to these experiments, if we denote the whole viral strain distribution in an infected host by $u^c(x,t)$, the initial condition in coinfection $u^c(x,0)$ is given by

$$u^c(x,0) = su_1^0(x) + (1-s)u_2^0(x)$$

with $s = 0.95$ and $s = 0.50$, respectively. In superinfection, there is an initial condition at each step, namely,

(1) $u_1(x,0) = su_1^0(x)$,
(2) $u^s(x,0) = u_1(x,\tau) + (1-s)u_2^0(x)$,

with $u_1(x,t)$ being the distribution of viral strains of the first infection for $0 \leqslant t \leqslant \tau$, i.e., before the second infection takes place. For both infectious processes, it can be assumed that $\int_a^b u_1^0(x)\,dx = \int_a^b u_2^0(x)\,dx = P_0 \ll 1$ (recall that the carrying capacity is normalized to 1). Hence, $\int_a^b u^c(x,0)\,dx = sP_0 + (1-s)P_0 = P_0$, i.e., the total initial viral population in coinfection is equal to P_0. In superinfection, the same amount of virus P_0 is introduced into the infected host but at two different times.

Therefore, in coinfection, $u^c(x,t) = u_1^c(x,t) + u_2^c(x,t)$ with $u_1^c(x,0) = su_1^0(x)$ and $u_2^c(x,0) = (1-s)u_2^0(x)$. In superinfection, after the second infection, we have $u^s(x,t) = u_1^s(x,t) + u_2^s(x,t)$ with $u_1^s(x,0) = u_1(x,\tau)$ and $u_2^s(x,0) = (1-s)u_2^0(x)$.

In contrast to what happens in coinfection, in superinfection the resident viral population will be larger and fitter than the viral population of the second infection if τ is big enough, as in our case. In particular, since $\tau = 6$ h (while the whole passage is 24 h long), we assume that

$$\int_a^b r(x)u_1(x,\tau)\,dx \gg \int_a^b r(x)u_2^s(x,0)\,dx. \tag{5}$$

With respect to the dynamics of each infectious process, the viral population densities $u_i(x,t)$ satisfy, in both cases, the equation

$$\partial_t u_i = Au_i - \mathcal{N}(u_1 + u_2)u_i, \quad i = 1,2.$$

Hence, integrating with respect to x, it follows that the total size of each population (resident and invader) satisfy, respectively,

$$\frac{dP_i(t)}{dt} = B_i(t) - B(t)P_i(t), \quad i = 1,2, \tag{6}$$

where $P_i(t) := \int_a^b u_i(x,t)\,dx$ and $B_i(t) := \int_a^b r(x)u_i(x,t)\,dx$. Note that (2), which governs the dynamics of the whole viral population, follows immediately from these equations because $P(t) = P_1(t) + P_2(t)$ and $B(t) = B_1(t) + B_2(t)$. In particular, a monotonous increase of $P(t)$ occurs whenever the total size of the initial condition $P_1(0) + P_2(0) < 1$. For instance, in superinfection, this means that $P'(t) > 0$ as long as $\int_a^b [u_1(x,\tau) + (1-s)u_2^0(x)]\,dx < 1$.

However, according to (6) and whenever $P'(t) > 0$, it follows that an initial decline of the invading viral population ($P'_2(t) < 0$) can occur while the resident population undergoes a positive growth ($P'_1(t) > 0$). In such a case, we can consider that the second infection fails, i.e., there is no establishment of the invading viral population, since its initial size is assumed to be very small ($(1 - s)P_0 \ll 1$). In fact, as long as $P'(t) > 0$, such an increase of the resident population should be always the case when the viral population of the second infection declines since $P'(t) = P'_1(t) + P'_2(t)$.

