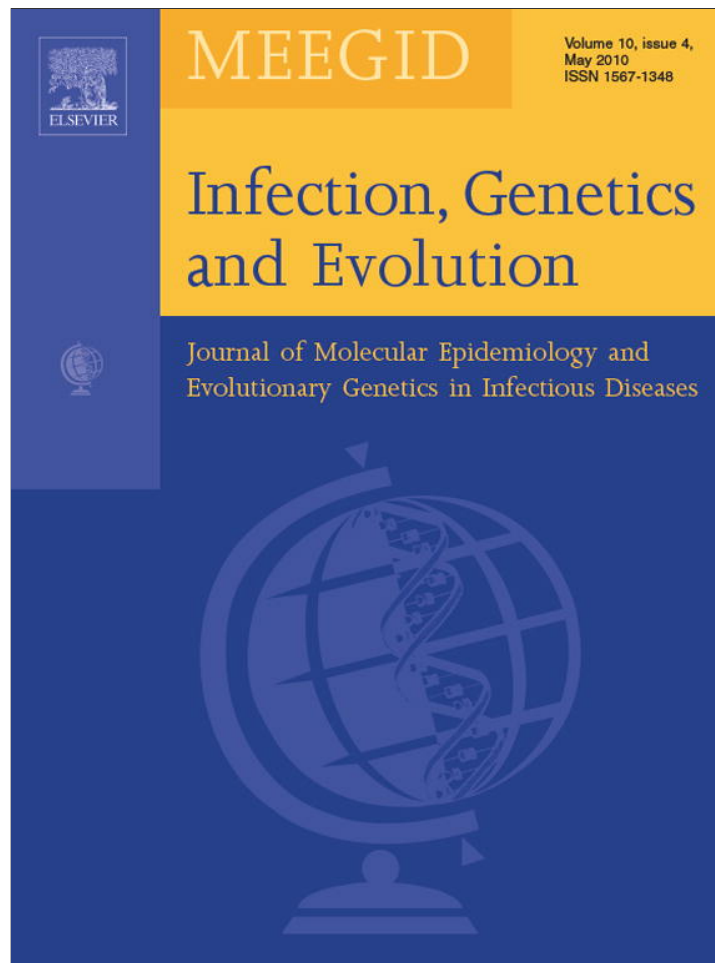


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

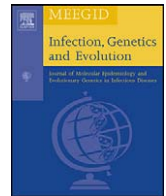
In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Regulated superinfection may help HIV adaptation on rugged landscape

Vladimir Leontiev^{a,*}, Lilach Hadany^{b,1}^a Department of Biology, University of Iowa, 143 Biology Building, Iowa City, IO 52242-1324, United States^b Department of Plant Sciences, University of Tel Aviv, Ramat Aviv, Tel-Aviv 69978, Brittainia 409, Israel

ARTICLE INFO

Article history:

Received 1 December 2009

Received in revised form 16 February 2010

Accepted 18 February 2010

Available online 11 March 2010

Keywords:

HIV

Rugged fitness landscape

Computational models

Recombination

Superinfection

Phenotypic rescue

ABSTRACT

Human immunodeficiency virus (HIV) is highly adaptable to a, changing environment, including host immune response and antiviral drugs. Superinfection occurs when several HIV proviruses share the same host cell. We previously proposed that HIV may regulate the rate of its superinfection, which would help the virus to adapt (Leontiev et al., 2008). In this paper we, investigate the effect of regulated superinfection in HIV on complex, adaptation on rugged fitness landscapes. We present the results of our *in silico* experiments that suggest that regulated superinfection facilitates HIV, adaptation on rugged fitness landscapes and that the advantage of regulated, superinfection increases with the ruggedness of the landscape.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

The adaptability of HIV to drugs and host defense systems is attributed to its ability to evolve at a very high rate (Martinez-Picado and Martinez, 2008). Superinfection – the infection of a single host cell by multiple virions (Tanaka et al., 2003) – might play an important role in HIV evolvability. In a previous paper (Leontiev et al., 2008) we showed that if HIV superinfection is regulated it may increase the adaptability of the virus to a changing environment. In this paper we extend our model to include the case of HIV evolution where complex interactions between loci are possible.

The effect of epistasis, when fitness contribution of one locus is modified by one or several other loci had been experimentally measured for HIV (Bonhoeffer et al., 2004) and some other RNA viruses (Sanjuan, 2006; Sanjuan et al., 2004). Sanjuan and Nebot (2008) had proposed that the sign and strength of epistasis is determined by the amount of multi-functionality, connectivity, and redundancy in an organism's molecular machinery. The variations in sign and/or value of epistasis between loci mean that there are favorable and unfavorable combinations of gene alleles; favorable combinations form fitness “peaks” unfavorable form fitness “valleys”. This led Wright (1932) to introduce the metaphor of “fitness landscape”. The fitness landscape is the N-dimensional

virtual plot of the fitness as a function of the genotype. A simple fitness landscape is smooth and has a single peak; in contrast, a rugged fitness landscape has many fitness peaks of different heights separated by fitness valleys. Combinations of mutations, recombination and selection tend to push genotypes up the fitness gradient; therefore the general tendency of populations is to move toward the nearest fitness peak. When the landscape is rugged, getting to the global maximum – corresponding to the best adaptation to the environment – might require crossing one or more “fitness valleys”. Consequently, the population may get temporarily or permanently trapped in one of the many local fitness peaks. Kauffman and Weinberger (1989) introduced the $N-K$ model of rugged landscapes which has since become widely adopted in theoretical population genetics. This model affords control over the ruggedness of the fitness landscape through the tuning of two parameters— N and K (see below).

