A trade-off between neutrality and adaptability limits the optimization of viral quasispecies

Jacobo Aguirre, Ester Lázaro, Susanna C. Manrubia *

Centro de Astrobiología, INTA-CSIC, Ctra. de Ajalvir km. 4, 28850 Torrejón de Ardoz, Madrid, Spain

A B S T R A C T

Theoretical studies of quasispecies usually focus on two properties of those populations at the mutation-selection equilibrium, namely asymptotic growth rate and population diversity. It has been postulated that, as a consequence of the high error rate of quasispecies replication, an increase of neutrality facilitates population optimization by reducing the amount of mutations with a deleterious effect on fitness. In this study we analyse how the optimization of equilibrium properties is affected when a quasispecies evolves in an environment perturbed through frequent bottleneck events. By means of a simple model we demonstrate that high neutrality may be detrimental when the population has to overcome repeated reductions in the population size, and that the property to be optimized in this situation is the time required to regenerate the quasispecies, i.e. its adaptability. In the scenario described, neutrality and adaptability cannot be simultaneously optimized. When fitness is equated with long-term survivability, high neutrality is the appropriate strategy in constant environments, while populations evolving in fluctuating environments are fitter when their neutrality is low, such that they can respond faster to perturbations. Our results might be relevant to better comprehend how a minority virus could displace the circulating quasispecies, a fact observed in natural infections and essential in viral evolution.

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1. Introduction

The evolution of heterogeneous populations of genomes replicating at high error rate was first theoretically described in the context of molecular evolution at prebiotic times. Upon replication for a sufficiently long time in a constant environment, infinitely large populations reach a stationary state, characterized by mutation-selection equilibrium (Kimura and Maruyama, 1966), where each molecular type maintains a constant frequency (Eigen, 1971; Eigen and Schuster, 1979; Schuster and Swetina, 1988). At the stationary state, those populations are referred to as quasispecies. Their structure and organization have been the subject of a large number of studies (Eigen et al., 1988; Biebricher and Eigen, 2006; Vignuzzi et al., 2006; Stich et al., 2007). Quasispecies theory has provided insight into the behaviour of other heterogeneous populations such as RNA viruses and viroids (Holland et al., 1992; Eigen, 1993; Domingo and Holland, 1997; Ambrós et al., 1999, Domingo et al., 2006). Among them, we highlight the existence of cooperative properties (Perales et al., 2005; Vignuzzi et al., 2006), the extinction of virus populations through increased mutagenesis (Crotty et al., 2001), or the

existence in the population of minority genomes with adaptive properties that become relevant when new selective pressures arise (Charpentier et al., 2004; Mullins and Jensen, 2006).

Each individual in a quasispecies is quantitatively characterized by its fitness, often defined as the capacity to generate viable progeny in a particular environment and in competition with the rest of the population (Domingo and Holland, 1997). According to this definition, fitness could be used as a synonym of *reproductive rate*. However, the average fitness of a population cannot be obtained simply by averaging the reproductive abilities of individuals at a fixed time. Rather, if we define fitness as the ability to survive in the long run, its nature as a multi-trait quantity that involves different features is made explicit (Lewontin, 2003). Three relevant traits of fitness that we are going to consider in this work are reproductive rate, adaptability, and genetic robustness—i.e. the ability to withstand the effect of new mutations (Hermisson and Wagner, 2004; Wagner, 2005). Among the many reported ways to increase genetic robustness, we can mention complementation (Montville et al., 2005), gene redundancy, epistasis, overexpression of chaperones, and *neutrality* (de Visser et al., 2003). Increasing neutrality has been postulated as the main mechanism to increase genetic robustness in asexual populations of replicators (Schuster and Swetina, 1988; van Nimwegen et al., 1999; Wilke 2001; Wilke and Adami, 2003), such as RNA viruses (Montville et al., 2005; Sanjuán et al., 2007),

* Corresponding author.
E-mail address: cuevasms@inta.es (S.C. Manrubia).

viroids (Codoñer et al., 2006), or populations of digital organisms (Wilke et al., 2001).

Apart from reproductive rate and genetic robustness, a third component of fitness deserving attention is adaptability. Adaptability is mainly determined by the degree of phenotypic and genetic diversity that can be either maintained in the population or produced in response to external changes. Amongst frequent perturbations that populations have to deal with, we find environmental changes and fluctuations in population size. A particular extreme case of the latter is represented by population bottlenecks, a situation holding when a reduced number of individuals are randomly chosen to found a new population. Population bottlenecks occur very frequently during the inter-host or intra-host transmission of RNA viruses (Escarmís et al., 2006), and strongly affect the fitness of viral populations (Novella et al., 1995; Lázaro et al., 2003). In constant environments, a population is expected to perform optimally at the mutation-selection equilibrium. Thus, in the face of perturbations, it can be stated that the shorter the time to recover the asymptotic equilibrium, the fitter the population (Elena et al., 1998).

