

Information catastrophe in RNA viruses through replication thresholds

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Abstract

RNA viruses are known to replicate at very high mutation rates. These rates are actually known to be close to their so-called error threshold. This threshold is in fact a critical point beyond which genetic information is lost through a so-called *error catastrophe*. However, the transition from a stable quasispecies to genetic drift and loss of information can also occur by crossing replication thresholds, below some replication rates, the viral population is suddenly unable to survive. Available data from hepatitis C virus population analysis [Mas, A., Ulloa, E., Bruguera, M., Furčić, I., Garriga, D., Fábregas, S., Andreu, D., Saiz, J.C., Díez, J., 2004. Hepatitis C virus population analysis of a single-source nosocomial outbreak reveals an inverse correlation between viral load and quasispecies complexity. *J. Gen. Virol.* 85, 3619–3626] can be interpreted through this theoretical view, providing evidence for such a replication threshold. Here a simple model is used in order to provide evidence for such a phenomenon, consistent with available data. © 2005 Elsevier Ltd. All rights reserved.

Keywords: RNA virus dynamics; Error threshold; Hepatitis C; Quasispecies

1. Introduction

RNA viral populations are extremely heterogeneous and have been labelled *molecular quasispecies* (Eigen et al., 1988, 1989). The quasispecies structure has numerous implications for the biology and associated pathology of RNA viruses (Novella et al., 1995; Domingo et al., 1998). The heterogeneous population structure is a reservoir of variants with potentially useful phenotypes in the face of environmental change. However, mutation rates cannot reach arbitrary values: there is a threshold to mutational change beyond which no selection is possible. This threshold has been dubbed the *error threshold* (Domingo and Holland, 1994; Domingo et al., 1995; Nowak and May, 2000). Roughly, it is predicted that the mutation rate μ per nucleotide and replication round should be $\mu_c \approx 1/v$, v being the sequence length. The theory predicts that, beyond

μ_c , genomic information is lost as the population enters into a drift phase (the information catastrophe, see Eigen, 1971; Schuster, 1994). The transition from the quasispecies domain ($\mu < \mu_c$) and the drift phase ($\mu > \mu_c$) is sharp. This error catastrophe actually corresponds to a phase transition phenomenon (Eigen et al., 1989; Solé et al., 1996; Solé and Goodwin, 2001).

RNA viruses are known to replicate close to their error threshold (Domingo and Holland, 1994; Holland et al., 1990). Several theoretical approaches have been developed in order to understand the presence and implications of this threshold (Swetina and Schuster, 1982; Eigen et al., 1988, 1989; Pastor-Satorras and Solé, 2001; Kamp and Bornholdt, 2002). The presence of a sharp error threshold has been a matter of debate over the years. Indirect, but compelling evidence of such a threshold comes from a number of sources, particularly mutational studies. In this context, increased mutagenesis acting on cell cultures has shown that—as predicted by the theory—viruses are unable to persist, at least in vitro (Loeb et al., 1999; Crotty et al., 2001). Here, we consider a less known scenario in which a threshold from viable to non-viable populations

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takes place as a consequence of changes in replication instead of mutation. The starting point of our analysis is a recent study by Mas et al. (2004) in which quasispecies features of hepatitis C virus (HCV) were analysed. HCV is a single-stranded RNA virus with a positive-sense genome of approximately $v \approx 10^4$ nucleotides. Infection with HCV is a major health problem because nearly 170 million people worldwide are estimated to be infected with. Only about 15–30% of HCV infections are spontaneously cleared in the first 6 months after infection, the remaining result in virus persistence with subsequent development of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (Alter et al., 1999).

The infection history of the HCV-infected patients described by Mas et al. (2004) is particularly interesting because (i) all patients involved in the outbreak were infected on the same day from a single-seropositive person, (ii) samples were available at an early time point (11–17 weeks) after infection, and (iii) the infection dose was lower than in previously studied transmissions via blood transfusion. This latter aspect was inferred by two observations, first the infection source was a vial of heparin presumably contaminated by a needle bearing HCV-positive blood, and second, the low HCV load in both patients that were the possible transmitters.