Since the evolution on rugged fitness landscapes is qualitatively different from the evolution on smooth landscapes, we aimed to compare the effect of regulated superinfection on the adaptation of HIV on both types of landscapes. In studying the effect of superinfection on HIV adaptation, at least three distinct effects should be considered.

First, superinfection is required for productive recombination. Recombinant forms are very common among HIV variants, which points to the importance of recombination in HIV evolution (Ramirez et al., 2008). The effect of recombination on HIV evolution depends on the effective population size and other factors such as the sign of epistasis (Althaus and Bonhoeffer, 2005; Bocharov et al., 2005; Leontiev et al., 2008; Vijay et al., 2008).

* Corresponding author. Tel.: +1 319 384 1858; fax: +1 319 335 1069.

E-mail addresses: vladimirleontiev@gmail.com (V. Leontiev),lilach.hadany@gmail.com (L. Hadany).¹ Tel.: +972 3 640 9831; fax: +972 3 640 9380.

Second, superinfection may affect HIV adaptation through phenotypic rescue: viruses lacking a certain gene can survive by using the product of this gene supplied by other viruses sharing the same cell. Phenotypic rescue allows viruses with mutated or truncated genes to survive as long as there is sufficient level of superinfection. The presence of low-fitness mutants in HIV populations (Fernandez et al., 2007) may be attributed to phenotypic rescue. Phenotypic rescue leads to partial hiding of deleterious mutations from selective pressure (Wilke and Novella, 2003) which may conceivably help crossing the fitness valleys in the rugged landscape.

A third aspect of superinfection is the competition for resources experienced by viruses infecting the same host cell. When several viruses occupy the same cell this may result in less progeny virions *per infecting genome*. If this is the case, then there is a fitness cost associated with competition for resources (hereafter termed cost of competition— C_c). Regulated superinfection leads to redistribution of the cost with bias toward less fit individuals (Leontiev et al., 2008) which may play an important role in HIV adaptation.

HIV possesses several mechanisms for downregulating superinfection. Several HIV genes, such as early expressed Nef1, act to decrease the levels of HIV receptors on the surface of the host cell (see Burtey et al., 2006 for details). In Michel et al. (2005) it was proposed that HIV (and other primate lentiviruses) have evolved time windows – time intervals after infection and prior to early HIV gene expression – during which superinfection is more likely to occur because more HIV receptors are available. Downregulation of receptors decreases the possibility of superinfection. Earlier, we suggested (Leontiev et al., 2008) that this “window of opportunity” for superinfection, i.e. the time between the first infection and receptor downregulation, would be shorter for fitter, faster reproducing viruses and longer for unfit viruses. Furthermore, unfit viruses may have deficiency in their mechanism of receptor downregulation. Downregulation of receptors may be delayed or not happen at all, and the window of opportunity for a secondary infection would be even wider. Thus, the rate of superinfection would be governed by fitness, or at least by the component of the fitness that depends on the early expressed HIV genes. This would constitute a simple mechanism of fitness-associated superinfection.

Despite the existence of a specific mechanism for decreasing superinfection the experimental evidence indicates that a large percentage of an HIV population is located in superinfected cells (Jung et al., 2002) suggesting that superinfection is very common during HIV infection. In the following we investigate the effects of regulation of superinfection and the associated recombination, phenotypic rescue, and cost of competition on the adaptation of HIV on rugged fitness landscapes.

2. Models

To model a rugged fitness landscape with different levels of ruggedness we used the N – K model (Kauffman and Weinberger, 1989). In the N – K model a “chromosome” is modeled by an N -vector of loci, each of which has a value denoting one of a set of possible alleles. We used the most common two-allele model where each locus can have the value of either 0 or 1. The contribution of each gene i in a chromosome to the fitness depends on the value of the allele a_i , as well as the values of alleles at $K-1$ additional loci. Therefore for $K=1$ fitness depends only on value of allele of gene itself. For $K=3$ fitness depends on allele of gene itself and on 2 other alleles in chromosome. Additional alleles, affecting the fitness of given allele initially chosen at random, but dependency does not change during the simulation.

This dependency on other loci represents the epistatic interactions between genes: higher K values mean more epistatic interactions, and higher K/N ratios result in a more rugged landscape. In particular, when $K=1$ the fitness landscape is smooth, with a single maximum at which each gene has its fittest allele selected. For each gene i , and for each combination of the K alleles at the affecting loci we chose a fitness value drawn randomly from a uniform distribution between 0 and 1. The fitness of a genotype is then defined as the normalized sum of fitness values of all its genes.

Superinfection adds complexity to the definition of fitness. When two viruses co-inhabit the same cell there is no separation of viral products of different origins, and all the superinfecting viruses use a common pool of gene products. All combinations of alleles present in both viruses are possible and will contribute to fitness. Therefore we calculate the fitness of double-infected cell as an average fitness of all possible combinations of alleles present in the cell.