Populations can increase their fitness by optimizing any of the above-mentioned traits: individual reproductive ability, genetic robustness, or adaptability. What is not known is whether these traits can be simultaneously optimized or whether there is some constraint in the form of a trade-off among them. The presence of trade-offs preventing populations from attaining an optimal performance in all traits affecting fitness has been addressed in several studies. Growth rate and yield (Novak et al., 2006), virulence and reproductive ability in competition assays (Herrera et al., 2007), or phage productivity and competitive ability (Kerr et al., 2006) have been pondered as characteristics that act in opposite ways and simultaneously affect the survivability of a viral population. The degree to which robustness hinders adaptability (Lenski et al., 2006) is still controversial. Simple models describing the evolution of populations of RNA sequences on secondary structure neutral networks seem to indicate that many different structures are easily accessible from each neutral network. This model, a proxy for more complex populations, indicates that evolvability—understood as the ability to locate new solutions in the space of sequences under changing selection pressures—would not be strongly constrained by neutrality (Huynen et al., 1996; Fontana, 2002). However, it has been observed that too high values of neutrality might eventually decrease diversity and restrict accessibility in that same system, thus preventing the population to innovate in short time spans. These simple models, which mimic in several ways the behaviour of viral quasispecies, also show that high neutrality is a trait under continuous selection in constant environments, since sequences with a higher number of neutral neighbours are at an advantage with respect to sequences more sensitive to mutations (van Nimwegen et al., 1999).

In this study we focus on the interplay between neutrality and adaptability in two antagonistic situations. High neutrality will be observed in environments that remain constant for a sufficiently long time, whereas adaptability (i.e. fast recovery after perturbations) will be optimized only in changing environments. We will analyse the consequences that an increase of neutrality has on the optimization of the growth rate and on the recovery time from extreme bottleneck events. To this end, we use a simple model which simulates the evolution of quasispecies reproducing under different degrees of neutrality and different probabilities to suffer deleterious and beneficial mutations. These parameters combine in a non-trivial way to yield different mutant distributions at the stationary state. Our results demonstrate that, if the ratio between beneficial and deleterious mutations is fixed, populations cannot optimize at the same time their growth rate at equilibrium and

the speed of regeneration of that same equilibrium after a bottleneck. The compromise reached by each population will depend on the relative weight of each selective pressure in the natural environment where it evolves.

2. Model for quasispecies evolution

We consider a population formed by at most N individuals, each with a reproductive ability (or reproductive rate) $r = 0,1,\ldots,R$. At each time step, every individual produces r offspring with a reproductive ability identical to that of her mother or differing in one unit. Our underlying assumption is that mutations in the genome take place during replication and cause changes in the reproductive ability: with probability p a mutation has a deleterious effect and lowers the reproductive rate in one unit, and with probability q it has a beneficial effect and r increases in one unit. There is a maximum value R for the reproductive ability of individuals, which corresponds to the absolute maximum number of offspring that an optimally adapted individual can yield per generation. Dynamics proceeds in discrete time steps (or generations), and at each time step a new population replaces the old one. The new population is a subset of N individuals extracted through usual Wright–Fisher sampling from the set formed by the old population plus their offspring. This procedure maintains the population size bounded. The dynamics of the population will be described through deterministic equations, as described in the next section. Thus, our results represent expected values for the relevant quantities tracked that might slightly differ from results obtained by means of stochastic models, especially when considering small populations. This nonetheless, our qualitative results are robust to that modification (Aguirre and Manrubia, 2007).

The mutation rates p and q can take any value between 0 and 1 with the condition that their sum does not exceed unity ($p+q \leq 1$), and that the fraction of deleterious mutations is higher than or equal to the fraction of beneficial mutations: $p \geq q$. The neutrality of a population is defined as $n = 1-p-q$. The class of largest reproductive ability is fixed in our numerical simulations to $R = 10$. This does not change any of our results or conclusions in a qualitative way.

Our model does not include a microscopic description of genomes and mutations, but only considers the effect that an overall genotypic mutation rate μ has on the reproductive ability of individuals. For the sake of this work, we assume that μ is essentially constant and produces on the average one mutation per molecule and replication cycle, in agreement with typical mutation rates of RNA viruses (Drake and Holland, 1999). Thus, the degree of neutrality attained by a quasispecies conditions the amount of mutations with effect on the phenotype and different values of our parameters p and q would describe different positions of the quasispecies in the genome space under the same value of μ. Some of the effects described in the forthcoming sections could be attained as well through variations in the mutation rate, which also affects p and q. In a more realistic model, changes in the mutation rate and changes in the degree of neutrality should be simultaneously considered. The limitations of the scenario studied in this work and possible extensions to overcome them are further addressed in the Discussion section.

2.1. Definition of relevant quantities

Reproductive rate or reproductive ability r—Number of offspring produced per individual and generation. In heterogeneous populations, individuals have different reproductive rates.

