Infection, Genetics and Evolution 10 (2010) 505–510

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.

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.

1567-1348/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2010.02.010

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.