Regulated and random superinfection strategies were compared by letting two populations – one employing random superinfection and one employing regulated superinfection – to evolve on the same fitness landscape from the same starting point. Population employing better superinfection strategy is expected to achieve better average fitness. Since each rugged landscape has its own peculiarities which can bias the result, we generated multiple random landscapes, each time allowing the two compared populations to evolve on the same landscape, and averaged the results over all the landscapes. For each combination of parameters, pairs of simulation runs were repeated at least 200 times for $K=1$, 400 times for $K=3$, and 1000 times for $K=7$.

In our model the HIV genome is represented by a set of 10 genes ($N=10$), each having 2 alleles. Reproduction occurs in discrete, non-overlapping generations. Simulations start with a fixed-size population of infected cells, each carrying a single HIV provirus. Infected cells produce virions (not represented explicitly), which are used to infect the cells in the next infection cycle. The steps performed at each infection cycle are the following:

- a. *Calculating fitness.* From an evolutionary viewpoint the fitness is a measure of the capacity to reproduce. HIV exploits cellular machinery for its reproduction, so the quantity and quality of progeny depends on the ability of the HIV genome to exploit the host cell. The fitness is therefore the virion-producing capacity of the infected cell. HIV fitness depends on its own genotype and, in case of superinfection, on the genotypes of the other proviruses present in the same cell. For single-infected cell the fitness is taken from a lookup table representing the N – K rugged fitness landscape described above. In view of phenotypic rescue, the fitness of viruses residing in superinfected cells could be defined either as the fitness of the best combination on all available alleles or as the average fitness of all combinations. We tested both approaches and got very similar results. Here we present data based on the averaging approach. For superinfected cells the fitness is therefore calculated as an average fitness of all possible combinations of alleles present in the cell; fitness for each combination is taken from the lookup table as described above. Neglecting cells infected by more than 2 virions, superinfected cells contain 2 virions instead of 1. The fitness of superinfected cell, i.e. its probability to send at least one of the viruses they contain to the next generation, is therefore doubled. To account for the cost of competition, the fitness of double-infected cells was multiplied by a factor of $2 - C_c$, where C_c is a cost of competition. Since there is no direct experimental evidence for the cost of competition (Dixit and Perelson, 2005) we ran our simulation experiments with the two extreme values of C_c : 0 and 1.

- b. *Primary infection.* For each infected cell in the next cycle an infecting virus is chosen in the following way: a “parent” cell where the infecting virus is produced is chosen at random with a probability proportional to its fitness. An infecting virus is then chosen at random from the possible forms of the progeny virus: if the cell contains two proviruses then one of the proviruses is chosen at random. If the cell contains only one provirus then its genotype is chosen for infection.
- c. *Secondary infection.* The choice of the cells being infected by a second virus is at the core of our model. The fraction of cells that undergo the secondary infection was kept the same for unregulated and regulated superinfection. In the case of unregulated superinfection target cells were chosen at random, with all cells having equal chance of secondary infection. In the case of regulated superinfection, only the cells infected by the least fit viruses, i.e. cells that produce viral products less efficiently, were subject to superinfection. To simplify the model we allowed a maximum of two infecting viruses per cell.
- d. *Mutation and recombination.* Mutation and recombination occur randomly during reverse transcription. The number of mutation events per genome per generation was sampled from a Poisson distribution with expectation $\lambda_m = 5 \times 10^{-2}$, i.e. 0.05 mutations per genome per generation. The number of template switching (recombination) events per genome per generation was either 0 (no recombination) or was sampled from a Poisson distribution with expectation $\lambda_r = 0.5$, i.e. 0.5 recombination events per genome per generation.

The combination of mutation and selection leads to increased average population fitness until the population either reaches the global maximum fitness or becomes trapped in a local maximum. For a single simulation run it is impossible to tell if the equilibrium that the population has reached at a given time is the final one. However, by repeating the simulation multiple times we can define a number of generations after which the changes in the average fitness of the populations, averaged over multiple runs, become sufficiently small to ignore. We define ‘stabilization’ of a population as the first time when the average fitness over 50 consecutive generations (averaged over all simulation runs) does not change by more than 0.1%. In our simulations stabilization was reached within 1000 generations. Average population fitness values presented here are the averages over 50 generations after the population has stabilized.

BOOST C++ libraries (<http://www.boost.org>) were used for random number generation.

3. Results

We compared the average population fitness for two superinfection strategies—regulated and random superinfection. Fitness was measured after the populations have evolved on a given fitness landscape and stabilized as described above. To reduce the effect of specific landscapes and starting points we generated a new rugged landscape for each pair of simulation runs, and ran two simulations on the same landscape: one with random superinfection, and one