To see more precisely the condition for a failure of the second infection, let us write (6) in the form

$$\frac{\mathrm{d}P_i(t)}{\mathrm{d}t} = B(t)\left(\frac{B_i(t)}{B(t)} - P_i(t)\right), \quad i = 1, 2.$$

So, the sign of $P'_i(t)$ depends on the difference between $B_i(t)/B(t)$, a measure of the relative fitness of the i-population, and $P_i(t)/P^*$, the fraction of the carrying capacity occupied by this population (recall that $P^* = 1$). This implies that if the invading viral density $u_2(x, 0)$ is mainly distributed around low values of the replication rate $r(x)$ so that $B_2(t)/B(t) \ll 1$, then it is possible to have a negative growth even for small values of $P_2(0)$. In this case, $P'_2(t) < 0$ and, using that $B(t) = B_1(t) + B_2(t)$, it follows that

$$\frac{\mathrm{d}P_2(t)}{\mathrm{d}t} < 0 \iff \frac{B_2(t)}{B(t)} < P_2(t) \iff \frac{B_1(t)}{B(t)} > 1 - P_2(t). \tag{7}$$

Hence, whenever $P_1(t) + P_2(t) < 1$, the last inequality implies that $B_1(t)/B(t) > P_1(t)$ and, so, that $P'_1(t) > 0$. That is, $P'_2(t) < 0$ implies the increase of the resident viral population. In particular, if (5) holds at the moment of the second infection, then

$$\frac{B_1(0)}{B(0)} \approx 1 \quad \text{and} \quad \frac{B_2(0)}{B(0)} \approx 0.$$

That is, under (5) almost all the invading populations with initial sizes $P_2(0) = (1 - s) \int_a^b u_2^0(x)\,\mathrm{d}x$ will have a negative initial growth ($P'_2(0) < 0$). In other words, at the moment τ of the second infection, the fitness of the resident population (measured by $B_1(0)$) is so high that most of the second infections will fail in superinfection.

Therefore, the success of the second infection depends critically on two main factors, namely,

(i) the total replication rate of the resident population at the moment τ of the second infection, $B_1(0) = \int_a^b r(x)u_1(x, \tau)\,\mathrm{d}x$, and
(ii) the total replication rate of the initial viral population of the second infection, $B_2(0) = (1 - s) \int_a^b r(x)u_2^0(x)\,\mathrm{d}x$.

Note that, when $r(x)$ is strictly increasing, the previous integrals are a measure of the fitness of the resident and invading viral populations, respectively. This measure depends on the size of the total population and on the distribution of its density $u(x, t)$ in the fitness space Ω.

Now let us compare conditions experienced by an invading population under coinfection and superinfection processes. For this purpose, we consider the equation for $u^s(x, t)$, the viral density in superinfection. In fact, as the operator A is the same for both infectious process (and so are λ^*

and A^*), the solution in each case can be written as $u^s(x,t) = T(t)e^{A^*t}u^s(x,0)$ and $u^c(x,t) = T(t)e^{A^*t}u^c(x,0)$, respectively, with the initial condition being the only difference between them. Proceeding as in the previous section and using the expression of $u^s(x,0)$ given at the beginning of this section, one obtains that $T(t)$ satisfies

$$\frac{T'(t)}{T(t)} = \lambda^* - T(t) \int_a^b r(x)e^{A^*t}[u_1(x,\tau) + (1-s)u_2^0(x)]\,\mathrm{d}x$$

with $T(0) = 1$. Hence, it follows that, when $T'(0) > 0$, both viral populations, the resident and the invader, have a positive growth since they are given by $u_i^s(x,t) = T(t)e^{A^*t}u_i^s(x,0)$, $i = 1,2$, respectively. On the contrary, if $T'(0) < 0$, both populations undergo a decline in their sizes. (Note that since the dominant eigenvalue of A^* is equal to 0, the term $e^{A^*t}u(x,0)$ of the solution is mainly responsible of the shift of the profile of $u(x,t)$ to a shape which is proportional to that of φ^* (see Section 5.2) and, therefore, changes in $P(t)$ are mainly due to changes in $T(t)$.)