Asymptotic growth rate, λ, *and asymptotic distribution of reproductive classes*, u_r—The characteristics of the mutation-selection equilibrium of the population can be analytically calculated. The elements of the mean matrix, $M_{rr'}$, give by definition the expected number of individuals with class r arising from an individual of class r' under reproduction with conservation of the parental population. The non-zero elements of $M_{rr'}$ (for $0 \le r' < R$) are

$$M_{rr'} = \begin{cases} r(1-p-q)+1 & \text{for } r' = r \\ (r+1)p & \text{for } r' = r+1 \\ (r-1)q & \text{for } r' = r-1 \end{cases}$$

There are two different boundary conditions. Note that, for $r' = 1$ individuals with replicative ability $r = 0$ are produced. Since they do not replicate, they are unable to generate other types in the population. For $r' = R$ we have the condition $M_{RR} = R(1-p)+1$, which sets an upper bound to the replicative ability of individuals. The dynamical equations for the evolution of the population can thus be written as

$$n_r(g+1) = \sum_{r'=r-1}^{r+1} M_{rr'} n_{r'}(g)$$

where $n_r(g)$ is the number of individuals of class r at generation g. Note that although the dynamics are deterministic, we work with finite populations. At early times after the bottleneck, the number of individuals in classes different from the founder is small and initially below one. In agreement with our deterministic description, these populations are also allowed to replicate and mutate, thus generating adjacent classes in subsequent generations. The total population expected at generation g is $n_T(g) = \sum_{i=0}^{R} n_i(g)$, for any g. After a sufficiently large number of generations (in the limit $g \to \infty$), the population attains a mutation-selection equilibrium, where the fraction of individuals in each reproductive class stabilizes and the growth rate of the population is the same for all classes. The asymptotic distribution of reproductive classes $u_r = n_r(g \to \infty)/\sum_{i=0}^{R} n_i(g)$ corresponds to the eigenvector associated to the largest eigenvalue of the mean matrix. The largest eigenvalue yields the asymptotic growth rate λ (Manrubia et al., 2003; Aguirre and Manrubia, 2007). In the case of discrete time dynamics, growth of the population implies $\lambda > 1$. Both λ and u_i are functions of R and of the two mutation rates p and q. They are related through

$$\lambda = \sum_{i=1}^{R} i u_i + 1$$

where $\sum_{i=1}^{R} i u_i$ is the average reproductive rate of the population.

Diversity, σ—The phenotypic diversity of the population is quantified as the dispersion σ of the distribution of reproductive classes at the mutation-selection equilibrium. The growth rate λ and the diversity of a population are asymptotic properties of the dynamics. In order to calculate this diversity, we make use of the definition of standard deviation, and we obtain

$$\sigma = \sqrt{\sum_{i=0}^{R} u_i (i - \lambda + 1)^2}$$

Neutrality, n—The neutrality of a population is defined as $n = 1 - p - q$, that is the fraction of mutations that do not have an effect on the phenotype. In our model, high neutrality means high robustness, and vice versa.

Recovery time, T—Average number of generations elapsed between an extreme bottleneck event (where a single individual randomly drawn from the equilibrium distribution founds a new population) and the regeneration of the mutation-selection equilibrium distribution. In practice, we assume that the asymptotic equilibrium distribution has been attained when the difference between the average reproductive ability in the numerical simulation and the analytical solution (for infinite time and exactly known) is $< 10^{-4}$ at the current state and subsequent generations. The recovery time is a property relevant out of the mutation-selection equilibrium.

Adaptability—The ability of an individual to regenerate the quasispecies. In this work, it is inversely proportional to the recovery time T.

Robustness—The ability to withstand the effect of mutations. One way to increase robustness is to evolve towards higher neutrality.

Fitness—The ability to survive in the long run. It is a multi-trait quantity that involves, among others, reproductive rate, adaptability, and robustness.

3. Results

Diversity and asymptotic growth rate are two properties of populations at the mutation-selection equilibrium. Both depend on the degree of neutrality and on the values of the mutation rates p and q. Diversity is usually associated to the ability of populations to adapt to environmental perturbations. We will analyse to what extent diversity is related to adaptability to population bottlenecks, a situation that pushes populations out of mutation-selection equilibrium. One of our main results is the observation that the amount of diversity that the population can generate immediately after the bottleneck (related to the diversity it bears) strongly determines its survivability.

3.1. Quasispecies optimization as a function of the mutation rates p and q

The mutation rates p and q determine three important quantities describing the population. Two of them are equilibrium properties (growth rate and phenotypic diversity) and are defined once mutation-selection equilibrium is attained. The third is a non-equilibrium property (recovery time after bottlenecks) and characterizes the transient behaviour between the population bottleneck and the equilibrium.

Growth rate—We investigate first the dependence of the asymptotic growth rate λ on the model parameters. The results are summarized in Fig. 1(a), where we show the value of λ obtained for each pair of possible values of the mutation rates p and q. At fixed p, an increase in q favours the production of faster reproducing variants and thus λ augments. At fixed q, increases in p cause reductions in λ, since it enhances the production of slower reproducing types. The lowest absolute values of the growth rate are obtained in the limit $p \to 1$, $q \to 0$, where $\lambda \to 1$, while the largest absolute values of λ correspond to the non-trivial limit $p \to 0$, $q \to 0$, where neutrality attains its maximum value and $\lambda \to R+1$.