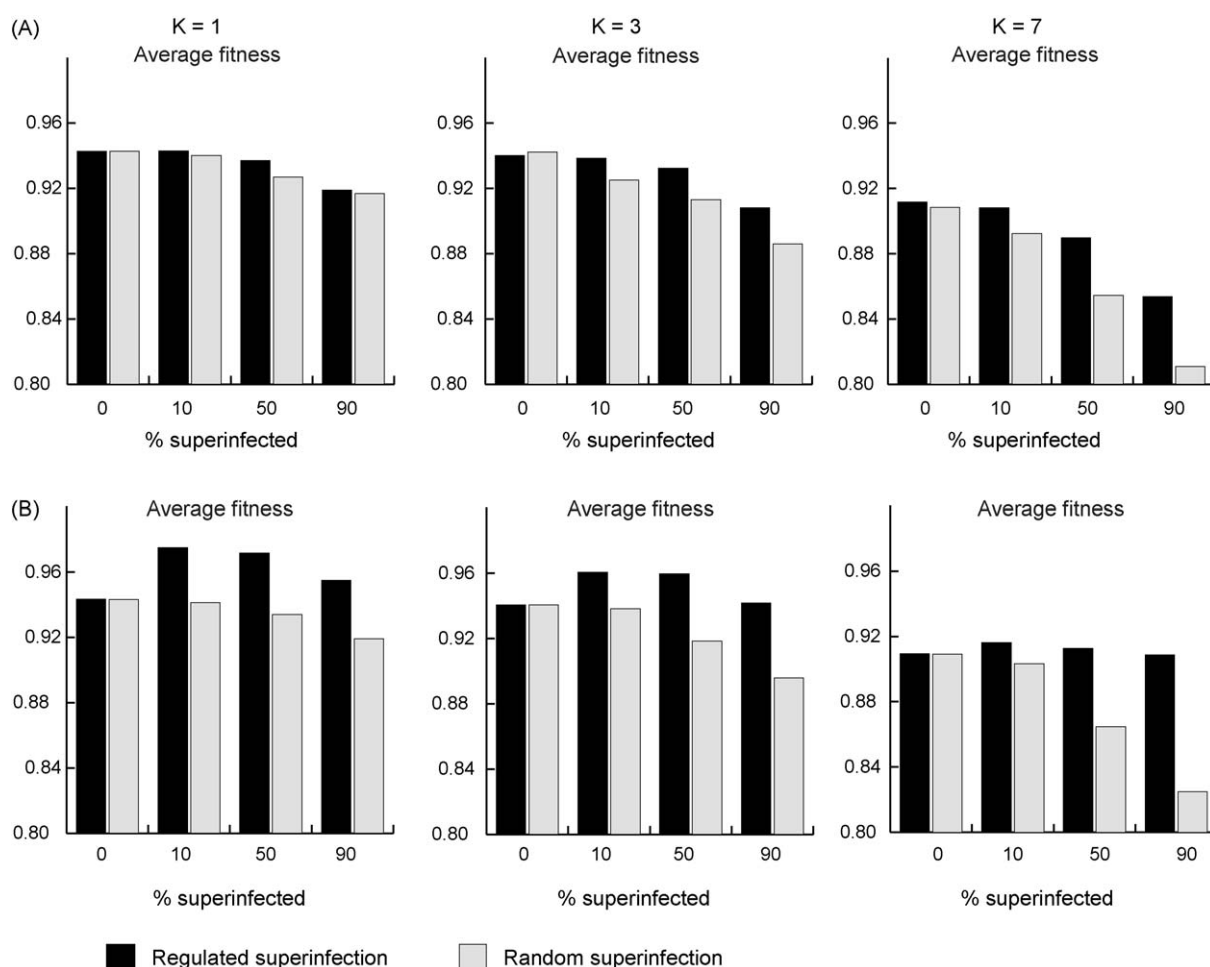


Fig. 1. Effect of the ruggedness of landscape and the fraction of superinfected cells on average population fitness. Vertical bars represent the average population fitness calculated over multiple simulation runs. (A) Cost of competition = 0; (B) cost of competition = 1. Confidence intervals are less than 0.1% (not shown). Simulation parameters: $\lambda_m = 5 \times 10^{-2}$, $\lambda_r = 0.5$, population size = 5000.

with regulated superinfection. Results of multiple simulation runs were averaged.

Average population fitnesses for different simulation parameters are shown in Fig. 1. As one can see, the regulated superinfection strategy in most cases leads to increase in average population fitness. The exceptions are the controls with superinfection rate = 0 and evolution on a smooth landscape with no cost of competition, at a low rate of superinfection (Fig. 1A, $K = 1$).

To better represent the effect of regulated superinfection we calculated the difference in average fitness between the two populations and divided that difference by the fitness of the population with random superinfection. We termed this parameter “advantage of regulation” or AR:

$$AR = \frac{\omega_{\text{regulated}} - \omega_{\text{random}}}{\omega_{\text{random}}}$$

where $\omega_{\text{regulated}}$ is the average population fitness with regulated superinfection and ω_{random} is the average population fitness with random superinfection.

Fig. 2 demonstrates the effects of recombination on the advantage of regulated superinfection. In the presence of recombination there is an increase in the advantage of regulated superinfection. On rugged landscapes ($K = 7$) both the value of AR and the increase due to recombination are greater than on smoother landscapes ($K = 3$ and $K = 1$). The cost of competition increases the effect of recombination on the rugged landscapes; on the smooth landscape it adds equally to AR with and without recombination.

