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Emerging viruses: why they are not jacks of all trades?

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In order to limit the impact of the recent pandemics ignited by viral host jumps, it is necessary to better understand the ecological and evolutionary factors influencing the early steps of emergence and the interactions between them. Antagonistic pleiotropy, that is, the negative fitness effect in the primary host of mutations allowing the infection of and the multiplication in a new host, has long been thought to be the main limitation to the evolution of generalist viruses and thus to emergence. However, the accumulation of experimental examples contradicting the hypothesis of antagonistic pleiotropy has highlighted the importance of other factors such as the epistasis between mutations increasing the adaptation to a new host. Epistasis is pervasive in viruses, affects the shape of the adaptive landscape and consequently the accessibility of evolutionary pathways. Finally, recent studies have gone steps further in the complexity of viral fitness determinism and stressed the potential importance of the epistatic pleiotropy and of the impact of host living conditions.

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Introduction

Many emerging viral diseases are caused by viruses that acquired the capacity to infect a previously non-susceptible host population [1,2]. The newly accessed population can be constituted of host individuals of a new genotype, ecotype, variety, or species that now becomes part of the virus' host range. Such recent emergences have had tremendous repercussions for human and animal health and agricultural production. Approaches identifying emerging viruses before they become pandemic are thus needed [3]. This requires a better understanding of the independent and concomitant effects of the evolutionary and ecological factors influencing the early steps of emergence, in order to tentatively ameliorate our ability to predict the emerging potential of viral genotypes or isolates [3,4]. In this review, we use examples from DNA and RNA viruses infecting animal, plant, or bacteria. Host type and genetic material are associated with specific constrains (e.g. mutation rate is higher in RNA virus; the animal immune system is much more specific than the plant one), but we want to give a broad panorama of the factors affecting viral emergence and hopefully draw some general mechanisms ruling it.

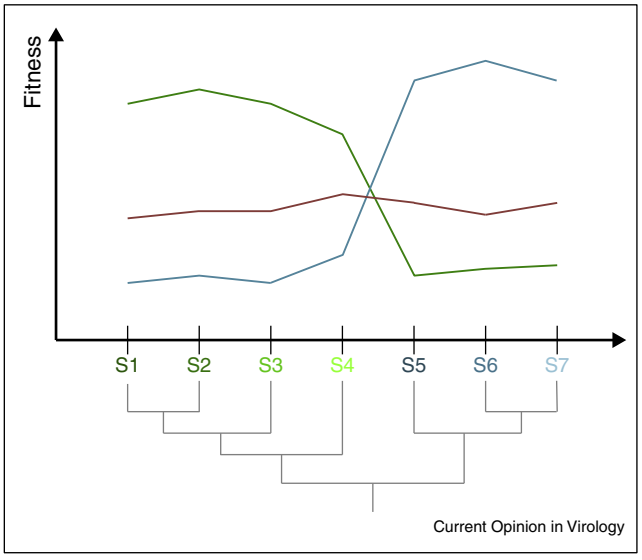
Generation of genetic diversity as an a priori condition for emergence

A first and necessary condition for emergence is the existence in the viral population replicating within the primary host of standing genetic variation making possible the infection and multiplication in the new host after occasional and often repeated spillovers [2,5]. Viruses, and in particular RNA viruses, have a strong evolutionary potential as a consequence of their fast and error-prone replication [6] and large population sizes [2,5]. Consequently, mutant generation should not be a limitation to their emergence. The only studies systematically investigating the rate of spontaneous host range mutations [7•,8•] did so for the phage Φ6 and its ability to infect new hosts closely related to the ancestral one. In this system, the equilibrium frequency of mutations that enable infection of a novel host was high (3×10^{-4}) [7•] and likely higher than in other systems including more distantly related hosts. The strong potential of viruses to generate host range mutants is also supported by the very rapid generation of viral escape mutants breaking RNAi mediated resistance in a plant RNA virus [9,10].

The antagonistic pleiotropic effect of adaptive mutations

A second condition for emergence is that the host range mutant should be able to replicate sufficiently well both in the primary and the novel hosts. Indeed, it is assumed that the mutant is initially poorly adapted to the new host and its adaptation requires that it persists long enough in the new hosts to allow for evolutionary rescue and/or repeated spillovers from the ancestral host, acting as a source, to the new host, that acts as a temporary sink [11,12••]. It has long been thought that it is difficult to meet this second condition, because of fitness trade-offs between hosts, that is, because mutants performing well in one host will perform badly in another host (Figure 1). This phenomenon is usually referred to the 'jack of all