As we are interested in comparing initial growths in coinfection and superinfection processes, let us consider the condition which combines an initial decline of the invading viral population in superinfection, and a positive growth for the same population in coinfection. This happens if the resident viral population at the moment of the second infection, $u_1(x,\tau)$, is such that the following inequalities are satisfied (recall that $T(0) = 1$):

$$\int_a^b r(x)[su_1^0(x) + (1-s)u_2^0(x)]\,\mathrm{d}x < \lambda^* = \frac{\int_a^b r\varphi^*}{\int_a^b \varphi^*} < \int_a^b r(x)[u_1(x,\tau) + (1-s)u_2^0(x)]\,\mathrm{d}x. \tag{8}$$

The first inequality in (8) comes from imposing that, in coinfection, the initial growth of the viral population in the infected host is always positive as far as the total initial population is small and, clearly, the magnitude of λ^* is related to this (linear) initial growth where competition effects among viral strains can be neglected (see Section 5). In contrast, the second inequality in (8), which comes from imposing $T'(0) < 0$, is only satisfied if, at the moment of the second infection, the resident viral population is big enough and, moreover, it is distributed around high values of $r(x)$. In this case, the introduction of a second population implies an increase of the competition effects which causes a decrease of both populations, the resident and the invading one.

More precisely, it follows that, for a fixed $u_2^0(x)$, it is less and less feasible that, as τ decreases, the second inequality in (8) can be satisfied, i.e., it is more and more feasible the success of the second infection. Note that in the limit case $\tau = 0$, we have a coinfection process instead of a superinfection process, and the left and right terms of the previous inequalities are exactly the same since then $u_1(x,0) = su_1^0(x)$. On the contrary, as τ increases, $u_1(x,\tau)$ becomes fitter and fitter since its shape approaches to that of $\varphi^*(x)$, which tends to be concentrated in the highest values of $r(x)$ if mutation is not very high. Eventually, as we have seen when dealing with the asymptotic behaviour of solutions in Section 5, one has that, as $\tau \to \infty$,

$$u_1(x,\tau) \to \frac{\varphi^*}{\int_a^b \varphi^*}.$$

In this limit case, $\int_a^b r(x)u_1(x,\tau)\,\mathrm{d}x \approx \lambda^*$ and, so, the second inequality is always fulfilled since then it is equivalent to the (trivial) inequality $\lambda^* < \lambda^* + (1-s)\int_a^b ru_2^0$. In other words, in this limit case, the resident viral population has taken possession of all the carrying capacity and, so, the introduction of a second viral population results in negative competition effects for both of them.

Note that mutational effects are present in (8) throughout λ^* since its value depends on the particular election of the operator A which, in its turn, contains the representation of the mutation process.

On the other hand, consider a fixed $\tau > 0$ and a fixed duration time t_p for the infection passages. If $u_1^n(x,t)$ denotes the n-passage viral population before the second infection, then $u_1^n(x,t) = T(t)e^{A^*t}u_1^n(x,0)$, $0 \leqslant t \leqslant \tau$. At each single infection passage and if t_p is large enough, the shape of $u_1(x,t_p)$ approaches to that of φ^*, i.e., it shifts to higher values of $r(x)$. Therefore, since the size of the viral population at the beginning of each passage is always the same, namely, sP_0, the mean fitness of the viral population at the beginning of the next passage, measured by $s \int_a^b r(x)u_1^0(x)\,dx$, increases with n, the number of single infection passages. This implies that, as the number of passages increases ($n \to \infty$), inequalities given by (8) become

$$s\alpha \int_a^b r\varphi^* < \left(\lambda^* - (1-s)\int_a^b r(x)u_2^0(x)\,dx\right) < \frac{s\lambda^*\alpha \int_a^b r\varphi^*}{s[1 - e^{-\lambda^*\tau}]\alpha \int_a^b r\varphi^* + \lambda^*e^{-\lambda^*\tau}}, \tag{9}$$

where it is used that, if the second infection occurs at the n-passage at a fixed $\tau > 0$, then we have

$$u_1^n(x,0) = su_1^{n-1}(x,t_p) \to s\alpha\varphi^*(x) \quad \text{as } n \to \infty$$

and

$$u_1^n(x,\tau) = T(\tau)e^{A^*\tau}u_1^n(x,0) \to sT_\infty(\tau)\alpha\varphi^*(x) \quad \text{as } n \to \infty.$$

Here α is a positive constant depending on the initial condition at the beginning of the passage $u_1^n(x,0)$ (see Theorem 1 in Section 5.2) and $T_\infty(t)$ is given in Section 5.2.