Phenotypic diversity—The degree of phenotypic diversity maintained by a population at the mutation-selection equilibrium is evaluated as the dispersion σ of the distribution of reproductive abilities of its individual components. Fig. 1(b) summarizes the dependence of the population diversity at equilibrium with the model parameters. Diversity depends non-monotonically on the mutation rates in certain regions of the parameter space. The absolute minimum value of σ is obtained for $p \to 0$, $q \to 0$, where the absolute maximum growth rate λ was obtained (see Fig. 1(a)). In this limit, the fastest reproducing class $r = R$ accumulates all of the individuals, and the population becomes homogeneous. The limit $p \to 1$, $q \to 0$ also has vanishing diversity, though in this case the population accumulates at $r = 0$. The largest absolute diversity
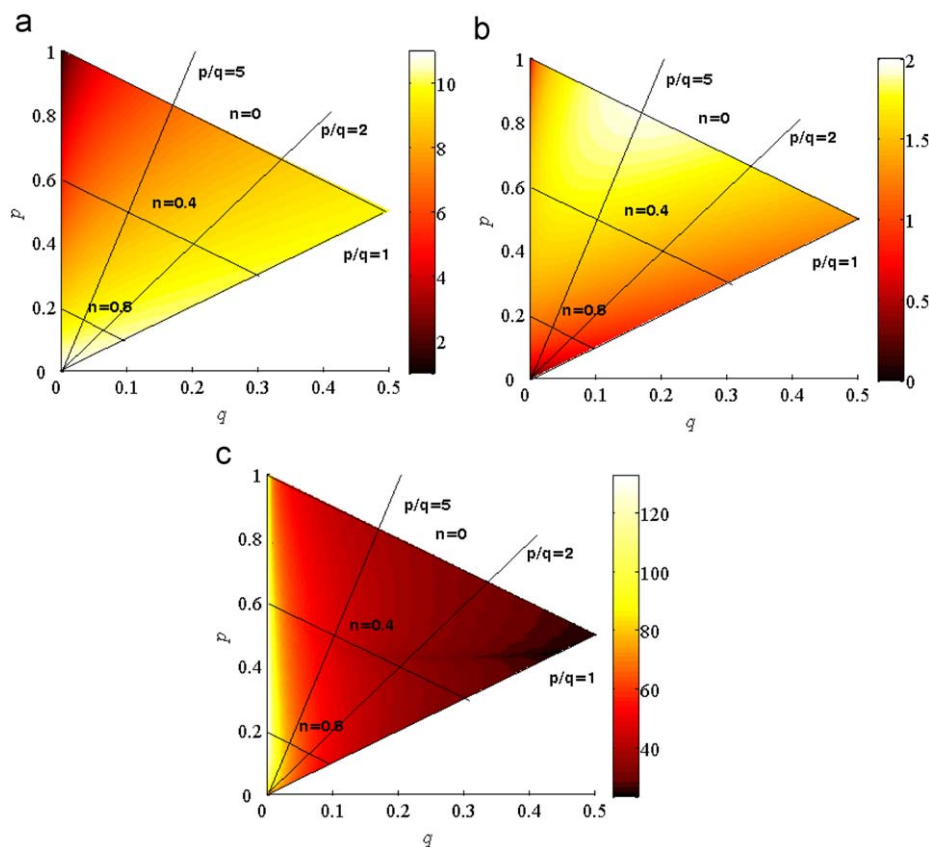
Fig. 1. Population properties as a function of the mutation rates p and q. Several curves corresponding to constant neutrality and constant p/q are explicitly shown. (a) Growth rate λ at the stationary state. (b) Diversity σ corresponding to the distribution of reproductive ability classes at the stationary state. (c) Recovery time T. Each point represents an average over 10^4 realizations starting with one founder individual randomly chosen from the mutation-selection equilibrium distribution. In practice, we consider that a population has attained equilibrium when its average reproductive rate is at a distance less than 10^{-4} from its asymptotic value at its current state and in subsequent generations.

is obtained, however, around $p = 0.83$, $q = 0.15$, a region characterized by intermediate values of the growth rate.

Recovery time after extreme bottlenecks—After an extreme bottleneck, a single individual of reproductive ability r has the task to regenerate the whole quasispecies. Fig. 1(c) shows the variation of the recovery time as a function of the parameters p and q. For a given value of p, an increment of q leads to large reductions in the recovery time while, for fixed q, the variation of p modifies only weakly the value of the recovery time.

3.2. Quasispecies optimization as a function of the ratio p/q and neutrality n

The effect of deleterious mutations can be counteracted by those with beneficial effect, thus we analyse the influence that a given degree of neutrality has in the optimization of populations as a function of the ratio p/q.