The study carried out by Mas et al. (Fig. 1) involved a molecular characterization of the underlying quasispecies complexity in a cohort of seven patients, that could be grouped by viral load values. Quasispecies structure from patients displaying high viral loads were less complex and dominated by one sequence. Instead, quasispecies isolated from patients showing low viral load values were characterized by a highly heterogeneous set of sequences, resembling a random set with similar frequencies. In other words, an inverse correlation was found between viral load and quasispecies complexity. The study offered a unique opportunity of exploring the quasispecies nature of HCV early (11 weeks) after the infection caused by a single donor.

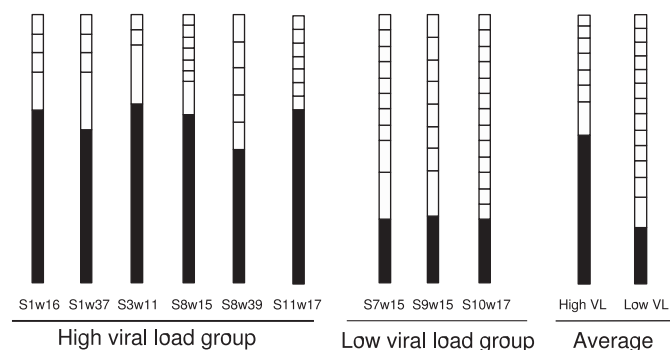


Fig. 1. Changes in RNA quasispecies complexity in the patient samples from the analysis by Mas et al. (2004). Here the vertical bars indicate the fraction of viral variants within each sample for nucleotide sequences. Each segment in a bar represents one different sequence, being the dominant viral variant indicated in black. Two well-defined groups are found, involving high and low viral loads (see Mas et al., 2004 for details).

The results from such analysis suggest that a shift between localized quasispecies and drifting swarms occurs early after infection. There is no reason to think that mutation rates have increased in those viruses with the highest heterogeneous set of sequences. Therefore, the existence of more mutagenic viruses should not be the reason for crossing the information threshold. Typically, HCV RNA appears in the serum within 1–2 weeks after infection and increases rapidly. Anti-HCV arises at the time of symptoms or shortly thereafter (after 5–6 weeks) and probably at this timepoint HCV infection is directed to become chronic or to be spontaneously clarified (Zoulim et al., 2003). Once chronic infection is established, serum HCV RNA levels tend to stabilize.

Since sample collection was as early as at week 11 after infection, patients with a high viral load could be carrying viruses that are chronifying the infection (those with one predominant sequence), whereas patients with low viral load values could be infected with those viruses that are still trying to establish a persistent infection (those without a predominant sequence). As we will show below, the observations by Mas et al. (2004) could be related to a theoretical interpretation intimately tied to the error catastrophe. Actually, our study provides support to the quasispecies view of RNA populations and to the existence of a well-defined transition from Darwinian dynamics to information loss.

We will address the problem by using two different, but complementary approaches: (a) a simple mean-field model allowing us to show that the experiment can be interpreted in terms of a threshold phenomenon and (b) a bit string model, which allows to simulate the genetic variability of the quasispecies under different parameter values related to the two different scenarios present in the real data set.

2. Mean-field quasispecies model

The dynamics of HCV infection is a complex one. Actually, little is known about the early phases of infection, although the immune system should be involved in some way in the outcome of the infection. Here we ignore the specific details of the HCV–immune interactions. Instead, we concentrate in a simplified picture of the HCV quasispecies structure, by reducing it to a minimal model. From the low quasispecies complexity seen within the group of high viral load patients, we can conclude that the population might be located close to a sharp peak in the HCV fitness landscape (Stumpf and Zitzmann, 2001). In other words, one sequence (the dominant one seen in the molecular characterization) has a large frequency, whereas the others have a much smaller, and perhaps not too different one. We will label as the master sequence the dominant one and consider the others as forming a homogeneous pool of sequences having a smaller replication. In its simplest form, we can consider a reduced system of equations defining a population as formed by two basic groups: the master sequence x_1 and the other sequences,

which we assume to be grouped into an “average” sequence with population x_2 (Swetina and Schuster, 1982). Let us also assume (as a first approximation) that mutations occur from the master to the second compartment but not in the reverse sense. The enormous size of the sequence space makes this assumption a good first approximation. The model is given by the next two ordinary differential equations set

