Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Virology

Virus population bottlenecks during within-host progression and host-to-host transmission

Serafín Gutiérrez[1,2], Yannis Michalakis[2] and Stéphane Blanc[1]

Despite rapidly growing to immense sizes, virus populations suffer repeated severe bottlenecks, both within hosts and when transmitted from host to host. The potential effect of bottlenecks has been theoretically and experimentally documented, but formal estimations of their actual sizes in natural situations are scarce. Bottlenecks during colonization of organs and during transmission are influenced by those occurring at the cellular level. The study of the multiplicity of cellular infection (MOI) thus appears central, and this trait may be differentially regulated by different virus species. The values of MOI and their putative regulation deserve important future efforts, in order to disentangle the complex interactions between the control of gene copy numbers and the populations dynamics/genetics of viruses.

Addresses
[1] UMR BGPI, INRA-CIRAD-SupAgro, TA-A54K, Campus International de Baillarguet, 34398 Montpellier Cedex 05, France
[2] Unité Mixte de Recherche GEMI 2724, CNRS-IRD, Avenue Agropolis, B.P. 64501, 34394 Montpellier Cedex 05, France

Corresponding author: Blanc, Stéphane (blanc@supagro.inra.fr)

Current Opinion in Virology 2012, **2**:546–555

This review comes from a themed issue on **Virus evolution**

Edited by **Raul Andino** and **Marco Vignuzzi**

For a complete overview see the Issue and the Editorial

Available online 22nd August 2012

1879-6257

http://dx.doi.org/10.1016/j.coviro.2012.08.001

Introduction

Short generation times and high mutation rates allow adaptation of viruses to selective pressures at a speed matched by no other organisms. The remarkable population dynamics makes viruses excellent experimental models for evolution studies, where evolution at work can be monitored in real-time on a lab bench. In the real world, fast and efficient adaptation allows these parasites to overcome natural or man-made barriers such as host defenses, host resistance, or drug treatments, by generating new emerging variants with altered biological properties. Consequently, viruses have successfully invaded all living organisms in all ecosystems, with virus–host interaction ranging from pathogenic to mutualistic [1].

Both fundamental and health/sanitary concerns have fuelled a wealth of research on virus evolution. A most important question that is often addressed is the relative action of two of the main forces driving evolution: deterministic natural selection and random genetic drift. When natural selection is the predominant force shaping the evolution a virus population, adaptation can be fast. On the contrary, adaptation is generally slowed down when genetic drift dominates because the resulting random variation in allele frequencies can distort the direction that would be driven by selection. There are important practical implications from evaluating the selection/drift balance in a particular virus–host interaction. For example, facing man-made barriers such as antiviral drugs or virus-resistant crop varieties, viruses must adapt or disappear. Whether and how fast these barriers will be overcome depends to a large extent on the intensity with which selection acts, as opposed to genetic drift.

Estimating the relative intensity of selection and drift in a given viral population is not a trivial task. It requires determining the size of the viral population replicating in the analyzed system. The population size is highly informative here because selection can act more intensely in large populations, whereas the random effects of genetic drift are more severe in small populations. It must be stressed that the important figure to be measured here is not necessarily the whole population, but only the fraction that multiplies. Since this parameter is obviously rather elusive, the effective size of the population is generally used instead. The effective population size can be defined as the size of an ideal population where stochastic variations in allele frequencies would be the same as those observed in the population under study (for a comprehensive review on this parameter and its estimation see [2]) Although not exactly equivalent to the number of replicating individuals in all circumstances the effective population size is a valuable quantity because (i) it captures the stochasticity involved in the observed changes in allele frequencies, and (ii) it is an experimentally accessible parameter.

The effective size of populations is logically sensitive to fluctuations in the census (total) size, especially to dramatic reductions sometimes experienced by populations, the so-called bottlenecks. A population bottleneck can be defined as a transient reduction of the number of viral genomes within a population and has two major effects. On the one hand, it induces a genetic bottleneck through its stochastic effect on the number of genotypes passed down to the next round of infection. This stochastic effect is inversely proportional to the size of the bottleneck. On the other hand, it determines the

Table 1

Comparison of the quantitative estimates of bottlenecks available in the literature