Growth rate—Fig. 2(a) represents λ as a function of p/q for different degrees of neutrality (curves for three cases, $n = 0$, 0.4, and 0.8 are shown in Fig. 1(a)). In principle, neutrality does not strongly condition the maximum growth rate that a population can reach, since λ attains large values in all cases when $p \rightarrow q$. For any value of the ratio p/q, the higher the neutrality, the higher the λ reached by the population. In addition, as the ratio p/q increases, populations with low neutrality are rapidly pushed towards lower growth rates. In contrast to this, the growth rate of populations

with high neutrality is more insensitive to variations in the ratio p/q. Consequently, high neutralities are only compatible with a narrow range of large values of λ, whereas populations evolving at low neutrality can reach stationary states with a much wider range of λ values. This behaviour is consistent with predictions derived from different theories where neutrality becomes critical in the optimization of the growth rate when most non-neutral mutations have deleterious effects (Codoñer et al., 2006; Sanjuán et al., 2007).

Increasing neutrality is an adaptive strategy with effects similar to decreasing the mutation rate, with the additional advantage that it is compatible with high genetic diversity. If the asymptotic growth rate of a population were equated with its fitness, two populations with the same λ would be evaluated as equally fit, despite the fact that the same growth rate can be attained with different values of neutrality and ratio p/q. However, since low neutralities demand proportionally less deleterious mutations (i.e. lower p/q) to achieve the same asymptotic growth rate, bearing low neutrality might become important to promote survival when other selective pressures, acting out of equilibrium, come into play.

Phenotypic diversity—The variation of σ with p/q is shown in Fig. 2(b) for different degrees of neutrality (three curves of constant neutrality are shown in Fig. 1(b)). Populations with low neutrality present a maximum of diversity at moderate values of the ratio p/q. However, for high values of neutrality, diversity grows monotonically with the ratio p/q, and the maximum disappears.
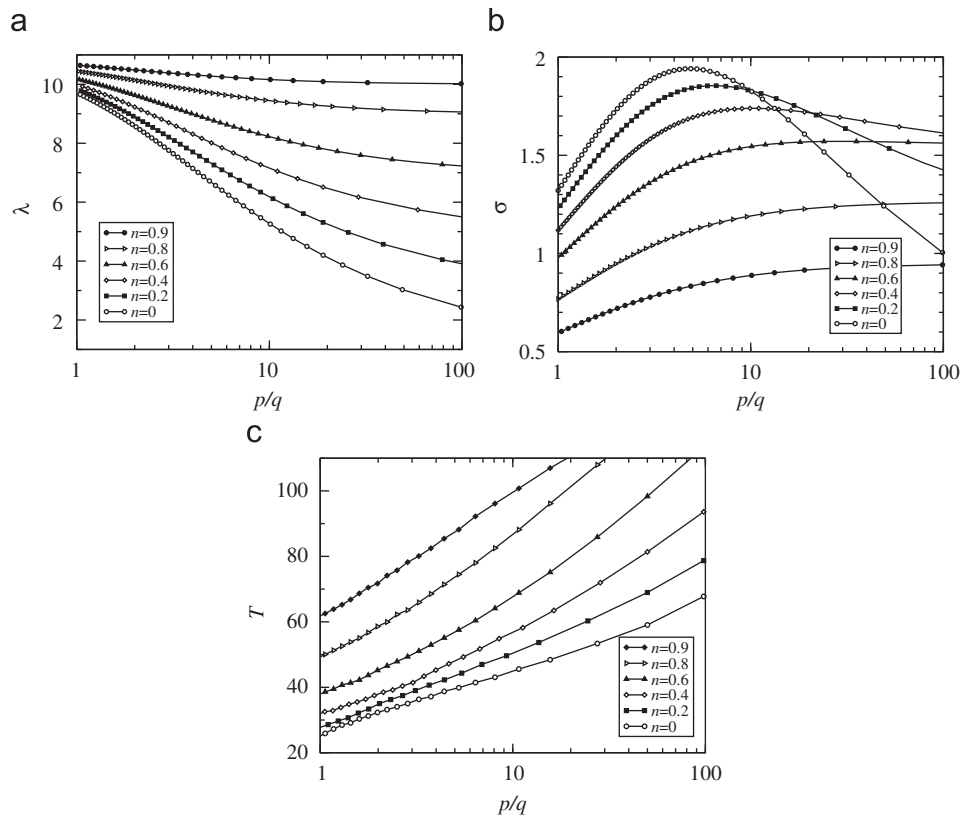
Fig. 2. Population properties as a function of the ratio p/q for different values of the neutrality $n = 1-p-q$. Compare these plots with those in Fig. 1. (a) Growth rate λ of the population at the stationary state. (b) Diversity σ corresponding to the distribution of reproductive ability classes at the stationary state. (c) Recovery time T. Each point is calculated as in Fig. 1(c).

Comparing Figs. 2(a) and (b) reveals that populations evolving at high neutrality maintain high growth rates, but their diversity is necessarily low. Attempts to maximize λ while maintaining high, constant neutrality imply decreases in σ, which are accompanied by decreases in the ratio p/q. On the other hand, populations evolving at fixed low neutrality can maximize their growth rate for $p/q \rightarrow 1$. There is, however, a region of intermediate values of the p/q ratio where diversity is maximized. The conclusion is that for a fixed neutrality value it is impossible to simultaneously maximize growth rate and the phenotypic diversity at the mutation-selection equilibrium.