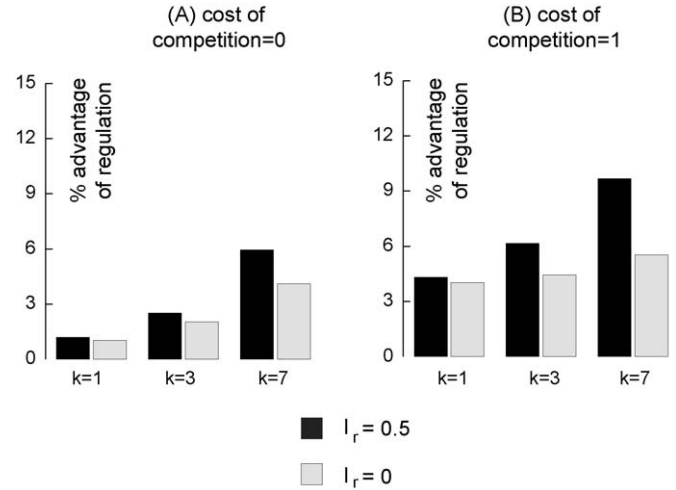


Fig. 2. The advantage of regulated superinfection: the effect of recombination. Vertical bars represent the advantage of regulated superinfection (AR). (A) Cost of competition = 0. (B) Cost of competition = 1. Confidence intervals are less than 0.1% (not shown). Simulation parameters: $\lambda_m = 5 \times 10^{-2}$, population size = 5000, level of superinfection = 50%.

Fig. 3 shows the dependence of AR on the ruggedness of the landscape and the fraction of superinfection. The value of AR is always positive which means that regulation of superinfection always leads to the increased average fitness of the HIV population compared with random superinfection. Conditions of high

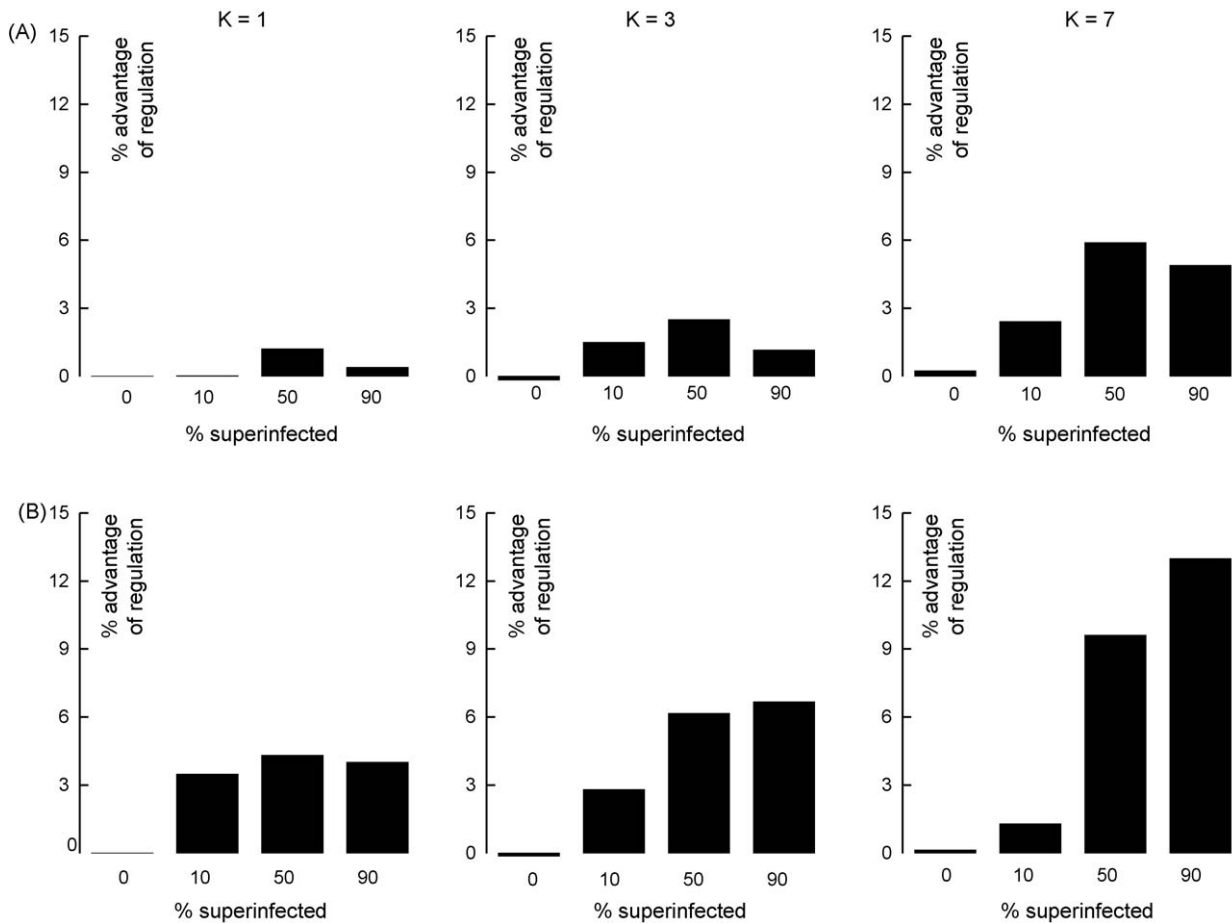


Fig. 3. The advantage of regulated superinfection: the effect of landscape ruggedness and fraction of superinfection. Vertical bars represent the advantage of regulated superinfection (AR). (A) Cost of competition = 0. (B) Cost of competition = 1. Confidence intervals are less than 0.1% (not shown). Simulation parameters: $\lambda_m = 5 \times 10^{-2}$, $\lambda_r = 0.5$, population size = 5000.

epistasis ($K/N = 0.7$) and high cost of competition ($C_c = 1$) yield over 10% advantage in average fitness for regulated superinfection.

