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Stochastic extinction of viral infectivity through the action of defectors

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Abstract – The high error rates of RNA viruses at replication suggest they might be close to the extinction threshold predicted by quasispecies theory. Hence, moderate increases in the mutation rate could drive them to extinction. In persistent infections of an RNA virus treated with a mutagen, it has been observed that infectivity eventually disappears, although the replicative ability of the virus is not affected. By means of a simple model that takes into account two phenotypic traits, we demonstrate that extinction is a purely stochastic phenomenon caused by the intermittent outbreaks of a defective, non-infective subpopulation. The transition between dynamics dominated by population fluctuations (finite system size N) and the mean-field behavior ($N \to \infty$) is characterized. We discuss the implications of this alternative pathway to viral extinction.

Introduction. – Large populations of replicating individuals evolving at high mutation rates form heterogeneous groups able to adapt to new environmental situations in relatively short time spans. The theory of quasispecies [1], a first attempt to formalize the collective behavior of such populations, predicts the existence of an error threshold above which the population cannot maintain its identity: As the threshold is crossed, the master sequence is lost from the population, which wanders in sequence space as an unstructured cloud of low fitness mutants. This situation is equated with the extinction of the quasispecies, understood as the disappearance of its viability. Quasispecies theory estimates the value of the critical mutation rate as the inverse of the length of the evolving molecules. Studies with different organisms, especially RNA viruses, seem to confirm that natural mutation rates are close to that value [2]. The evolutionary explanation of this fact argues that near the error threshold identity is still maintained, while diversity (assumed to determine adaptability) is maximal. Hence, a mechanism able to cause viral extinction could be the increase of the mutation rate to values above threshold.

At odds with those theoretical expectations, experimental observations in viruses [3] and ribozymes [4] reveal that they can withstand mutation rates 3 to 8 times above their natural ones and still maintain their viability. An important issue, not taken into account in the original quasispecies theory, is the very large number of potential genotypes expressing the same phenotype. An example is provided by the average number M_n of RNA sequences of length n whose folded state is compatible with a given secondary structure (a first step towards becoming functional molecules): $M_n = 1.402 n^{3/2} 1.748^n$ [5]. More realistic models of quasispecies distinguishing between genotype and phenotype (the true object of selection) predict a (phenotypic) error threshold at mutation rates several-fold higher than those expected only from considerations on the genotype [6]. This hints at the possibility that natural quasispecies are not that close to the error threshold. It has been suggested, instead, that the natural mutation rate might result from a process that minimizes adaptability time, the latter emerging from a compromise between minimizing search time in the genome space (this occurs at high mutation rates) and obtaining a rapid fixation of advantageous mutants (this takes place at low mutation rates) [7].

Though increased mutagenesis is a robust experimental way to produce the loss of viability of a viral population, a current matter of concern is whether extinction truly occurs through crossing an error threshold [8–11], as

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postulated by classical quasispecies theory. A different form of extinction is mutational meltdown, where all genotypes in the quasispecies disappear simultaneously. At present, this seems to be the mechanism that better describes experimental observations of viral extinction under a strong increase in the mutation rate.

Let us illustrate those two extinction mechanisms with a simple example. Consider a quasispecies formed by two phenotypes characterized by replicating at rates $\sigma_1 = \sigma > 1$ and $\sigma_2 = 1$. At time t, each type is represented by $\nu_1(t)$ and $\nu_2(t)$ individuals, whose abundances evolve according to

$$\begin{aligned} \nu_1(t+1) &= \sigma(1-\mu)\nu_1(t) + \mu'\nu_2(t), \\ \nu_2(t+1) &= (1-\mu')\nu_2(t) + \sigma\mu\nu_1(t) - \pi\nu_2(t). \end{aligned} \tag{1}$$