$$\frac{dx_1}{dt} = f_1(1 - \mu)x_1 - x_1\Phi(x_1, x_2), \quad (1)$$

$$\frac{dx_2}{dt} = f_1\mu x_1 + f_2x_2 - x_2\Phi(x_1, x_2), \quad (2)$$

where μ is the mutation rate, f_1 is the master sequence replication rate and f_2 the replication rate for the other strings. If we impose that the sum of the two populations is constant (i.e. $x_1 + x_2 = 1$) then it is not difficult to see that the population constraint is given by $\Phi(x_1, x_2) = \sum f_i x_i$ (i.e. the average replication rate). Assuming this constraint, we have a linear dependence $x_2 = 1 - x_1$ and thus we can analyse dynamics of the master sequence now given by

$$\frac{dx_1}{dt} = f_1x_1[\xi_1 - \xi_2x_1], \quad (3)$$

where $\xi_1 = 1 - \mu - f_2/f_1$ and $\xi_2 = 1 - f_2/f_1$. Here the fixed points are $x_1^* = 0$, $x_1^* = \xi_1/\xi_2$, and thus the non-trivial fixed point, representing a non-zero master sequence population, will be

$$x_1^* = 1 - \frac{\mu f_1}{f_1 - f_2}. \quad (4)$$

Actually, we can also compute the time evolution of the single-equation model, which gives a logistic-like solution

$$x_1(t) = \frac{\xi_1}{\xi_2} \left[1 + \left(\frac{\xi_1/\xi_2 - x_1(0)}{x_1(0)} \right) \exp(-\xi_1 f_1 t) \right]^{-1}, \quad (5)$$

thus showing that the approach to the steady state is a function of the replication and mutation rates. The non-trivial fixed point goes to zero as we approach to a critical replication rate, given (from $x_1^* = 0$) by

$$f_1^c(f_2, \mu) = \frac{f_2}{1 - \mu}. \quad (6)$$

In Fig. 2 we show the two possible phases, separated by the critical line, using a fixed f_2 value (here we use $f_2 = 0.25$). Once such boundary is crossed, we shift from one type of qualitative dynamics to the other. The standard error threshold condition is associated to an increase in mutation rate. Increased μ values crossing the critical line drive the master sequence into extinction. But another possibility becomes obvious by considering the second parameter: as the master sequence replication rate decreases, we can also perform the same type of phase transition. Such a scenario is consistent with a successful immune response against the dominant sequence, which leads to a decreased viability of the master sequence. As will be shown in the following section, this actually

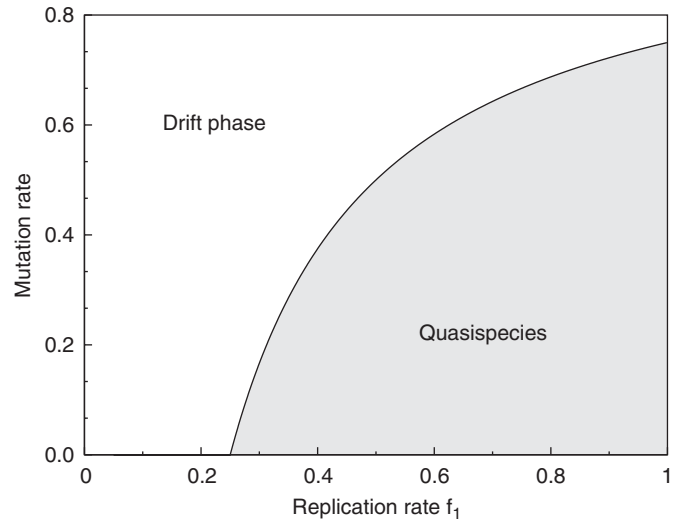


Fig. 2. Interpreting the changes in the RNA population structure observed in the patient sample. As the master sequence reduces its replication rate (due to its identification by the immune system) a rapid decay is observed once a replication threshold f_1^c is crossed.

explains several key features of the observed quasispecies complexity observed in the HCV infected patients.