Recovery time after extreme bottlenecks—For fixed neutrality, a raise in the ratio p/q increases the recovery time (Fig. 2(c)), reducing in this way the adaptability to perturbations caused by population bottlenecks. This happens both at high neutrality, when diversity grows as a function of the ratio p/q, and at low neutrality, when diversity presents a maximum at moderate values of p/q (Fig. 2(b)). These results show that diversity does not have a simple relationship with the recovery time. While the increments in diversity caused by increases in the ratio p/q are associated to longer recovery times, those that occur through reductions of the neutrality are accompanied by reductions in the recovery time. The important consequence is that keeping λ constant, the lower the neutrality, the lower the ratio p/q, and the lower also the recovery time.

Fig. 3 displays the results of different numerical simulations where extreme bottlenecks were applied to two independent populations with the same asymptotic growth rate but different neutrality values. In both cases, founder individuals with different reproductive abilities were chosen to reconstruct the quasispecies. As can be seen, any of the individuals within a population,
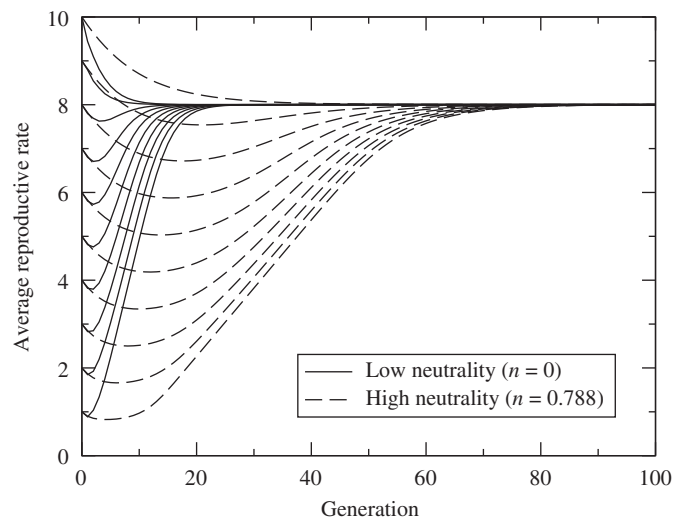

Fig. 3. Dynamics of the population towards equilibrium starting from different initial conditions. The process starts with one single individual of reproductive abilities r from 1 to 10 isolated from two populations with the same $\lambda = 9$ but differing in their neutrality: we use $n = 0$, $p = 0.614$, and $q = 0.386$ (low neutrality, recovers fast), and $n = 0.788$, $p = 0.208$, and $q = 0.004$ (high neutrality, recovers slowly).

independently of its initial reproductive ability, is able to regenerate it in approximately the same number of generations. This demonstrates that T is mainly a function of the degree of neutrality.

3.3. Out-of-equilibrium competition

In the scenario we have described, populations can optimize fitness through two different—and antagonistic—strategies. One implies increasing their neutrality, which permits high growth rates and makes the population highly robust against increases in the error rate. The second selects for higher adaptability through reductions in the recovery time, and requires a low degree of neutrality. For fixed λ, the latter strategy can only take place through selection of lower p/q ratios. Clearly, the best adaptive solution is context-dependent and will be selected as a function of the natural environment where the quasispecies evolves. These two strategies resemble r and K selection: an r-strategy is favoured in fluctuating environments, where long-term survival can be only guaranteed if high diversity is generated, while a K-strategy works better in constant environments, where high growth rates are attained (Pianka, 1970; Reznick et al., 2002).