Faster replicators mutate to lower replicators at a rate μ, while backward or compensatory mutations occur at a rate $\mu' < \mu$. Slower replicators can be hit by lethal mutations at a rate π. The extinction threshold is by definition the point where the high-fitness class is lost from the population while low-fitness classes are maintained. According to the model in eq. (1), this would happen when $\nu_1(t \to \infty) = \nu_1^*$ becomes zero, while $\nu_2^* \neq 0$. It can be easily shown that both populations maintain positive values for any $\mu' \neq 0$, irrespectively of the initial condition: There is no extinction threshold if the class of fast replicators can be regenerated by the slower class. For $\mu' = 0$, the extinction threshold occurs at $\sigma(1-\mu) \leqslant (1-\pi)$. Note that it always exists in the absence of lethal mutations while, for $\pi \geqslant \mu$, σ cannot simultaneously fulfill the previous inequality and be larger than one. The most relevant result is that the presence of backward mutations, unavoidable in any model describing the phenotype [6,12], precludes the existence of an error threshold.

The mutational meltdown takes place when the population cannot replicate fast enough to sustain itself, and both classes disappear simultaneously. Mathematically, this happens when the largest eigenvalue of the matrix describing the dynamics of the system becomes smaller than one, meaning that the average number of offspring is less than one per parent individual. In the previous example, its value can be exactly calculated. To first order in μ', mutational meltdown occurs when

$$\sigma(1-\mu) + \frac{\mu\sigma}{\sigma+\pi-1-\mu\sigma}\mu' < 1. \tag{2}$$

At odds with extinction through an error threshold, mutational meltdown seems to be a generic mechanism through which viral populations can undergo extinction.

There might be still other mechanisms behind the loss of viability of viral populations. It has been reported that also mild increases in the mutation rate can cause extinction of infectivity in viruses. Experiments with persistent infections of lymphocytic choriomeningitis virus (LCMV) treated with a small amount of mutagen revealed that the virus eventually loses the ability to produce infective particles, though replicative ability is not affected (see fig. 1). In a persistent infection, intracellular selection for higher replication rates is acting all the time. However, viral particles are slowly released to the external medium and the ability of the virus to jump from cell to cell is not selected for. This situation is remarkably different from lytic infections, where release of viral particles to the medium is a fast step that implies breaking the cell. In the latter case, selection for replicative ability cannot be decoupled from selection for infectivity.

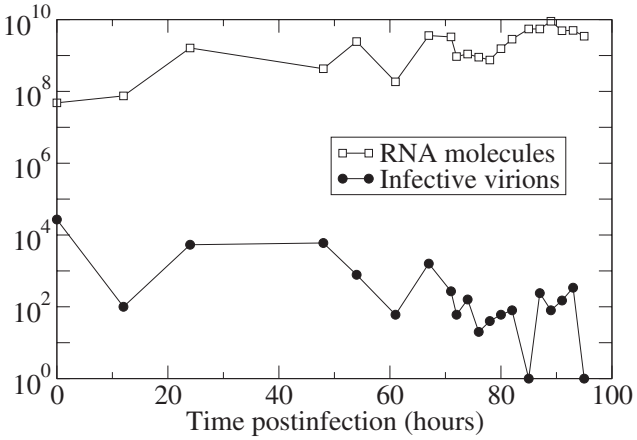

Fig. 1: Absolute number of RNA molecules and infective virions inside cells persistently infected with LCMV and treated with 100 μg/ml of the mutagen 5-fluorouracil. After about 90 hours of infection, the cells cease to produce infective virions, though RNA replication is not impaired [13].