As discussed in Section 2 above, the parameter K represents the number of loci affecting the fitness at the given locus. For a given genome length N , higher values of K lead to a more rugged landscape. Fig. 3 demonstrates that regulated superinfection is more advantageous on more rugged landscapes ($K = 7$) compared with a smooth landscape ($K = 1$), with intermediate effect at $K = 3$. The presence or absence of competition for resources by viruses infecting the same host cell (the cost of competition) has a substantial effect on the advantage of regulated superinfection: without the cost of competition (1A) regulated superinfection has less advantage over random superinfection, although the effect of regulation remains positive.

Fig. 3 also shows that the biggest advantage of regulation may occur at some intermediate frequency of superinfection. When there is no cost for superinfection, regulation is most advantageous at about 50% superinfection; with high cost of competition the optimal level of superinfection is shifted upwards.

4. Discussion

Experimental evidence suggests that the fitness landscapes of biological systems are often rugged, i.e. have multiple local maxima (Franco et al., 2007; Hayashi et al., 2006; Licht et al., 2003; O'Maille et al., 2008). To arrive at a new local maximum a life form often has to undergo several mutations. When some, but not all of these, are present, these mutations could be useless or even harmful. Natural selection is “shortsighted” (Clune et al., 2008), and an organism that had accumulated only some of the required mutations has a decreased chance of survival. This may be true, for example, for the adaptation of HIV to combinations of drugs that are used for HIV treatment. In terms of fitness landscape this means that moving between local maxima toward a global maximum would involve going through fitness valleys. As a result, on rugged fitness landscapes populations often become trapped in one of the local maxima. In some cases the global maximum cannot be reached and the fitness of the population remains suboptimal.

In our previous paper (Leontiev et al., 2008) we demonstrated that regulated superinfection in HIV is plausible and can increase the adaptability of HIV to antiviral drugs. Here, we expand our model to include the case of rugged fitness landscapes, using the classical N - K model of rugged landscapes (Kauffman and Weinberger, 1989). One of the advantages of the N - K model is the ability to adjust the ruggedness of the landscape by changing the K/N ratio. We compared the evolution of HIV on smooth ($K = 1$, $N = 10$), moderately rugged ($K = 3$, $N = 10$) and rugged ($K = 7$, $N = 10$) landscapes. In almost all tested cases regulated superinfection gave HIV a distinct fitness advantage, which increases with K . We shall now discuss some of the possible causes for the advantage of regulated superinfection.

4.1. Cost of competition

Cost of competition is the reduction in fitness of superinfecting viruses as a result of sharing host cell resources, which results in less yield of progeny *per infecting virion* than the yield in single-infected cells. To the best of our knowledge there is no experimental data about the cost of competition in HIV. Therefore we tested two extreme values for this parameter: no cost at all ($C_c = 0$) and full cost ($C_c = 1$). With no cost double-infected cells produce twice the number of virions as do single-infected ones. With full cost the double-infected cells produce the same number of virions as single-infected cells, thus reducing the number of progeny *per infecting virus* by a factor of 2. The real value of the cost is likely to be somewhere between these two extremes. Super-

infection decreases the overall productivity of an HIV population by imposing the cost of competition on the part of the population that resides in superinfected cells. This must have a negative effect on the population. However, when superinfection is fitness-dependent, only the cells carrying the less fit viruses get superinfected. Thus the burden of superinfection is redistributed away from the fittest individuals and towards the least fit ones. In our simulations this redistribution has a positive overall effect on the population: the time HIV requires to adapt to new drugs shortens (Leontiev et al., 2008), and the average fitness of the population increases. As shown in Figs. 1–3, the advantage of regulated superinfection usually increases with the cost of competition. A possible explanation for this is that shifting the burden of superinfection toward less fit individuals leads to stronger selection and, as a consequence, a fitter population.

4.2. Recombination

The effect of a high recombination rate on the evolution of HIV may be positive or negative, depending on the type and parameters of the model (Althaus and Bonhoeffer, 2005; Bocharov et al., 2005; Bonhoeffer et al., 2004; Bretscher et al., 2004; Carvajal-Rodriguez et al., 2007; Fraser, 2005). Quan et al. (2009) investigated the significance of recombination for the rescue of defective HIV genomes had suggested that cells harboring defective viruses may not die as quickly as those infected with wild-type viruses. They also suggested that such cells might be prone to superinfection. Their experiments show that superinfection leads to recombination and the recovery of fully functional genotypes. Recombination not only creates new and advantageous genetic combinations, but also breaks down existing good ones (Eshel and Feldman, 1970). This means that fitter genotypes always have less to benefit from recombination as compared with the less fit ones. Hadany and Beker (2003) have shown that for haploid sexual populations increasing the rate of recombination in less fit individuals and decreasing it in fitter ones leads to an increase in the average fitness of the population. In HIV the association between recombination rate and fitness may be achieved through regulation of superinfection: superinfection is required for productive recombination, therefore effectively only viruses residing in superinfected cells can recombine. Increasing the ruggedness of the landscape increases the number of fitness peaks which the population is unlikely to reach by mutation alone; however, these remote peaks can be discovered by recombination. By definition, on the rugged fitness landscape the least fit individuals reside in fitness valleys, while the fittest ones are closer to fitness peaks. Regulated superinfection leads to regulated recombination and as a result inhabitants of fitness valleys recombine more often. This may help suboptimal genotypes to discover new fitness peaks while the fittest individuals safely reside in single-infected cells and do not recombine. Fig. 2 demonstrates that the presence of recombination increases the advantage of regulated superinfection on rugged landscapes ($K = 3$ and $K = 7$) while having a less significant effect on a smooth landscape ($K = 1$).