3. Bit string model

The continuous, two-dimensional model provides a rationale for the qualitative pattern observed in the HCV data set. In order to go beyond this point, we need to improve the description of the population used. Let us consider now a set of in silico viruses to be represented as strings of bits. Such strings will be the genomes, to be compared with those analysed from the HCV sample. In our previous mean field analysis, μ was defined as the probability with which, at least, one bit will mutate. Now, considering sequences explicitly, μ must be function of v and μ_b (mutation rate per bit). It is not difficult to see that they are related through

$$\mu = 1 - (1 - \mu_b)^v \quad (7)$$

and then Eq. (6) is now

$$f_1^c = \frac{f_2}{(1 - \mu_b)^v}. \quad (8)$$

If $\mu_b \approx \mu_c$ (where μ_c is the mutation error threshold), we have

$$f_1^c(f_2, v) = \frac{f_2}{[1 - \frac{1}{v}]^v} \quad (9)$$

and it can be shown that Eq. (9) follows, in the limit,

$$f_1^c(f_2, v \rightarrow \infty) = e f_2. \quad (10)$$

We have thus a well-defined prediction for the threshold replication rate below which the quasispecies is no longer stable.

Using a string description of the population structure (Solé et al., 1998), we can measure the expected effects of changing replication rates on the distribution of mutants and their frequencies. Here each string S_k ($k = 1, \dots, N$) is a small genome of size v , i.e.

$$S_i = (S_i^1, S_i^2, \dots, S_i^v), \quad i = 1, 2, \dots, N, \quad (11)$$

where $S_k^i \in \{0, 1\}$ represents a vertex $S_k \in \mathcal{H}^v$ of a v -dimensional hypercube (see Fig. 3). The algorithm starts with an initial population of master sequences and repeats, at each generation, N times the following set of rules:

1. We take a string S_i at random from the population and replicate it with probability $f(S_i)$. Here two replication probabilities are also defined, one for the master sequence (where $S_k = 1$ for all $k = 1, \dots, v$) and the other for the rest of strings, to be indicated as f_1 and f_2 , respectively.
2. Replication takes place by replacing one of the strings in the population (also chosen at random) say $S_j \neq S_i$ by a copy of S_i . The copy mechanisms present error

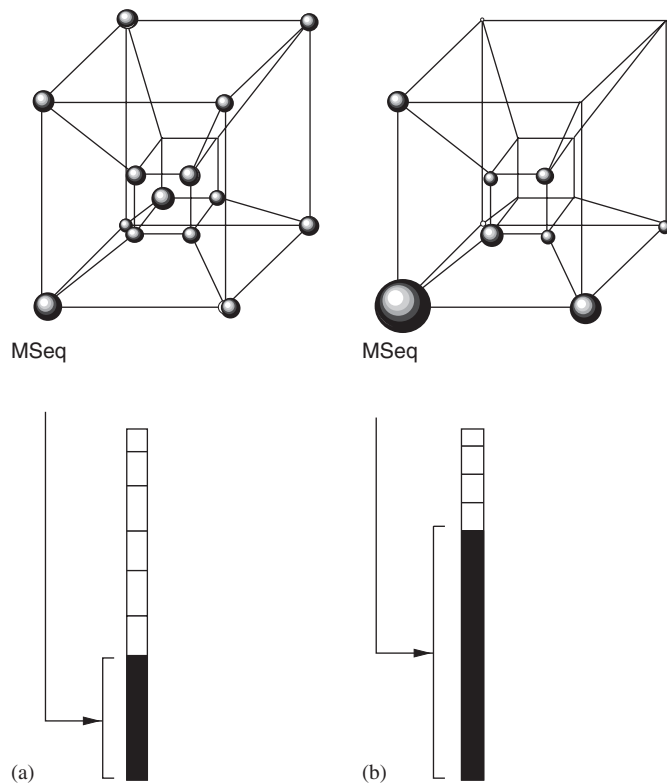


Fig. 3. Sequence space for a small-sized, four-bit ($v = 4$) strings. All but one of the sequences have the same fitness f_2 . The bottom left node has a fitness f_1 . The replication rates thus define a fitness landscape. Two situations are indicated here, namely: low (a) and high (b) master sequence replication rates. At low replication of the master sequence, other sequences are equally able to occupy the space, whereas for higher master replication it shows a localized quasispecies centred around the master. The lower drawings indicate what should be expected to observe in terms of the abundances of each string. Here the master sequence frequency is indicated with a black bar.