The extinction of infectivity in persistent infections of LCMV cannot be understood in the framework of present quasispecies theory. The molecular mechanisms behind the observed behaviour can be summarized as follows. The replicative ability of a genome is related to its capacity to bind to and be copied by replication enzymes (polymerases). This depends strictly on the sequence of the genome. Unavoidable mutations in the copying process can affect the binding and copying of a sequence. On the other hand, infectivity depends on the performance of proteins codified by that same genome. Hence, changes in infective ability are conditional on the genome experiencing a mutation, though not all mutations have an effect in proteins, and thus only a fraction of those will affect infectivity. The result is that, in a persistent infection, the two traits evolve under different selection pressures: genomes able to replicate compete inside each cell, while infectivity behaves as a neutral trait. A neutral trait, by definition, is not useful in the current environment and thus can accumulate random mutations. Those mutations may result in a loss of viability in the long run. It was conjectured [13] that the role of the mutagen is to enhance the appearance of a class of defective mutants, able to replicate but unable to infect susceptible cells. This parasitic subclass eventually induces the extinction of the whole population.

A step forward towards modelling real systems is to consider that phenotype is a multi-trait feature that can be only rarely reduced to a single variable. Actually, there are abundant examples in the literature where two phenotypic traits need to be considered in order to appropriately describe the evolution and adaptation of heterogeneous populations. Among them, growth rate and yield [14], robustness and evolvability [15,16], or virulence and replicative ability in competition assays [17] have been pondered as characteristics that simultaneously affect the survivability of a viral population. Motivated by the experiment of extinction of infectivity in LCMV, we here introduce a model for the evolution of a population whose individuals are characterized by two traits subject to positive and neutral selection pressures, respectively.

Model of a quasispecies with a two-traits phenotype. – We consider a quasispecies formed by four different classes. Fast replicators have an average of R offspring per replication cycle; slow replicators have r. Either type can take a viable or a defective form. We assume that viable forms maintain the integrity of their genomes and correctly code for the proteins that permit replication and infection. Thus, replication of either type is only possible if individuals of the viable type are present. The four types and the corresponding transition rates are depicted in fig. 2. The replicative ability decreases (increases) with probability p (q). Changes in this trait fix new mutations that can affect viability. With probability w, an individual mutating to the class of slow replicators can simultaneously lose its viability; with the same probability, viability is recovered conditional on experiencing a mutation increasing the replicative ability. The model includes lethal mutations with rate p affecting slowly replicating individuals. The rates p, q, and w, actually stem from a microscopic mutation rate characteristic of each virus. They can be treated as constant on the average for a given genome (*i.e.* population, species, or organism). Dynamics proceeds through discrete generations and the population size N is constant. The matrix M characterizing the mean-field dynamics of the system reads

$$M = \begin{pmatrix} R(1-p) & q & 0 & qw \\ Rp(1-w) & 1-p-q & 0 & 0 \\ 0 & 0 & R(1-p) & q(1-w) \\ Rpw & 0 & Rp & 1-p-q \end{pmatrix}. \quad (3)$$

We set $r=1$ without loss of generality, thus fixing the time scale. The vector describing the evolution of the fraction of individuals in each type, $\mathbf{n}(g) = \{n^V(g), n^v(g), n^D(g), n^d(g)\}$ obeys

$$\mathbf{n}(g+1) = \alpha(g) M \mathbf{n}(g)/[\alpha(g)\lambda], \quad (4)$$

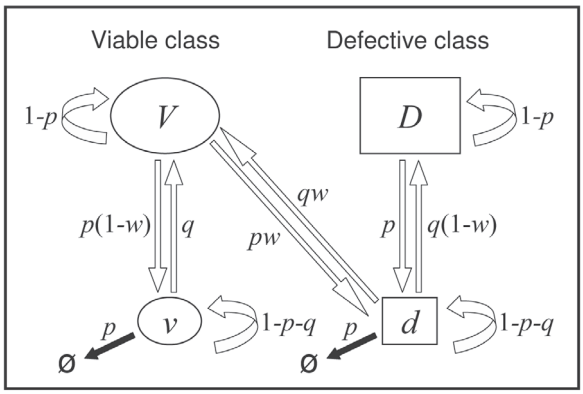

Fig. 2: Schematic representation of the four types forming the quasispecies. Permitted transitions between types are indicated as arrows, with their corresponding rates.

where $\alpha(g) = n^V(g) + n^v(g)$ is the fraction of viable individuals at generation g, and λ is the largest eigenvalue of M. As initial condition we assume $\mathbf{n}(0) = \{1,0,0,0\}$. Note that though $\alpha(g)$ actually does not affect the average composition of the population, it is the cause of extinction, since disappearance of the viable types means disappearance of the whole population.