4.3. Phenotypic rescue

Fig. 2a shows that even in the absence of both recombination and cost of competition, the regulation of superinfection carries considerable advantage to the population, and that this advantage tends to increase with K . One possible explanation for that is that phenotypic rescue associated with superinfection benefits less fit individuals but not the fittest ones. When superinfection is regulated, individuals inhabiting fitness valleys may rescue one another, effectively making fitness valleys shallower while leaving fitness peaks unchanged. Thus phenotypic rescue may help the

populations to cross fitness valleys, which leads to faster adaptation on rugged landscapes.

4.4. Fraction of superinfected cells

We compared the average fitness of populations with fitness-dependent superinfection to the populations with random superinfection; the overall fraction of superinfected cells was the same in both populations. When the fraction of superinfection is at one of the extremes (no superinfection or full superinfection) regulation is not possible. Consequently, one can expect the existence of an optimal level of superinfection, at which the population benefits most from its regulation. In our experiments such optimum is found at high levels of superinfection. With full cost of competition on rugged landscapes the advantage of regulation (AR) increased throughout the range tested (up to 90%); without the cost maximum effect of regulation is observed at the rate of superinfection of 50% (Fig. 3A and B). This is likely due to superposition of two effects: First, with full cost of competition (Fig. 3B) in the regulated populations the average fitness first increases with the rate of superinfection then decreases again to about its level with zero superinfection. Second, the fitness of the unregulated populations either remains unaffected or, at higher superinfection rates, decreases. The combination of the 2 effects shifts the AR maximum towards higher levels of superinfection (Fig. 3B). With no cost of competition, superinfection has either zero or negative effect on the fitness in both types of populations (Fig. 3A); however the negative effects on regulated populations are less pronounced. The maximum effect of regulation under these conditions is observed at a superinfection rate of 50% (Fig. 3A). Additionally, at high levels of superinfection phenotypic rescue leads to excessive accumulation of low-fitness genotypes, which can negatively affect the average fitness of the population. This may be the reason why the highest values of fitness for regulated superinfection correspond to superinfection rates between 0 and 10%, depending on the cost of competition (Fig. 3A and B).

Despite the existence of complex mechanisms that can decrease superinfection, the actual rate of superinfection in HIV may be quite high (Jung et al., 2002). Total avoidance of superinfection may be too costly for HIV or have some negative effect, such as suppression of productive recombination or other processes beneficial for the virus. Here we show (Fig. 3B) that moderate levels of superinfection may actually be beneficial for the virus in terms of average population fitness, provided that superinfection is regulated and that competition carries a cost. Our computational models suggest that instead of eliminating superinfection HIV may have acquired the ability to regulate it. A crude type of regulation involving a “window of opportunity” mechanism (Michel et al., 2005) seems plausible, while more sophisticated mechanisms may exist as well. If our prediction is true, this may have practical implications. The effectiveness of HIV entry inhibitors, such as the recently approved maraviroc (Lieberman-Blum et al., 2008), may depend on such factors as the level of superinfection, the titer of HIV in the patient and the degree of HIV adaptation to the host. Relatively small, less adapted HIV populations that need superinfection to adapt to a new host may be a better target for entry inhibitors.

Acknowledgements

We wish to thank Tuvik Beker and Wendy J. Maury for many helpful comments. The research was supported in part by NSF grant 0639990 (L.H.) and by ISF grant 840/08 (L.H.).