(mutation rate μ_b) per bit and replication cycle, respectively.

Using digital genomes of size $v = 32$ we represent, in Fig. 4, the evolution of the master sequence frequency at decreasing fitness values for such sequence. For this particular run, we have used $\mu_b = 0.04$ and $f_2 = 0.05$ in a population of $N = 500$ strings. Using Eq. (8), we have a theoretical estimate for a replication threshold of $f_1^c = 0.184$, which fits well the simulation results. As expected, beyond the critical rate f_1^c the master sequence (thick line) is not found at all in the population, whereas the other sets drift at random through sequence space. In Fig. 4, we also display the frequencies for the sequences with 1, 2, 3, \dots , v different bits from the master one (narrow lines). Beyond such replication threshold the whole population consists of a wide spectrum of mutants with different sequences and thus a larger population diversity, as seen in the HCV cases with low viral load. The time evolution of the master sequence is represented in Fig. 5.

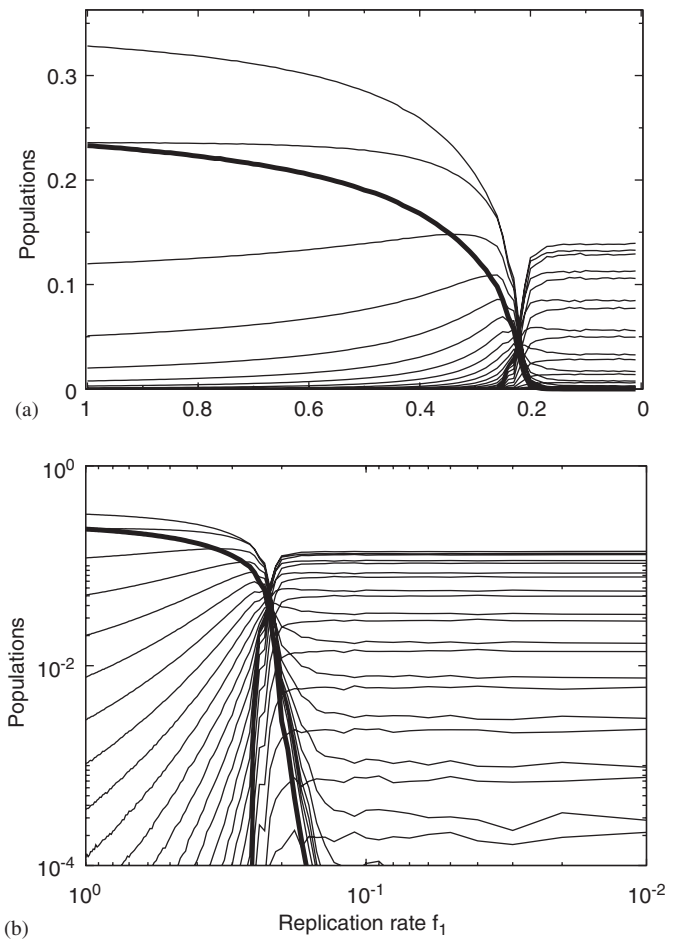


Fig. 4. Phase transition in the bit string model (here linear (a) and log-log (b) plots) with $\mu_b = 0.04$ and $f_2 = 0.05$ in a population consisting of $N = 500$ strings of length $v = 32$. Frequencies for the master sequence (thick line) and for sequences with 1, 2, \dots , v different bits from the master sequence (narrow lines) are computed from the mean taken over $T = 500$ generations (averaged over 50 replicas) after $\tau = 4500$ generations are discarded.