	Type of bottleneck[a]	Virus	Host	*N*	Ref.	Vector/transmission mode
Bottleneck during transmission	pop	PVY	Pepper	0.5–3.2	[14]	*Myzus persicae*
	pop	CMV	Tomato	1.2–2	[15]	*Aphis gossypii*
	pop	TMV	Tobacco	1.3–3.3	[17]	Leaf contact
	gen	HIV-1	Human	1	[19]	Several[b]
	gen	HCV	Human	1–2	[20]	N.S.[c]

	Type of bottleneck	Virus	Host	*N*	Ref.	Organ/tissue
Bottlenecks during organ colonization	pop	WSMV	Wheat	3–5	[47]	Tiller
	pop	TMV	Tobacco	3.1–5.6	[46]	Leaf
	pop	CaMV	Turnip	298–484	[39]	Leaf
	pop	PVY	Pepper	1–4	[45]	Leaf
	pop	TEV	Tobacco	1.2–47.9	[44]	Leaf
	pop	TEV	Pepper	1.1–5.4	[44]	Leaf
	pop	CaMV	Turnip	8.8–131	[43]	Leaf
	gen	HIV-1	Human	10^3–10^5	[34]	Blood
	gen	HCV	Human	2	[20]	Blood

	Virus	Host	*N*	Ref.
Multiplicity of cellular infection	phi6	Pseudomonas	2–3	See ref in [38]
	HIV-1	Spleen cells	3[d]	See ref in [38]
	HIV-1	Blood cells	1[d]	[42]
	TMV	*Nicotiana benthamiana*	1–6	[63]
	SBWMV	*Chenopodium quinoa*[d]	5–6	[64]
	CaMV	Turnip	2–13	[38]

[a] Pop: population bottleneck, number of viral genome; gen: genetic bottleneck, number of genotypes.
[b] Transmission modes include sexual, mother–child and syringe exchange.
[c] N.S.: not specified.
[d] Average provirus number per cell.

viral 'gene copy number' at the onset of infection, an utterly important aspect in the biology of any organism, that is too often overlooked in virology (further discussed later).

Virus populations are perceived as being extremely large, and this is usually the case for the census population within a host. For example, the size of the virus population in a tobacco leaf infected by *Tobacco mosaic virus* (TMV) has been estimated to approximately 10^{12} genome units [3]. Similar figures have been reported in the plasma of patients infected by *Human Immuno-deficiency virus-1* (HIV-1) during viremia peaks [4,5]. However, virus populations can endure very severe bottlenecks during the infection cycle. To start with, viruses must migrate repeatedly from one host to another, a perilous journey during which only a fistful usually make it from the trillions in the donor host. Once inside the recipient host, the situation does not improve. A large number of limiting factors – like host defenses, intrinsic decay rates of virions [6], limited availability of susceptible cells or of receptors at their surface [7] – reduce the number of individual viral genomes actually contributing to the expansion of the population. Interestingly, viruses themselves restrict their effective population size through what can be considered a territorial behavior. Once a cell becomes infected, the 'resident' virus usually launches molecular mechanisms precluding any new infection from incoming closely related genomes. This process is known as superinfection exclusion and seems largely spread among viruses infecting animals, plants and bacteria [8–12]. Superinfection exclusion thus reduces the effective size of viral populations by limiting the number of genomes that can actually enter and replicate in individual susceptible cells.

Population size can thus greatly drop at various steps of the viral cycle, and the impact of such bottlenecks on virus evolution has been addressed in many theoretical studies, plus several experimental demonstrations of an associated decrease of the viral fitness (reviewed in [13]). Quantitative data on bottleneck sizes from natural situations, however, remain surprisingly scarce (Table 1). In this review, we present an overview of the information available. Despite being largely fragmentary, a blurred picture starts to emerge where, as it could be expected from the virus diversity, drastically different population dynamics seem to be associated to diverging virus life styles.

Getting in: Are severe bottlenecks the rule during host-to-host transmissions?