References

- Althaus, C.L., Bonhoeffer, S., 2005. Stochastic interplay between mutation and recombination during the acquisition of drug resistance mutations in human immunodeficiency virus type 1. *J. Virol.* 79, 13572–13578.
- Bocharov, G., Ford, N.J., Edwards, J., Breinig, T., Wain-Hobson, S., Meyerhans, A., 2005. A genetic-algorithm approach to simulating human immunodeficiency virus evolution reveals the strong impact of multiply infected cells and recombination. *J. Gen. Virol.* 86, 3109–3118.
- Bonhoeffer, S., Chappey, C., Parkin, N.T., Whitcomb, J.M., Petropoulos, C.J., 2004. Evidence for positive epistasis in HIV-1. *Science* 306, 1547–1550.
- Bretscher, M.T., Althaus, C.L., Muller, V., Bonhoeffer, S., 2004. Recombination in HIV and the evolution of drug resistance: for better or for worse? *Bioessays* 26, 180–188.
- Burtey, A., Rappoport, J.Z., Bouchet, J., Basmaciogullari, S., Guatelli, J., Simon, S.M., Benichou, S., Benmerah, A., 2006. Dynamic interaction of HIV-1 Nef with the Clathrin-mediated endocytic pathway at the plasma membrane. *Traffic* 8 (1), 61–76.
- Carvajal-Rodriguez, A., Crandall, K.A., Posada, D., 2007. Recombination favors the evolution of drug resistance in HIV-1 during antiretroviral therapy. *Infect. Genet. Evol.*
- Clune, J., Misevic, D., Offria, C., Lenski, R.E., Elena, S.F., Sanjuan, R., 2008. Natural selection fails to optimize mutation rates for long-term adaptation on rugged fitness landscapes. *PLoS Comput. Biol.* 4, e1000187.
- Dixit, N.M., Perelson, A.S., 2005. HIV dynamics with multiple infections of target cells. *Proc. Natl. Acad. Sci. U.S.A.* 102, 8198–8203.
- Eshel, I., Feldman, M.W., 1970. On the evolutionary effect of recombination. *Theor. Popul. Biol.* 1, 88–100.
- Fernandez, G., Clotet, B., Martinez, M.A., 2007. Fitness landscape of human immunodeficiency virus type 1 protease quasispecies. *J. Virol.* 81, 2485–2496.
- Franco, S., Parera, M., Aparicio, E., Clotet, B., Martinez, M.A., 2007. Genetic and catalytic efficiency structure of an HCV protease quasispecies. *Hepatology* 45, 899–910.
- Fraser, C., 2005. HIV recombination: what is the impact on antiretroviral therapy? *J. R. Soc. Interface* 2, 489–503.
- Hadany, L., Beker, T., 2003. Fitness-associated recombination on rugged adaptive landscapes. *J. Evol. Biol.* 16, 862–870.
- Hayashi, Y., Aita, T., Toyota, H., Husimi, Y., Urabe, I., Yomo, T., 2006. Experimental rugged fitness landscape in protein sequence space. *PLoS One* 1, e96.
- Jung, A., Maier, R., Vartanian, J.P., Bocharov, G., Jung, V., Fischer, U., Meese, E., Wain-Hobson, S., Meyerhans, A., 2002. Multiply infected spleen cells in HIV patients. *Nature* 418, 144.
- Kauffman, S.A., Weinberger, E.D., 1989. The NK model of rugged fitness landscapes and its application to maturation of the immune response. *J. Theor. Biol.* 141, 211–245.
- Leontiev, V.V., Maury, W.J., Hadany, L., 2008. Drug induced superinfection in HIV and the evolution of drug resistance. *Infect. Genet. Evol.* 8, 40–50.
- Licht, S.S., Sonnleitner, A., Weiss, S., Schultz, P.G., 2003. A rugged energy landscape mechanism for trapping of transmembrane receptors during endocytosis. *Biochemistry* 42, 2916–2925.
- Lieberman-Blum, S.S., Fung, H.B., Bandres, J.C., 2008. Maraviroc: a CCR5-receptor antagonist for the treatment of HIV-1 infection. *Clin. Ther.* 30, 1228–1250.
- Martinez-Picado, J., Martinez, M.A., 2008. HIV-1 reverse transcriptase inhibitor resistance mutations and fitness: a view from the clinic and ex vivo. *Virus Res.* 134, 104–123.
- Michel, N., Allespach, I., Venzke, S., Fackler, O.T., Keppler, O.T., 2005. The Nef protein of human immunodeficiency virus establishes superinfection immunity by a dual strategy to downregulate cell-surface CCR5 and CD4. *Curr. Biol.* 15, 714–723.
- O'Maille, P.E., Malone, A., Dellas, N., Andes Hess Jr., B., Smentek, L., Sheehan, I., Greenhagen, B.T., Chappell, J., Manning, G., Noel, J.P., 2008. Quantitative exploration of the catalytic landscape separating divergent plant sesquiterpene synthases. *Nat. Chem. Biol.* 4, 617–623.
- Quan, Y., Liang, C., Brenner, B.G., Wainberg, M.A., 2009. Multidrug-resistant variants of HIV type 1 (HIV-1) can exist in cells as defective quasispecies and be rescued by superinfection with other defective HIV-1 variants. *J. Infect. Dis.* 200, 1479–1483.
- Ramirez, B.C., Simon-Loriere, E., Galetto, R., Negroni, M., 2008. Implications of recombination for HIV diversity. *Virus Res.* 134, 64–73.
- Sanjuan, R., 2006. Quantifying antagonistic epistasis in a multifunctional RNA secondary structure of the Rous sarcoma virus. *J. Gen. Virol.* 87, 1595–1602.
- Sanjuan, R., Moya, A., Elena, S.F., 2004. The contribution of epistasis to the architecture of fitness in an RNA virus. *Proc. Natl. Acad. Sci. U.S.A.* 101, 15376–15379.
- Sanjuan, R., Nebot, M.R., 2008. A network model for the correlation between epistasis and genomic complexity. *PLoS One* 3, e26663.
- Tanaka, M., Ueno, T., Nakahara, T., Sasaki, K., Ishimoto, A., Sakai, H., 2003. Down-regulation of CD4 is required for maintenance of viral infectivity of HIV-1. *Virology* 311, 316–325.
- Vijay, N.N., Vasantika, Ajmani, R., Perelson, A.S., Dixit, N.M., 2008. Recombination increases human immunodeficiency virus fitness, but not necessarily diversity. *J. Gen. Virol.* 89, 1467–1477.
- Wilke, C.O., Novella, I.S., 2003. Phenotypic mixing and hiding may contribute to memory in viral quasispecies. *BMC Microbiol.* 3, 11.
- Wright, S., 1932. The roles of mutation, inbreeding, crossbreeding, and selection in evolution. In: *Proceedings of the Sixth International Congress on Genetics*. pp. 355–366.