Figure 1



$G \times E$ interactions and host phylogenetic relationship. Fitness of three theoretical viral genotypes across a panel of susceptible hosts that differ in their degree of genetic relatedness. The genotype represented by the green line has evolved on and adapted to species 2 (S2) and is a specialist in host species belonging to the 'green' clade, but has very low fitness in species belonging to the 'blue' clade. Likewise, the genotype represented by the blue line has evolved on and adapted to species 6 (S6) and is a specialist of high fitness in the 'blue' clade but pays a fitness cost in host species belonging to the 'green' clade. Finally, the brown line illustrates the situation for a generalist virus that is paying a fitness costs in both hosts: on average it performs well across both clades of potential host species but its fitness on each host is always lower than the one shown by the corresponding specialist.

trade' hypothesis [13], or to $G \times E$ interaction (where G designs the virus genotype and E designs the environment in which the virus replicates, otherwise said, the host). More recently, this same phenomenon was also renamed 'sign pleiotropy' [14••] in the conceptual framework of $G \times G \times E$ interactions (see below). At the mechanistic level, it can be due to the antagonistic pleiotropy of host-range mutations [15] or to the accumulation of mutations neutral in one host, because they are in a gene whose product is not required in the new host, and detrimental in the other host [16]. This second mechanism is, however, unlikely in viruses with small genomes, overlapping genes, and encoding for multifunctional proteins.

The existence of $G \times E$ interactions has been verified in various viral experimental systems by two types of approaches. First, negative correlations between fitness in the primary host and in the new host have been established (e.g. [8•]). Second, experimental evolution approaches where the same virus isolate or genotype is passaged in different hosts (either different species of the host range or successive hosts of the life cycle) usually show a pattern of specialization, that is, virus lineages evolved in one host performed better in this host than lineages evolved in other hosts and this specialization comes to a cost in terms of fitness in alternative hosts in part of the cases [17–23,24•,25•]. Recently, another approach has brought both confirmation and refinement of the antagonistic pleiotropy hypothesis: Lalić *et al.* [26••] measured a component of fitness, the multiplication rate, for 20 point mutants of *Tobacco etch virus* (TEV) in eight host species. The full factorial design of this experiment allowed to partition the variance in fitness in its different components, showing that most of the observed variation (66.82%) was attributable to the $G \times E$ interaction, whereas 26.13% resulted from differences among host species and only 4.29% to genetic differences among mutants. Additionally, it showed that the mode and shape of the distribution of mutational fitness effects (DMFE) varied with the host species: mutations were either neutral or deleterious in hosts that are close relatives to the primary one (*Nicotiana tabacum*), and as hosts' taxonomic relatedness to the primary one decreased, the distribution became flatter with larger expected deleterious fitness effect but also a certain fraction of mutations being beneficial.

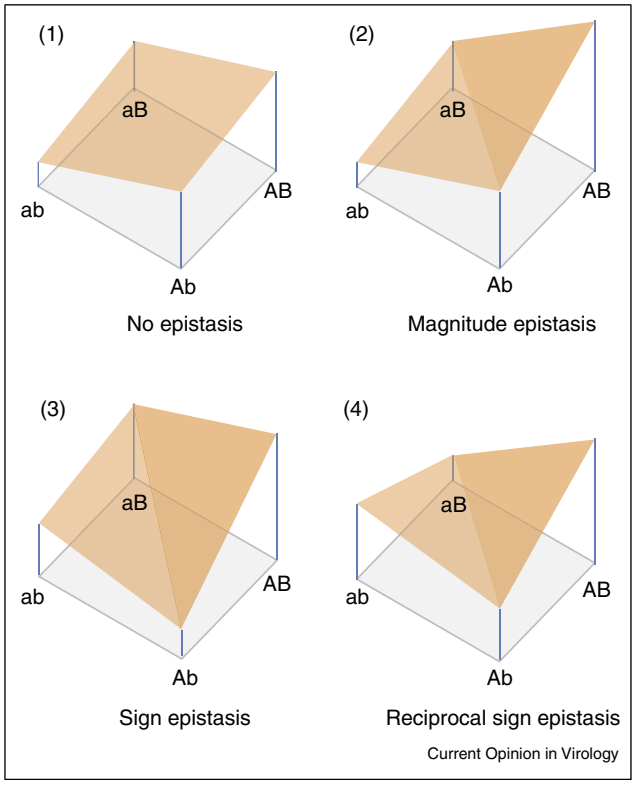
Along these multiple experimental confirmations of the existence of fitness trade-offs between hosts, there are also a number of examples of adaptation to a new host, or specialization, without any cost on alternative hosts [24•,27–30]. This has important consequences for the understanding of the host range evolution because if broadening of the host range can occur at no cost, it would mean that the idea that generalists are evolutionary disadvantaged because they are outcompeted by specialists in every hosts is not always true and that no-cost generalist should emerge a lot more often than they do. Probably a first step in understanding better what limits viral emergence is to realize that the antagonistic pleiotropy model is useful but overly simplistic and that more realistic models taking into account the complexity of host-range evolution are needed. A first aspect of this complexity is actually revealed by the effect of host relatedness on variation of the DMFE shape and mode. Indeed the E in $G \times E$ interaction designates differences between hosts ranging from different host genotypes, host ecotypes or different host species with various degrees of phylogenetic relatedness. The data from Lalić *et al.* suggest that the $G \times E$ interactions are more pronounced and frequent when the different hosts are phylogenetically distant, as sketched in Figure 1. This makes sense at the mechanistic level: related host species are more likely to share cell receptors and defence mechanisms, thus the ability to infect and replicate in related species is more likely to be positively correlated. This relationship between host jump ability and host phylogenetic relationship also opens the possibility that a virus initially unable to infect a host becomes able to infect it after adaptation to an intermediate host in terms of phylogenetic distance.