The first quantitative estimate of a population bottleneck during transmission was obtained for an insect-borne plant virus, the *Potato virus Y* (PVY), transmitted in a

non-circulative way by aphids [14•]. In this transmission mode, the virus does not replicate within the insect vector. It simply reversibly interacts with putative receptors in the mouthparts (stylets), where it can be retained infectious for only a few minutes [3]. Moury and colleagues used a simple and elegant method to determine the size of the population sample that is actually taken up and transmitted by aphids. First, they fed aphids on virus suspensions containing a mixture of virus particles from two different variants, one infectious in a given variety of pepper and the other one not. Aphids fed on virus suspensions with different relative concentrations of the two variants had different transmission success rates on these pepper plants: the higher the concentration of the non-infectious variant, the lower the success rate. From these data, a stochastic model was then used to estimate that each individual aphid could efficiently transmit an average of 0.5–3.2 viral genomes. In a comparable approach, similar figures were calculated for *Cucumber mosaic virus* (CMV), another plant virus also transmitted in a non-circulative way by aphids [15]. An important difference of the latter study, is that aphids did not acquire the virus from homogeneous artificial mixtures, but from leaves co-infected by the two CMV variants. Because CMV variants have been shown to spatially segregate in co-infected plants, a large fraction of leaf cells are generally infected by a single variant [16]. Aphids usually acquire CMV after feeding from one or a few cells. They may thus only access to the variant infecting the sampled cells, indeed generating a very strong genetic bottleneck reminiscent of what happens in natural situations, although it may or may not exactly correspond to the actual number of genomes transmitted.

Population bottlenecks during contact transmission have also been quantitatively analyzed in TMV, with a similar approach [17]. Surprisingly, though the transmission mechanism is totally different, the number of viral genomes initiating infection in the recipient host plants has been calculated to also lie between 1 and 4.

In animal viruses, the genetic bottleneck during HIV-1 transmission has been quantitatively examined using an approach initially developed by Keele *et al.* [18•]. In this approach, a model of random viral evolution is implemented with the phylogeny data obtained from deep-sequencing the virus population early in infection. All following studies consistently suggest that a single HIV genotype is usually at the origin of the virus population within the patient (reviewed in [19]). Such an extreme genetic bottleneck has been evidenced in 70–90% of the cases of mother to child transmission, in 60–90% of both heterosexual and homosexual transmissions, and even in 40–70% of transmissions through syringe exchanges. A similar approach has also been used to estimate the founder population in four individuals infected with *Hepatitis C virus* (HCV), another blood-borne virus, with very similar results of 1 or 2 founder genotypes per infected host [20].

To the best of our knowledge, these are the only formal quantitative estimates of population/genetic bottlenecks during viral transmission, currently available in the literature. One could conclude indeed to a very limited exploration of this question among the described viral diversity. Nevertheless, though they do not represent actual quantifications, additional studies have detected drops in genetic diversity upon transmission of several viral species, in diverse genera and families (e.g. [21–23]). Despite this consistent trend, narrow bottlenecks might not always be the inevitable outcome of virus transmission. Aaskov *et al.* have shown that defective genotypes of Dengue virus are transmitted among humans and mosquitoes in nature [24]. Two phenomena acting together can explain this observation: complementation of defective genotypes by functional ones, and relaxed transmission bottlenecks allowing the presence of both types in the recipient hosts. Likewise, a recent study analyzing changes in population diversity of the Equine influenza virus between donor and recipient horses also suggests an absence of a severe transmission-associated bottleneck [25•]. Finally, it has been logically postulated that when the number of transmission events per recipient host is large, a large viral population can be transmitted whatever the bottleneck associated to single events. Though it has never been directly investigated, this has particularly been suggested for host plants visited by large numbers of insect vectors [26].

In any case, there is a clear need for more quantitative data on bottlenecks during natural viral transmission. As it stands, the current literature on the subject is not diversified enough to allow any inference of general trends which could reliably relate a specific mode of natural transmission to severe or to relaxed transmission bottlenecks.

Beyond the average size of a bottleneck, the distribution of these sizes among transmission events should also be documented further. It must be stressed that even a small number of multiply infected hosts could matter most in virus evolution. Multiply infected hosts are the melting pot where genetic exchange can take place between genotypes, a phenomenon that can radically change virus properties (e.g. host range expansion) with important implications, even if rare, in ecology and epidemiology. Moreover, the detailed analysis of distribution of the bottleneck sizes during transmission could also reveal unknown aspects of the transmission mechanisms. For example, a bimodal distribution of the transmission-associated bottleneck sizes might reveal two different transmission routes for a given virus, as suggested by infection of cells with HIV [27].