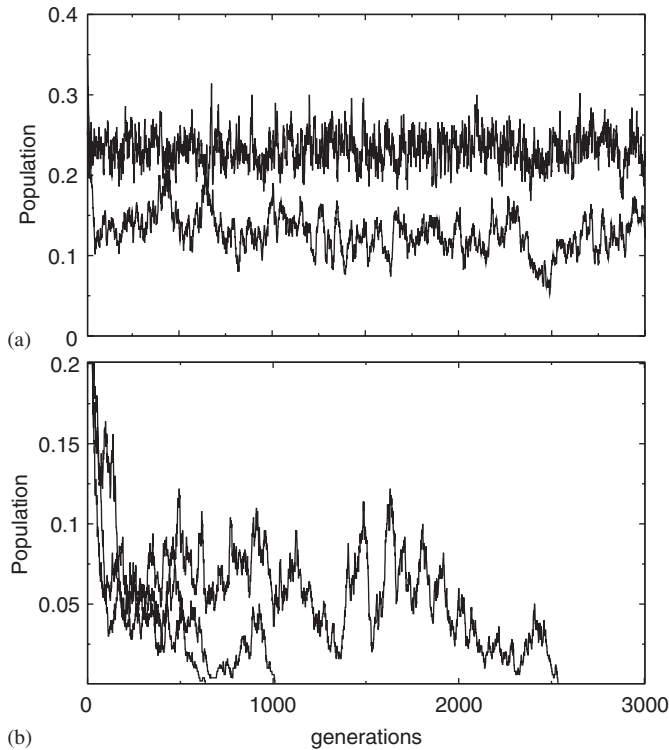


Fig. 5. Time evolution of the master sequence above (a) and below (b) the critical replication rate f_1^c in the population with $N = 500$ strings of length $v = 32$. Here $\mu_b = 0.04$ and $f_2 = 0.05$. From down to top: in (a) $f_1 = 0.3$ and $f_1 = 0.95$; in (b) $f_1 = 0.18$, $f_1 = 0.2$ and $f_1 = 0.22$.

Here two scenarios are considered: the first (a) taking $f_1 > f_1^c$ and the other (b) taking $f_1 < f_1^c$. Here, as shown in Fig. 4, crossing the replication threshold involves the extinction of the master sequence and the entry in the drift phase.

In order to analyse the impact of the transition on population complexity (how much variance is present) we compute the stationary entropy $H(f_1)$ for changing replication rates f_1 . Such entropy is given by

$$H(\{p_i\}, f_1) = -\frac{1}{\ln N} \sum_{i=1}^N p_i(f_1) \cdot \ln p_i(f_1). \quad (12)$$

Here p_i is the probability to find a given sequence in the population of N strings.

In the data obtained from the HCV infected patients, a higher viral load was always related to a lower quasispecies complexity and vice versa. Entropy in the population of strings is shown to increase at decreasing master sequence replication rates. Hence, the interpretation of the decrease in the viral load due to a decrease of the master sequence replication rate and its associated higher quasispecies complexity is also obtained from this populational approach. Such phenomenon is displayed in Fig. 6, where is possible to see that beyond the critical replication threshold f_1^c , entropy rapidly increases undergoing a transition towards maximum and maintained values. This transition actually means that no information can be stored

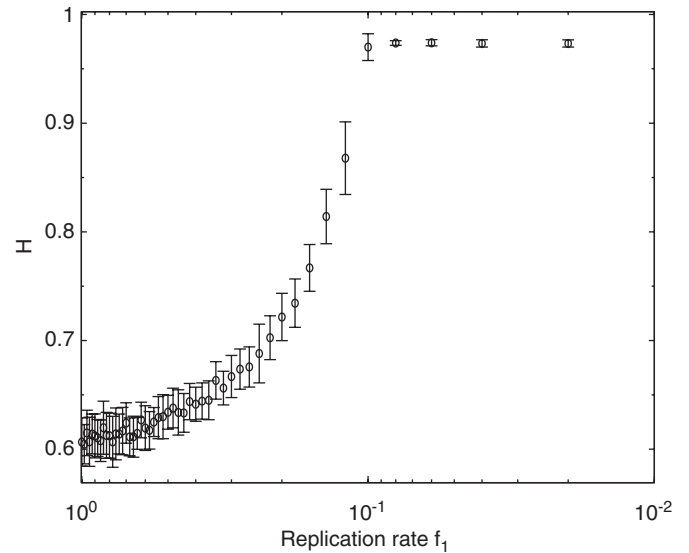


Fig. 6. Entropy H (here log-linear plot) at decreasing master sequence replication rates (f_1) with $\mu_b = 0.02$ and $f_2 = 0.05$ computed from the population (here also consisting of $N = 500$ strings of size $v = 32$) after $\tau = 5000$ generations. Each data point represents the mean \pm standard deviations (error bars) taken over 25 replicas.

in the system having a wide cloud of different and heterogeneous mutants in the population structure.