Complex interactions between mutations

Another level of complexity that has to be integrated is what hides behind G in $G \times E$ interactions. Again, depending on the experiment, G can represent point mutants, isolates or experimentally evolved lineages but in any case, the genotype is considered as a whole. Full genome sequences of experimentally adapted virus lineages showed that they frequently differ from their ancestors by several mutations (e.g. [24•,25•,31]), opening the possibility of epistasis between them. Epistasis (or $G \times G$ interaction) designates the fact that effect of mutations is not multiplicative but that there are interactions among them. This definition of $G \times G$ interaction is the one classically used in quantitative genetics and it should not be confused with the interaction between host genotype and pathogen genotype that plant pathologist also name 'genotype' by 'genotype' interaction. Epistasis is known to be a key determinant in adaptive processes as it determines the ruggedness of the adaptive landscape [32,33], and thus the accessibility of adaptive pathways throughout the landscape [34–36] and the probability of trajectories to end up at suboptimal fitness peaks. A measure of epistasis can be derived from experimental fitness measures of single and double mutants [37] and epistasis can be divided in various types depending on the actual effects of the interaction (Figure 2): magnitude epistasis refers to cases where the magnitude effect of a mutation depends on the background while its sign is constant. Magnitude epistasis is positive when the double mutant is fitter than expected from the multiplicative effect of the individual mutations and negative in the opposite case. Sign epistasis refers to cases where the background affects the sign of the effect of a mutation. Reciprocal sign epistasis is a particular case where the sign of the effect of a mutation depends on the allele present at another locus and reciprocally. Reciprocal sign epistasis is a necessary condition for an adaptive landscape to be rugged [33]. The pervasiveness of epistasis is revealed by studies directly investigating the level of epistasis (references in [38] and [39–43,44•,45•]) as well as by the importance of historical contingency and compensatory evolution in viral evolution [46,47], compensatory evolution being a special case of reciprocal sign epistasis. Additionally, recent studies in plant viruses [43,44•,45•], bacteriophages [39] and human viruses [42] highlighted that sign epistasis, and in particular reciprocal sign epistasis, are more frequent than it was previously thought. This suggests the existence of rugged fitness landscapes in these species and that reciprocal sign epistasis is actually a factor partially limiting their emergence potential.

$G \times E$ and $G \times G$ interactions are thus probably two evolutionary mechanisms limiting viral emergence, but it is more and more clear that they do not capture all the complexity of viral emergence and of the interactions between ecological and evolutionary factors determining its success.