Finally, we believe it is of prime importance to keep in mind that, although approaches described above on HIV-1, HCV and CMV are reliably quantifying genetic bottlenecks during transmission [15,18•,20], they might be partly blind to the actual number of transmitted viral genomes, as opposed to transmitted genotypes. This is an important point which deserves to be specifically addressed in the future because, as further discussed below, this number matters much more than usually considered. It sets the (viral) gene copy number at initial phases of the host infection. Both theoretical and experimental investigations have revealed the importance of the number of copy of a gene inside a cell [28,29,30••,31]. Even in very simple gene networks with diverse putative reciprocal regulations, slight changes in gene copy numbers can dramatically modify their relative expression and so the phenotypic outcome of the network. This phenomenon applies to viral genes and can change the fate of the infected cell, and thus of the viral cycle [28–31] (see below the Section on MOI for further discussion).

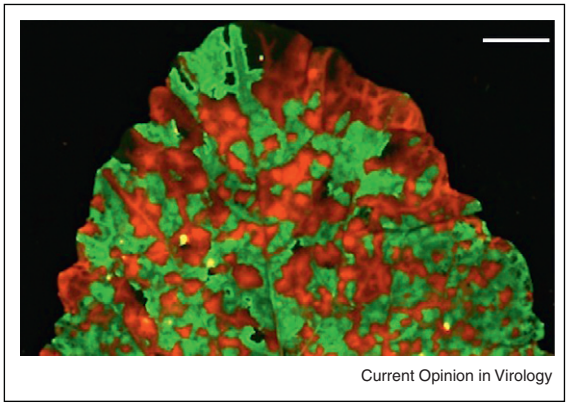
How host colonization shapes population structure and evolution

As briefly mentioned above, the genetic bottleneck associated to transmission may largely depend on the structure of the virus population in the donor host. If this population is genetically heterogeneous, transmission bottlenecks could be determined partly by the genetic diversity available in the host compartment where the transmitted population is sampled. The extent of compartmentalization of viral populations is a question that requires detailed analysis at various steps of host colonization. These analyses inform on dynamics of disease progression, on within-host evolution processes, and ultimately on the history and composition of the subpopulation available for transmission.

Highly structured viral populations within individual hosts

After primary infection of a host, viruses replicate to large numbers, numerous mutants appear and, in many cases, recombination shuffles mutations and generates new genotypes. This diversity is most often loaded into the vascular system and transported away before entering new cells and replicating further. Each cell type and/or organ can be considered as a different host compartment that viruses infect with varying success, and where the developing viral subpopulations are more or less isolated and may diverge from the initial source population [22,23,32–34] (Figure 1). Empirical evidence of population compartmentalization in different virus models has transformed the previous panmictic view of within-host populations into a metapopulation view [35,36]. Nevertheless, recent reports in both animals and plants have mitigated the situation and shown that extremely different degrees of compartmentalization may exist in different virus species [37–39].