The advantages of reducing the recovery time are obvious when several populations subjected to bottleneck events compete for the same ecological niche. In this case, a population with higher asymptotic growth rate can be displaced by a second one replicating at a lower pace if the latter has the ability to regenerate faster. The relationships between neutrality, diversity, p/q ratio, length of the recovery time, and λ make it difficult to predict the winner population in out-of-equilibrium competitions (Aguirre and Manrubia, 2007). In a different model, it has been shown that a viral strain whose reproductive ability is substantially affected by deleterious mutations can displace a second strain, less affected by deleterious mutations, when the populations are subdivided and compete for empty environmental patches (O'Fallon et al., 2007). This only occurs when the time allowed for population growth within each patch (able to sustain a finite number of individuals) is short enough that mutation-selection equilibrium is not reached.
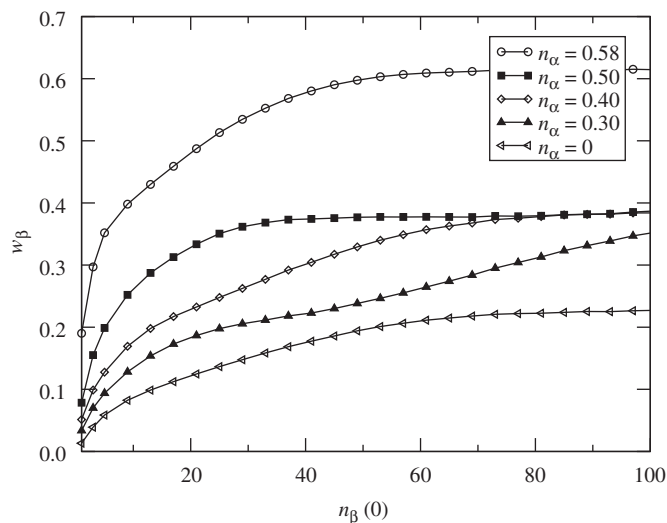
As an illustration of this phenomenon in the framework of our model, Fig. 4 shows the result of many simulated competition experiments where an individual of a population with $\lambda = 7$ competes with an increasing number of individuals of a population with $\lambda = 6$ and $n = 0$. In these cases, a population is termed extinct when the number of individuals falls below one. All individuals from both populations were randomly chosen from their mutation-selection equilibrium distributions. Different curves correspond to different neutralities in the population with $\lambda = 7$. There is always a non-zero probability that the winner quasispecies is that with lower λ, due to the shorter recovery times associated to lower neutrality. In this situation, it is clearly seen that growing faster at equilibrium does not guarantee winning in out-of-equilibrium situations. The higher the number of individuals in the initial group sampled from the population with the lower λ, the higher the probability that it eventually wins. This behaviour agrees with experimental results showing that a variant clone of vesicular stomatitis virus with higher growth rate can displace the parental population only when seeded above a certain concentration (de la Torre and Holland, 1990).

4. Discussion

We have analysed the interplay of two equilibrium properties, diversity and growth rate, and one out-of-equilibrium feature—recovery time from extreme bottleneck events—by means of a simple model in which the transient dynamics and mutation-selection equilibria of the populations only depend on the degree of neutrality and the mutation rates p and q. The results of our simulations show that growth rate can be optimized at high neutrality. Moreover, if the ratio between deleterious and beneficial mutations is kept constant, the higher the neutrality, the higher the growth rate that the population can attain. These results seem to indicate that populations should evolve towards reproducing at the highest attainable neutrality. However, we have also shown that reproduction at high neutrality can be unfavourable for populations suffering frequent bottlenecks. Fixing the ratio p/q, the lower the neutrality the shorter the recovery time, a fact related to the generation, early after the bottleneck, of a larger absolute number of beneficial mutants. Interestingly, the unavoidable concomitant increase in the number of deleterious mutants has a weak influence in the value of the recovery time. Finally, an increase in the ratio p/q at constant neutrality is associated to longer recovery times, this being now a consequence of the reduction in the absolute number of beneficial mutations.

Depending on the environmental characteristics, populations adopt one out of these two different adaptive strategies—or a compromise in between. If they are not subjected to frequent bottlenecks, the growth rate can be optimized through an increase in their neutrality, thus minimizing in particular the frequency of deleterious mutations. In contrast, populations subjected to frequent bottlenecks should have as adaptive priority the reduction of the time required to regenerate the equilibrium population starting with one or a few individuals. Actually, optimization of the recovery time can be attained without costs in λ in cases when a reduction of the ratio p/q is possible, though that optimization is unavoidably associated to decreases in the growth rate when the ratio p/q cannot be modified.

There are observations of increased neutrality in eukaryotic microRNAs as compared with other sequences folding into the same stem-loop secondary structure: the neutrality of the native molecules is more than twice higher (Borenstein and Ruppin, 2006), according to the definition used in this work. Plain changes

Fig. 4. Result of the competition between two quasispecies experiencing bottleneck events. A quasispecies β with growth rate $\lambda = 6$ and neutrality $n = 0$ competes with different populations α with growth rate $\lambda = 7$ and different degrees of neutrality, as shown in the legend. The horizontal axis shows the number of individuals of population β that compete with a single, randomly chosen individual of population α. The two populations grow independently until the total number of individuals reaches the maximum population size 10^3. Competition starts at that moment, and the total population is maintained constant until one competitor becomes extinct (i.e. its population falls below one). The vertical axis shows the probability w_β that the competitor with the lower asymptotic growth rate, i.e. population β, displaces its competitor. Averages are performed over 10^6 independent realizations.

in neutrality at fixed mutation rate may thus cause broadly varying phenotypic effects. Obviously, reductions in the mutation rate should translate into decreases in the values of p and q, and will affect quasispecies fitness. Recent experiments with polio-virus variants endowed with higher fidelity polymerase have demonstrated that the wild type form is at an advantage when it comes to generating higher-fitness genotypes (Vignuzzi et al., 2008). This gives indirect support to our statement that more neutral variants would be slower at recovering from perturbations. In the same way that reduced viral polymerase fidelity enhances adaptation, so would do a limited degree of neutrality.