From these inequalities it follows that the left- and right-hand terms in (9) are the same for $\tau = 0$ while, even for not very large values of τ, the right-hand term becomes close to λ^* when n increases. This means that, when the number n of passages increases, the second infection can succeed in invading the resident viral population only for small values of τ since only then $T'(t) > 0$.

As usual in infectious processes, there is a bottleneck in this sort of evolution experiments which is related to sampling from the final viral population at the end of a passage ($u_1^{n-1}(x,t_p)$) to obtain the initial population for the next passage ($u_1^n(x,0)$) since, then, stochastic effects become more important if the (normalized) size of the initial population P_0 is small. In our case and as far as the sampling renders approximately the 'shape' of the viral distribution at the end of the previous passage, this could mean a slower (or, at least, a non-uniform) approximation with respect to the number of passages n of (8) to the inequalities given by (9) but, eventually, we arrive at the same conclusions as before.

In summary, as far as (7) holds or the second inequality in (8) is satisfied at $t = 0$, the viral population of the second infection will decline. In practical terms, as the invading population size is very small, we are talking about a failure of the second infection. In the reported experimental results in [30], such decrease of the invading viral populations goes in parallel with the fact that, for the value of $\tau = 6$ used in the experiments, the resident viral population is in the exponential phase of the logistic growth curve. This combination of facts, namely, an exponential growth of the resident viral population, an initial decreasing of the viral population of the second infection, and a finite duration of the infection passages, make that the total invading viral population cannot achieve a significant level at the end of the infection passage. This could be the reason why

there were not differences between the average increase of fitness after a series of recurrent su-superinfection passages and the average increase of fitness after a similar series of single-infection passages in the evolution experiments reported in [30]: they are essentially equivalent infectious processes when τ is big enough. This conclusion remains true in spite of the approach one adopts to model the mutation process as far as the resulting operator has properties (i)–(iii) in Section 5.1.

7. Discussion and conclusions

In this paper we have presented a general abstract formulation of selection–mutation models in a one-dimensional continuous fitness space which includes those based on the reaction kinetics approach (i.e., reaction–diffusion models) as well as alternative models based on a transport-like approach. This formulation provides a useful unifying approach to study the basic scenarios of modeling different pathways of multiple infection, following the basic protocols defined in a previous paper [30], and allows us to give an analytical understanding of both the reported experimental and simulation results. From this approach it follows that, although having different assumptions on the mutation process, there are not qualitative differences among the predictions obtained from the classical diffusion approach to model mutations with respect to those obtained using other approaches.

Under natural hypotheses on the model ingredients, we have characterized the asymptotic behaviour of solutions to the general model, which is given by a convergence to a globally and asymptotically stable non-trivial equilibrium which is proportional to φ^*. Moreover, it has been established conditions for the failure of the second infection in a superinfection scenario (see (7) and (8)). In the first case, the failure is due to an initial decrease of the invading population when its initial distribution is biased to fitness values lower than those around of which the resident population is mainly distributed. In fact, since mutations provoke that both the resident and the invading viral distributions have all the fitness space Ω as support, in the long run both populations would coexist because the joint distribution tends to φ^* as $t \to \infty$. However, this eventual coexistence is compatible with an initial decline of the invading viral population which does not allow the population to recover and attain a significant level during an infection passage because of its finite duration. In the second case (see (8)), there is a decline of the whole population, resident and invader, caused by the increase of competition effects after the second infection. In both cases, as the size of the invading viral population is smaller than that of the resident when τ is big enough, such an initial decline implies the failure of the superinfection.