4. Discussion

RNA virus extinction by crossing the error catastrophe threshold has been recently discussed as an antiviral strategy (Domingo, 2005). Mutagens have been recently used to extinguish positive RNA viruses, such as HCV (Crotty et al., 2001), HIV (Loeb et al., 1999), or FMDV (Sierra et al., 2000), and negative RNA viruses such as LCMV (Grande-Perez et al., 2002; Ruiz-Jarabo et al., 2003; de la Torre, 2005). However, virus extinction remained in some cases elusive, although it is possible by using the mutagenic agents in combination with antiviral compounds (reviewed in Domingo et al., 2005; and modelled in Gerrish et al., 2003).

In this paper, we show that the transition from maintained information to informational loss in RNA viruses populations can be achieved not only by mutation but also by replication thresholds (Figs. 2, 4 and 5). This replication-induced catastrophe is analysed by a simple mean-field quasispecies model and a populational bit string approach. Our results are consistent with available data obtained by Mas et al. (2004). These authors reported the quasispecies structure of HCV populations isolated from a group of patients infected during a single-source nosocomial outbreak. During this episode, patients were infected with HCV viruses from a single donor with low viral load, and this fact allow us to propose the clonal nature of the infection. Therefore, just a few viral particles initiated the infection. From this starting point, viruses replicate until

host develops antiviral strategies, mainly immune response, and then the most adapted viruses will be the most replicative ones following Darwinian dynamics. If the virus finds a mutant spectrum that can replicate with high efficiency under these conditions, the quasispecies will evolve to a predominant master sequence.

This evolution is represented in Mas et al. (2004) by viruses isolated from the high viral load group of patients. On the other hand, quasispecies distributions shown by the viruses isolated from patients with low viral load could be representing viruses unable to find a mutant spectrum that replicate at high efficiency rates, then mutations will accumulate without effective selection, and finally viral quasispecies will be more heterogeneous until extinction. This transition from the quasispecies to the drift phase can be achieved due to a low viral replication ability as we show in Figs. 4 and 5. If the replication rate of the master sequence decreases below a threshold (represented as the line that separates the drift phase from the quasispecies domain in Fig. 2), the proportion of the master sequence also decreases and the mutant cloud consists of a wide spectrum of different mutant sequences (Fig. 4). The decrease in the proportion of the master sequence as a consequence of the decrease in the replication rate is followed in parallel by a rapid increase in the entropy as we show in Fig. 6. Thus, the inverse correlation among viral load and quasispecies complexity seen in the HCV data is also predicted by the bit string model.

HCV needs viral enzymes to infect and replicate itself. Two of these enzymes, the protease and the polymerase, are the main candidates to develop antiviral compounds with clinical applications. Given our mathematical approach, strategies to inhibit HCV replication rates (acting, for instance, on such enzymatic machinery) could force such error catastrophe phenomenon and the consequent genetic meltdown. Hence, the entry into the drift phase could be achieved quickly by the combination with antiviral compounds that show mutagenic activity, as reported for ribavirin (Crotty et al., 2001). Thus, forcing viruses to cross the error threshold would be a matter of two forces: one directed towards increasing the error rate and the other towards reducing viral replication rate. This strategy could promote viral extinction due to a combination of antiviral compounds and mutagenic agents. Recently, Pariente et al. (2005) have reviewed the effect on FMDV infection in vitro of mutagenic agents and antiviral compounds either alone or in combination. In this context, Tapia et al. (2005) achieve HIV extinction by using the mutagenic deoxyribonucleoside analogue 5-hydroxydeoxycytidine, but the extinction becomes systematic and more effective when adding the mutagenic agent in combination with the reverse transcriptase inhibitor AZT.

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