Interactions of mutations with the genomic context and their relative value in different external environments (presence of drugs, differences in physical parameters, and others) make the effect of most mutations unpredictable. Their phenotypic value is context-dependent, though any variation in the current conditions typically reduces the fraction of neutral mutants (Korona, 1999; Remold and Lenski, 2001; Kishony and Leibler, 2003). Up to now, there have been few quantitative studies evaluating the amount of beneficial and deleterious mutations suffered by a population (Rokyta et al., 2008; Eyre-Walker and Keightley, 2007; Sanjuán et al., 2004). Even the estimation of the mutation rate is not a simple issue. Lethal or strongly deleterious mutations, for instance, might be rapidly eliminated from the population, such that the overall count yields fewer mutations than those that have actually occurred. Also the fixation of a given beneficial mutation can lead to the loss of others that were simultaneously spreading in the population.

Directed mutagenesis in Tobacco Etch virus yielded 41% lethal mutations and 36% deleterious mutations. No advantageous mutations were found among 66 mutated clones, which indicates that the virus was highly optimized (Carrasco et al., 2007). Point mutations in HIV protease caused a complete loss of enzymatic activity in 54% of 107 mutants, a decrease of activity in 32% of cases, and only 2% of beneficial mutations (Parera et al., 2007). A study with *Escherichia coli* determined that the frequency of advantageous mutations is about 4×10^{-9} per cell and generation (Imhof and Schlotterer, 2001). These and other studies have led to the consensus that beneficial mutations are approximately 1000-fold less common than neutral or deleterious ones (Miralles et al., 1999; Keightley and Lynch, 2003; Orr, 2003), a situation corresponding to a ratio $p/q \approx 10^3$. This describes populations well adapted to constant environments; they have likely attained high growth rates and low phenotypic diversity, and would perform poorly when faced with reductions in the population size. According to our model, in these populations, a true adaptation to the latter should be accompanied by reductions in the neutrality, with the concomitant reduction in the growth rate. On the other hand, experimental estimations of the ratio p/q in populations poorly adapted to the environment (thus far from the mutation-selection equilibrium) yield values around 5 or 10 (Sanjuán et al., 2004).

Our results might be relevant to determine the eventually dominant viral quasispecies when several variants cocirculate in the same geographical region. When the host-to-host transmission takes place through drastic bottlenecks, as it happens in many respiratory diseases caused by viruses (Artenstein and Miller, 1966; Gerone et al., 1996), there is a high probability that the virus variant that establishes in the host population is the one able to regenerate faster the equilibrium quasispecies, even if its asymptotic growth rate is lower. This out-of-equilibrium property has to be taken into account when aiming at predicting whether an emergent, potentially dangerous viral mutant, could substitute the previously circulating one (Smolinski et al., 2003; O'Fallon et al., 2007). Substitution is actually a regularly observed phenomenon in viral infections, such as influenza (Earn et al., 2002). The

size of the bottleneck at the transmission event and the length of the recovery time would also be important factors to determine when the reinfection of an individual could lead to the substitution of the resident quasispecies. This situation is frequent in infections such as those caused by HIV and HCV that can remain latent for a long time, without causing visible symptoms (Smith et al., 2005; Blackard and Sherman, 2007). An extension of our results applies to the evolution of natural populations subjected to a strong selective pressure favouring a minority variant, as it happens when drug-resistant mutants emerge and compete for dominance (Coffin, 1995; Loveday and Hill, 1995). This situation is partly equivalent to a population bottleneck, since only a few individuals can replicate optimally under the new conditions—though in this case they do it in competition with the rest of the quasispecies. The factors determining which virus sub-population eventually dominates are as yet uncertain, although our results suggest that a variant of lower neutrality could be at an advantage, similarly to the suppression of an *a priori* superior subpopulation described by de la Torre and Holland (1990).

An interesting extension of the work here presented is to permit explicit variation in the parameters p and q along evolution. In particular, the probability of experiencing beneficial mutations could depend on the fitness of individuals (Novella et al., 1995; Poon and Otto, 2000; Lázaro et al., 2002, 2003). Eventually, it must be the feedback between the enrichment in more successful individuals in a given environment and the characteristics of the latter what should lead to an emerging optimal strategy. One could then follow evolutionary trajectories in the p–q plane that would tell about the life history of the population and the selective pressures it has experienced. More sophisticated models could include an explicit description of the genomic level in order to derive the error rates p and q from the microscopic mutation rate. One possible scenario for this lower level of description is yielded by populations of RNA sequences with selection acting on their folded states (Fontana, 2002). The dynamics of such populations on the space of genomes under a variety of environments permits an explicit quantification of changes in neutrality while keeping the mutation rate μ constant (Stich et al., 2009). An additional improvement might come from considering as well variations in the mutation rate. In this extended framework, it should be possible to design appropriately varying environments and to study the selection of n and μ under suitable dynamical rules. This entails, however, an additional difficulty, which is the knowledge of the relative time scales at which μ and n change. The latter is a highly non-trivial issue which likely needs to take into account, among others, the degree of evolvability that organisms can achieve (which limits variation in the mutation rate) and the geometrical characteristics of neutral networks (which determine how rapid the increase in neutrality can be). All these are fascinating open questions of high relevance to understand evolution and adaptation of populations, and which will be the subject of future investigation.

Acknowledgements

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