Assuming that viability is a neutral trait implies that cell-to-cell transmission is not represented in the model. Hence, eq. (4) describes intracellular dynamics, with a typical time scale shorter than that of transmission of the infection. The size of the system thus corresponds to the number of viral genomes inside a single cell.

This model has an explicit solution $\mathbf{n}^* = \mathbf{n}(g\to\infty) \equiv \{n^V, n^v, n^D, n^d\}$,

$$\mathbf{n}^* = \mathcal{N}^{-1}\{2q, (1-w)(c-a_+), 2q(1-w), c-a_+\}, \quad (5)$$

with $\mathcal{N} = (2-w)(c-a_-)$, $a_\pm = (R-1)(1-p) \pm q$, $c = [(1-p)^2(R-1)^2 + 2(R(1+p)-(1-p))q + q^2]^{1/2}$, and $\lambda = 1/2[(R+1)(1-p) - q + c]$. As with the example discussed in the introduction, no extinction threshold is found for $q \neq 0$, that is, when backward mutations exist. Mutational meltdown is possible and holds for an asymptotic growth rate at the mutation-selection equilibrium below one, that is $\lambda < 1$.

The solution given in eq. (5) represents well the dynamics of the quasispecies only for sufficiently large populations. For small population sizes the dynamics are qualitatively different and dominated by the intermittent appearance of class D. In this regime, stochastic extinction is a common event.

There are different limits of the model worth mentioning. The case $w=0$ corresponds to a quasispecies described only by its replicative ability where back and lethal mutations are considered (only classes V and v sustain finite populations). The case $w=1$ is formally identical, with classes V and d surviving. This model has been analyzed for instance in [9]. The case $q=0$ is particularly interesting. Since class D can only be

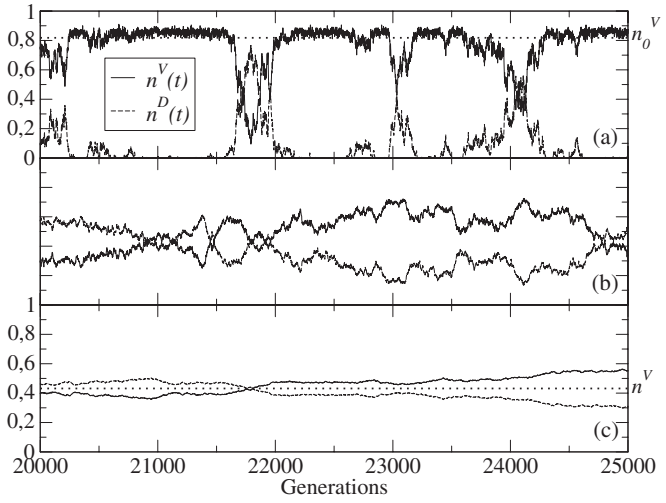

Fig. 3: Dynamical regimes of the model. (a) Stochastic regime, $N = 200$. For small system sizes, the dynamics are dominated by the intermittent appearance of class D. The dotted line corresponds to the value obtained in the approximation $q = 0$, $n_0^V \simeq 0.8182$. (b) Transition regime, $N = 2500$. For system sizes $N \simeq N_m$, the population of D individuals is always above zero, though fluctuations are still large. (c) Mean-field regime, $N = 10^5$. For $N \to \infty$, the population in each class approaches the mean-field value. The dotted line corresponds to the asymptotic solution, $n^V \simeq 0.4317$. Parameters for all simulations are $p = 0.1$, $q = 0.01$, $w = 0.1$, $R = 2$, which yield $N_m \simeq 2116$ according to eq. (8).