Figure 2

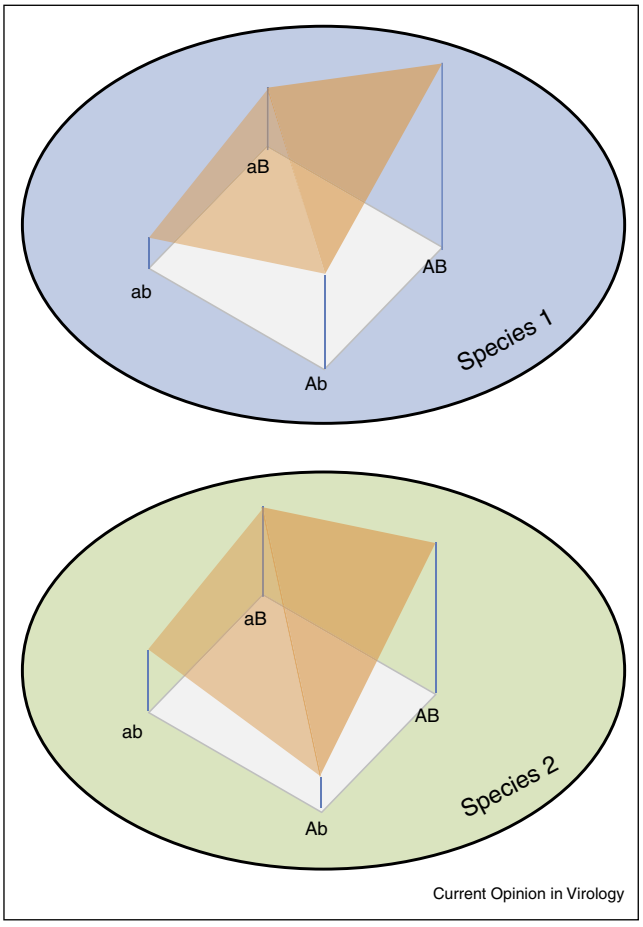


Different types of epistasis between two loci. Two loci define the fitness of a genotype. Small letters indicate the wildtype alleles and capital letters the mutant alleles. **(1)** In the case of no epistasis the fitness of the double mutant *AB* simply results from multiplying the fitness effects of mutations *A* and *B* on the wildtype genetic background (i.e. the fitnesses of genotypes *Ab* and *aB*). **(2)** If magnitude epistasis exists, the fitness of the double mutant *AB* is different from the multiplicative expectation. In the example, the observed fitness of *AB* is larger than expected (positive epistasis). Both in the cases of no epistasis or of magnitude epistasis, the effects of mutations *A* and *B* are unconditionally beneficial. If the effect of one of the mutations is conditionally beneficial (i.e. beneficial in one genetic background but deleterious in another), then we are in the situation of sign epistasis **(3)**. Finally, if both mutations *A* and *B* are deleterious by themselves, but beneficial when combined, we are in the situation of reciprocal sign epistasis **(4)**.

Higher order interactions

A further source of complexity is the triple way interaction corresponding to the combination of the two previous two-way interactions. Concretely, it means that the type and magnitude of epistatis could depend on the host species (Figure 3). Recently, these $G \times G \times E$ interactions have been suggested by one study in HIV-1 [42] and directly demonstrated in a plant virus [48]: average epistasis was positive in the primary host, but on average negative in alternative hosts. Furthermore, the number of non-epistatic interactions was significantly larger in more phylogenetically distant hosts. $G \times G \times E$ interactions are equivalent to the recently introduced notion of epistatic pleiotropy

Figure 3



The type, strength and sign of epistasis among mutations in a viral genome may depend on the host species wherein fitness is evaluated. In this example, mutations *A* and *B* show positive magnitude epistasis in host species 1 but change to sign epistasis in host species 2 due to the deleterious effect of mutation *A* on the genetic background *Ab* when replicating in this host species.

[14••] which allows for the evolution of either specialist or no-cost generalist viruses, depending on the host in which the viral population evolves. Consequently, these third order interactions will increase or decrease the probability of host-range expansion depending on the specific host-switch.

So far, we have considered the host species or genotype as a constant environment. This assumption is not necessarily true because individual hosts can live in different conditions and these conditions might interfere with processes of transmission (e.g. [49]) or within-host multiplication (e.g. [50]) and, as such, affects virus fitness and potentially mutation rates. Integrating such environmental effects on virus emergence is going beyond the ecological considerations that traditionally are used to explain emergence on the basis of changes in the frequency of contacts among different species [51]. In the context of global warming, these effects are likely to be particularly important in poikilothermic species, such as insect vectors. This means that $G \times C$ interactions (C standing for host's environmental conditions) should also be taken into account to obtain a complete picture of factors affecting viral emergence, particularly when exploring its link with global climate change. $G \times C$ interactions could actually be a key determinant of geographic extension of viruses. A recent study suggests the existence of $G \times E \times C$ interactions. The triple interaction was shown in an experimental system with three ranavirus isolates, two frog species raised at two different temperatures [52•]. This triple-way interaction is actually what plant pathologists and epidemiologists have dubbed for long time as McNew's disease triangle [53].

Conclusions

Evidences for across-host fitness trade-offs in viruses are abundant, yet examples of the evolution of generalist viruses paying no cost also exist. It is often assumed that fitness trade-offs restrict the size of host species range and thus prevent the transformation of occasional spillovers into successful epidemics. Here, we have reviewed the genetic mechanisms that may cause such fitness trade-offs. First, antagonistic pleiotropy makes valuable mutations in the reservoir host detrimental in alternative ones. Second epistasis among beneficial mutations, in particular of the type showing reciprocal sign epistasis, makes certain evolutionary pathways inaccessible for the viral population. Third, epistatic pleiotropy makes epistasis dependent on the host species and traps viral populations in evolutionary dead-ends in alternative hosts. And, finally, the last level of complexity that has to be included is the disease triangle, in which the effects of all the above factors will depend upon the host's environment. In conclusion, recent studies reveal that factors impeding and favouring the adaptation of viruses to new hosts are numerous and intimately linked. This means that predicting the emerging potential of viral isolates requires a lot of experimental and environmental data that are not always accessible. In the end, the complexity of the factors determining the emerging potential renders it difficult to predict.

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