Addendum to: Ojosnegros S, Beerenwinkel N, Antal T, Nowak MA, Escarmis C, et al. Competition-colonization dynamics in an RNA virus. Proc Natl Acad Sci USA 2010; 107:2108–12. PMID: 20080701; DOI: 10.1073/pnas.0909787107.

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A single and purified clone of foot-and-mouth disease virus diversified in cell culture into two subpopulations that were genetically distinct. The subpopulation with higher virulence was a minority and was suppressed by the dominant but less virulent one. These two populations follow the competition-colonization dynamics described in ecology. Virulent viruses can be regarded as colonizers because they killed the cells faster and they spread faster. The attenuated subpopulation resembles competitors because of its higher replication efficiency in coinfected cells. Our results suggest a new model for the evolution of virulence which is based on interactions between components of the quasispecies. Competition between viral mutants takes place at two levels, intracellular competition and competition for new cells. The two strategies are subjected to density-dependent selection.

Viral populations are dynamic ensembles of genetically diverse genomes, referred to as viral quasispecies, that arise from of error-prone genome replication catalyzed by low fidelity RNA-dependent RNA or DNA polymerases. Genomes in the population are subjected to continuous competition, and both, individual replicative fitness and interactions among viruses within the population determine which variants will increase their relative abundance and which will remain minority components of the quasispecies (Fig. 1). RNA viruses are thus endowed with great adaptability to changing environments, and even single clones have the potential to rapidly generate explosive diversity after few rounds of replication. Studies with viral mutants that copy their genetic material with increased fidelity have documented that high mutation rates are essential for adaptation of the virus to a complex biological environment. In the case of our recent study with the important animal pathogen foot-and-mouth disease virus (FMDV), a single purified clone evolved during serial cell culture infections to give rise to two phylogenetically different progeny subpopulations. Remarkably, the subpopulation with higher fitness (replication capacity) was found to be the minority in the quasispecies. Four questions are raised by this result: (1) Which selective pressure favoured the divergence of a single purified clone into two defined evolutionary trajectories? The question is particularly intriguing if we consider that an established cell line is the most homogeneous environment available under laboratory conditions to support sustained viral replication. (2) Why did the high-fitness virus remain at low frequency over an extended course of serial infections?

Two interesting questions are raised by this result: (1) Which selective pressure favoured the divergence of a single purified clone into two defined evolutionary trajectories? The question is particularly intriguing if we consider that an established cell line is the most homogeneous environment available under laboratory conditions to support sustained viral replication. (2) Why did the high-fitness virus remain at low frequency over an extended course of serial infections?

A detailed phenotypic analysis of the two subpopulations revealed striking differences between them. The minority high-fitness subpopulation manifested a high-virulence phenotype, measured as the cell killing rate. The dominant subpopulation showed higher efficiency in progeny production in coinfected cells, and interfered with replication of the virulent variants resulting in a delay of cell
colonization defined in ecology. Virulent evolutionary strategies of competition and within the viral population resemble the subpopulation.

The dominance of the low-fitness within coinfected cells, thus explaining the low-fitness subpopulation.

Moreover, the evolution of virulence in viruses has typically been addressed applying models that either did not take into account coinfection of cells at all, or that assumed virulent viruses to be more efficient during coinfections. Consequently, many of these models predict an increase in the average virulence of the population. Based on our experimental results, we developed a new mathematical approach based on the SIR model that describes the dynamics of susceptible and infected cells and the production of the two viral subpopulations as a microepidemic in cell culture. Specifically, the model considered the existence of coinfected cells (Fig. 3). Analysis of the model revealed that the coinfection dynamics depends only on the two parameters that best describe the competition and colonization phenotypes, namely the difference in virulence between the two viruses and their intracellular competitiveness. Model simulations also predicted a density-dependent outcome of the coinfection dynamics. Under conditions of low MOI, virulent colonizers had an advantage because they spread faster through the unoccupied space of susceptible cells. In contrast, under high-density conditions, coinfections were more frequent and the competition strategy had higher success. Our new mathematical approach therefore establishes density-dependent selection as a link between fitness and virulence. The model is a promising tool to study the evolution of virulence, offering a broader scenario in which viral populations have the potential to evolve either towards increased virulence or towards attenuation.

Density-dependent selection of viruses and suppression of high-fitness clones has been described in other viral systems.
which strongly suggests that competition-colonization dynamics could be a general evolutionary mechanism for viruses. During coinfection, different members of the quasispecies can interact between them in the form of negative dominant mutants, also referred to as interfering viruses.10,15-19

A growing body of evidence indicates that mutant spectra of RNA virus quasispecies are ensembles of interacting mutants rather than mere collections of mutants ranked according to individual fitness and mutation rate as in the classic mutation-selection equilibrium. Fitness is a collective property of the virus population as also recognized for a number of cellular consortia.20 Evidence for RNA viruses includes the presence of memory genome subpopulations which can alter the evolutionary outcome of the ensemble, complementation and interference among components of the mutant spectra that were reproduced with reconstructed quasispecies, pathogenic potential of viruses or response to antiviral treatments which are influenced by the amplitude of the mutant spectrum and by its composition.1,13,21,22

The interplay between inhibitor-escape mutants and defector genomes produced by mutagenesis underlies a possible advantage of a sequential versus combination antiviral therapy.23 In our recent study,4 a link between quasispecies behavior and ecology can explain modulation of virulence. This phenomenon was mediated by high mutation rates and the potential of the viral system to select a differentiation trajectory for the benefit of the ensemble.

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