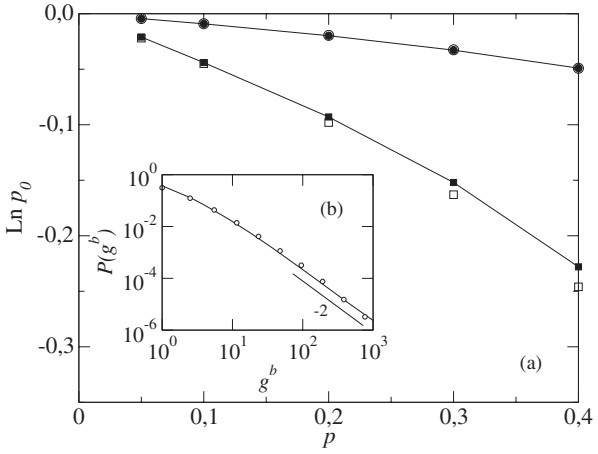

Fig. 4: Numerical and analytical results for the model in the stochastic regime. (a) Exponent of the distribution of interval lengths (in number of generations) without individuals of the D class. Two values of $q = 0.01$ (circles) and $q = 0.05$ (squares) are shown for $N = 100$, $R = 4$ and $w = 0.5$. Solid symbols are results from simulations; open symbols are analytical results as in eq. (7). (b) Probability $P(g^b)$ of a D-outbreak of length g^b generations. The solid line is the prediction of critical branching processes; circles correspond to numerical simulations for $p = 0.1$, $q = 0.01$, $w = 0.2$, $R = 2$, and $N = 100$.

generated through (rare) beneficial mutations appearing in class d, class D can remain empty for extended periods of time when the population size is small enough. Thus, in the biologically relevant limit of small N and $q \to 0$, the case $q = 0$ should approximate accurately the intervals where $n^D(g) = 0$. The stationary populations $\mathbf{n}_0^* = \{n_0^V, n_0^v, n_0^D, n_0^d\}$ in this limit are

$$\mathbf{n}_0^* = \mathcal{N}_0^{-1} \{\mathcal{N}_0 - Rp, Rp(1-w), 0, Rpw\}, \qquad (6)$$

with $\mathcal{N}_0 = R + p - 1$ and $\lambda_0 = R(1-p)$.

In order to check the accuracy of our analytical results, we have performed numerical simulations of the dynamical model. As initial condition, we take N individuals in class V. At each generation g, the population replicates deterministically (with rates R and 1) to generate the individuals at generation $g+1$, which then mutate according to the probabilities described. This step introduces stochasticity in the system. If the population $n(g+1) > N$, a random subset of N individuals is selected. This keeps the population size bounded. When, as a result of fluctuations, the number of viable individuals reaches zero, the population is considered extinct and the simulation halts.

The different dynamical regimes of the population are illustrated in fig. 3. As the size of the population N increases, the behaviour changes from a stochastic regime dominated by the intermittent appearance of class D and with average values well described by eq. (6) to a mean-field regime with average values following eq. (5). Though the transition is smooth, it will be shown that there exists a characteristic system size N_m where the stochastic regime crosses over to the mean-field regime.

Stochastic regime. – For small q and finite system size, the population of defective, fast replicating individuals appears in bursts that either are terminated after a finite number of generations or (also in finite time) invade the population, thus causing its extinction. In this limit, the probability p_0 that class d does not produce any individual of class D in one generation is $p_0 \simeq (1 - q(1-w)/\lambda)^{N n_0^d}$. Hence, the probability $P_0(g) = p_0^g$ of having an interval of g generations without individuals of class D reads

$$P_0(g) \simeq \exp\left\{-gN \frac{Rpw}{R+p-1} \ln\left(1 - \frac{q(1-w)}{R(1-p)}\right)\right\}. \qquad (7)$$