On the other hand, the simulation study in [30] revealed, in agreement with the in vitro results, that coinfection promotes higher fitness levels and that superinfection is not better than simple infection in terms of the final fitness generated through the evolution process. These results were shown to be a consequence of the advantage provided by the resident population size, and they are actually closely related to previous theoretical studies on community assembly models [27–29] in competitive communities. Although a given species might be a better competitor than others present in the resident population, it can be shown that the invasion probability of a newly arriving species is strongly context-dependent: it might invade under some circumstances and fail under some others. Once the community has reached some degree of complexity, it can hardly be invaded. A main difference between the model presented here and those previously proposed

[23–26] is that previous models are based in the generally accepted assumption of a trade-off between transmission rate and virulence. All these models optimize the basic reproductive rate of the parasite, R_0, instead of the within-host replication rate (equivalent to virulence for a lytic virus like VSV), as we have done here. In [30] transmission rate is uncoupled from virulence by artificially sampling the viral population at the end of the within-host growth cycle and by transmitting a constant amount of viral particles regardless of their transmission abilities. By doing so, it was possible to specifically explore the role played by within-host replication and competition between variants. As we stated before, our goal here was to provide a theoretical framework to explain the results obtained in [30] and, therefore, the model here presented is valid for the case of transmission rate not being subjected to the action of natural selection. Hence, this might result in a less general model than others previously presented. The next obvious step in our research will be to introduce differential transmission rates and explore the role of trade-offs between transmission rate and within-host replication and competence. This step will be taken both experimentally and theoretically.

The previous studies suggested a similar situation, in which the time delay before a new infection takes place under the superinfection scenario prevented the success of the new invading clone. Beyond the differences arising from the ecological and the host-parasite cases, it seems clear that some of the limitation introduced by community assembly rules in communities of competitors might well apply within our context. Here we further expanded the previous analysis using well-defined mathematical models of multiple infection in which viral strains are represented in terms of a time-dependent density function $u(x,t) \geqslant 0$ evolving on a simple one-dimensional fitness space. By using both standard diffusion and an integral operator with a continuous kernel $\gamma(x,y)$ (that represents the probability of mutation from the type y to the type in the interval $(x, x + dx)$) our previous predictions have been fully confirmed: coinfection dynamics are the most efficient mechanism in promoting increasing levels of viral replication. This is the result of competitive interactions under similar initial conditions, which enhances the selection of faster replicators. Under superinfection, the population advantage taken by the resident strain makes very difficult a successful invasion by the newly introduced strain and, as a consequence, it is typically not better than simple infection.

Our model has been developed to explain the within-host competition dynamics between variants of the same virus. However, it is a common and interesting problem in clinical virology the existence of mixed infections between different types of viruses. For example, coinfection between hepatitis B or C viruses (HBV or HCV, respectively) and human immunodeficiency virus type-1 (HIV-1) is extremely common in hemophiliacs and injection drug-user. Conflicting results had been reported for this case. Daar et al. in [55] found a significant increase in the rate of progression to clinical AIDS and AIDS-related mortality in patients coinfected with HCV and HIV-1 compared with patients singly infected with HIV-1, which might indicate a selection for faster replicating HIV-1 strains, as predicted by our model. In some other cases, the interaction between both viruses is indirect; HCV takes an advantage, increasing its load, as a consequence of the HIV-1 induced immunosuppression [56,57]. In contrast, it has been suggested than neither coinfection nor superinfection with HBV increases the rate of progression to AIDS [58]. Similarly, it has been reported than during coinfection with HIV-1 and human herpesvirus 6 (HHV-6), the presence of one virus appears not to modify the replication or cytopathicity of each other despite the fact than both infect and replicate in CD4^{+} T cells [59]. These apparently contradictory results

are clearly calling for models that help us to understand the population dynamics behind the observed phenomena and to clarify what parameters are responsible for such differences.

A potential weakness for the applicability of our model to real in vivo viral populations is that it ignores the density-dependence caused by immune responses as well as the spatial and temporal heterogeneity that represents the different tissues that constitute a multicellular organism. Under continuous mutation, changes in viral proteins that avoid elimination of the virus by immune responses (antigenic variation) should constantly arise. These changes lead to a dynamic steady-state of virus load with a rapid turnover of genotypes: genotypes are recognized by the immune system and cleared but scape mutants are constantly produced that avoid this clearance. It has been postulated that higher levels of antigenic variation produce higher virus loads constituted by extremely polymorphic populations [60]. Hence, the balance between strain-specific and cross-reactive immune responses controls and promotes the coexistence of viral strains [61]. Including in our models this effect is one of our future research avenues.

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