The exponent of this distribution, $\ln p_0$, is represented in fig. 4(a) together with the results of numerical simulations. The outbreaks of the D class for sufficiently small p and q start with a single individual and follow the dynamics of a branching process with branching ratio m. To a first approximation, the value of m is the average number of offspring of class D per individual in that class ($\approx R(1-p)$) divided by the asymptotic growth rate of the population λ_0. Hence, in this limit, where the contribution from class d is neglected, $m = 1$ and the dynamics follows a critical branching process [18]. The corresponding generating function, $f_1(s) = \mathrm{e}^{s-1}$,

allows to obtain a number of exact results. The probability of termination of the outbreak at any time in the future is the solution of $f_1(s^*) = s^*$, which has the known result $s^* = 1$. The probability of termination after g^b generations is $P(g^b) = f_{g^b}(0) - f_{g^b-1}(0)$, where $f_k(s) = f[f_{k-1}(s)]$. It can be iteratively obtained and, asymptotically, $P(g^b) \propto (g^b)^{-2}$. This function is compared with numerical simulations in fig. 4(b). The existence of a neutral trait and the critical branching dynamics of the defective class are two sides of the same coin: Any coupling between traits would imply deviations from neutral behaviour and values of the branching ratio different from one.

Mean-field regime. – As the size of the system increases, so does the duration of the outbreaks. At some system size N_m, the previously isolated bursts merge, and the approximation of the dynamics of D as a critical branching process is no longer valid. For $N > N_m$ all types are continuously represented in the quasispecies, albeit fluctuations in population sizes might still be large. The system size N_m can be estimated as the value of N where class d contributes on average one individual per time step to class D, $N_m n^d q(1-w)/\lambda \simeq 1$, which yields

$$N_m \simeq \frac{(R+p-1)}{q}\frac{(2-w)}{(1-w)}\frac{(R(1+p)+q-1-R-c)}{(R(1+p)+q-1+R-c)}, \quad (8)$$

using the value of n^d in eq. (5) and the corresponding λ. Series expansion of N_m in powers of q yields

$$N_m = \frac{2-w}{1-w}\left[\frac{1-p}{q} + \frac{R}{(1-p)(R-1)} + O(q)\right], \quad (9)$$

so N_m diverges as $q \to 0$. Hence, for finite p, w, and R, in situations where the probability of hitting beneficial mutations is small enough, the dynamics is systematically dominated by population fluctuations. The system size N_m separates the two relevant dynamical regimes. Below N_m, the dynamics is mostly determined by stochastic effects and well described by the solution $q = 0$ plus the probabilistic appearance of critical D-bursts: extinction is common. Above N_m, the dynamics is well described by the mean-field asymptotic solution. As N grows, extinction becomes increasingly unlikely.

The transition between the stochastic and the mean-field regimes can be further characterized through the distribution of probability densities for each of the four subpopulations. In the stochastic regime, the abundances of viable and defective types proceed in anti-phase, such that when the population of $V + v$ is high that of $D + d$ is low (as in fig. 3(a)). In this case the average population values agree with eq. (6): the distributions of V, v and d present a maximum near those values and the abundance of D is close to zero. When outbreaks of D appear, the populations of V and v decrease strongly while population D becomes abundant. Extinction supervenes if the number of $V + v$ attains zero, $\alpha(g) = 0$ in eq. (4). In the mean-field regime, the size of the system is large enough to sustain finite populations of all four classes. The maxima of the population size distributions move towards the average values predicted by eq. (5). In fig. 5 we plot the main quantities characterizing the transition.

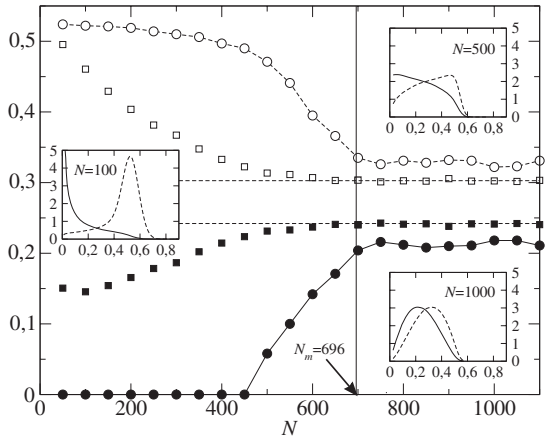

Fig. 5: Transition from stochastic to mean-field regimes. We show the position of the maxima for the distribution of populations D (solid circles) and V (open circles). Solid squares correspond to the average value of $n^D(g)$, open squares to that of $n^V(g)$. From eq. (6), $n_0^V = 0.53$, $n_0^D = 0$ (in agreement with the maximal values at the stochastic regime), while dashed lines signal asymptotic mean-field values obtained from eq. (5). The maxima of the distributions eventually converge to those values as fluctuations disappear in the limit $N \to \infty$. The insets show three representative probability distributions for $n^D(g)$ (solid line) and $n^V(g)$ (dashed line), below, during, and above the transition. The estimated system size separating stochastic from deterministic behavior is $N_m \simeq 696$, according to eq. (8). Parameters are $p = 0.3$, $q = 0.01$, $w = 0.2$, $R = 2$.

The average time to extinction $T_{\rm ext}$ grows exponentially with the system size, $T_{\rm ext} \propto \exp\{kN\}$, with k depending on the model parameters. For the case shown in fig. 5, $k = 0.0054(1)$, so $T_{\rm ext}$ increases more than a thousand-fold between $N = 100$ and $N = 10^3$. We do not have evidence that $T_{\rm ext} \to \infty$ at finite N, though its rapidly increasing value asserts that, in practice, extinction will be rarely observed once in the mean-field regime.

Discussion. – The transition between the stochastic and the deterministic regimes is reminiscent of the behavior observed in other dynamical systems with transitions characterized by qualitative changes in the populations distributions. Examples are the dynamics of particles in asymmetric potentials under the action of an external noise [19] or noise-induced transitions [20]. In our case, the source of noise is intrinsic and due to population fluctuations. Related collective behavior has been described for fluid neural networks, where the system experiences an ordering transition as the density of elements increases [21].

Fitness is a multitrait feature with different expression in different environments. In lytic infections, where cells are killed after a number of replication cycles, the requirement to maintain the ability of infecting susceptible cells acts as a positive selection pressure that regularly removes non-infective particles from the population. When infections are persistent, selection pressure over infectivity is released. Since the number of viral particles inside cells is relatively small (about 10^{2-3}), population fluctuations are large, and, in the presence of one trait not subject to selection, a defective subpopulation able to induce the extinction of the whole might appear. Stochastic extinction through lethal defection [13] becomes possible. From another viewpoint, stochastic extinction occurs only if the characteristic time between infections of susceptible cells is larger than the time to extinction $T_{\rm ext}$. Every new infection event acts as a filter cleaning the population from defectors, unable to infect, and thus resetting the dynamics to the initial condition. This mechanism can be generalized to situations where a previously essential trait is temporarily unneeded (not selected for) and then becomes essential again. This could be the case of genes that respond to uncommon environmental conditions or get rarely switched on: the absence of activity could lead to the loss of viability.

The model here presented shows how simple evolutionary mechanisms can cause the extinction of populations of fast mutating pathogens under environmental changes, and strongly suggests that one could devise strategies to take advantage of those mechanisms in fighting viral infections. In this context, tuning the balance among intracellular replication, frequency of infection of new cells and multiplicity of infection, or applying mild increases in viral mutation rate, appear as therapies alternative to the massive use of drugs. In a broader framework, a better understanding of the complex population dynamics typical of these organisms should make it possible to identify and manage selection pressures over target traits, resulting in the development of new control strategies at the host level for infectious diseases.

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