Figure 1

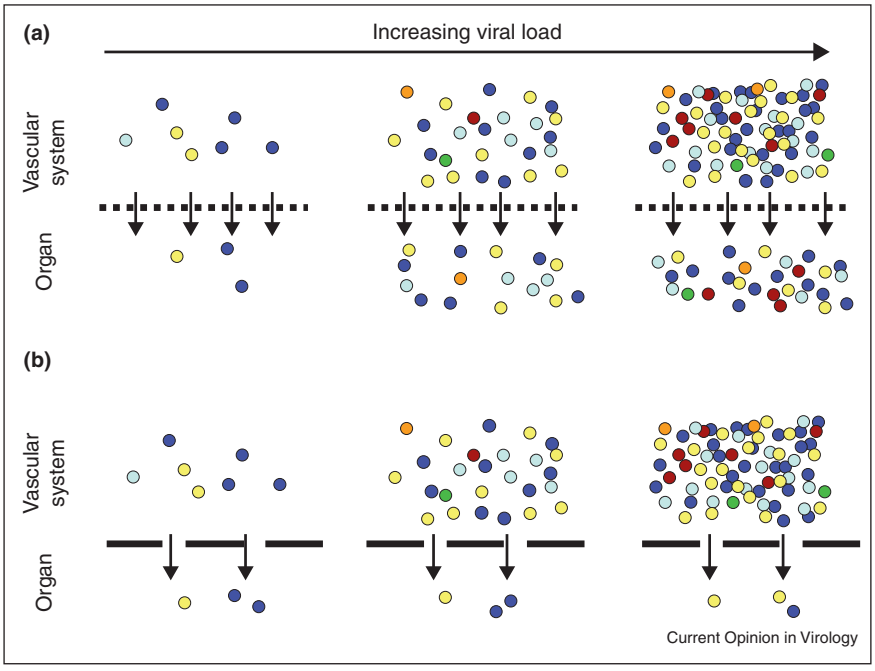


Compartmentalization of Turnip mosaic virus (TuMV) variants within a systemically infected leaf.
Each TuMV variant encodes and thus produces a different fluorescent protein (mGFP5 in green and mRFP1 in red). Compartmentalization is particularly easy to visualize here, as well as in many other plant viruses, owing to the possibility to express different fluorescent proteins via two otherwise identical clones, co-inoculated into the same host plant. Each clone can be observed to separately infect leaf cells, yielding a patchwork of infected regions with a single fluorescence. Bar = 0.5 cm.

Two initial parameters define the genetics of the population that invades a given compartment: the genetic diversity available in the source population and the size of the bottleneck during colonization. The population colonizing a new organ or tissue often derives from that in the vascular system. Unfortunately, the viral genetic diversity flowing in sap or plasma is not easy to characterize because it can amply vary along time. Spectacular changes of viral genetics in the vasculature are the recurring selective sweeps resulting from the attack of the host immune system on a virus serotype, which is then rapidly replaced by a new one [40]. The relationships between the viral dynamics within the vasculature and the viral migration within and in between the various host compartments remains to be investigated, although some recent studies start to tackle the question [41••,42•]. This paucity of data is particularly dramatic in the case of plant viruses, probably because simple methods for sampling the vascular system have long been missing [43•].

The extent to which the viral population passes from the vasculature to a given compartment depends on the mechanisms of entry. Two contrasted scenarios can be envisioned (Figure 2): (i) organs are highly permeable to virus infection and population bottlenecks depend only on the viral load (the viral dose or titer) in the vasculature, or (ii) limiting barriers lead to severe bottlenecks whatever the viral load in sap or serum. Deciding which of these scenarios most often takes place requires comparative data estimating in parallel the population bottlenecks during organ colonization and the viral load in the vasculature irrigating this organ. The only two examples available are

Figure 2



Two opposite scenarios during virus colonization of organs from the vasculature.
(A) The size of the population that invades the organ depends on the concentration of virus infectious units (coloured circles) in the vascular system. In this case, no limiting barriers exist and the virus can freely move from one compartment to the other.
(B) The size of the population invading the organ is constantly low, whatever the viral load in the vasculature, owing to barriers imposed by the host (e.g. limiting number of susceptible cells, limiting number of receptors, etc.), or by the virus itself (e.g. mechanisms inhibiting surperinfection).

dealing with plant virus models [43•,44•]. Both studies indicate that bottlenecks reported during leaf colonization by various plant viruses [39,45–47] could be in fact largely driven by the viral load in the sap. Zwart and colleagues have quantified the bottleneck sizes in populations of *Tobacco etch virus* (TEV) invading the first systemically infected leaf of tobacco hosts. Plants were initially inoculated with different viral doses, inducing different numbers of infection foci on the inoculated leaves (dose ranged from 1 to ~50 foci per plant). These numbers positively correlated with the size of the bottlenecks during ulterior systemic colonization of leaves, suggesting that the bottlenecks were in this case mainly determined by the viral load, though not directly demonstrated. With another virus species, the *Cauliflower mosaic virus* (CaMV), we have analyzed in parallel the bottlenecks at the entry of leaves successively appearing on the infected host plants and the virus titer in the sap flowing into these leaves [43•]. Our results show that successive leaves are colonized by increasing population sizes, ranging from units to hundreds of genomes, and that this colonizing population size directly depends on the virus load in the sap. In this situation, the most drastic bottleneck suffered by viruses during their life cycle might be the transmission bottlenecks. Indeed, these two studies together indicate that the limiting factor for the growth of the viral population is the viral load within the sap [43•], which is probably influenced by the initial inoculum [44•].

That physical barriers sometimes impose bottlenecks, and thus favor compartmentalization of the virus population, even when the viral dose available in the sap or blood is not limiting makes little doubt. For example, still in plant viruses, Li and Roossinck [23] observed a continuous loss of genetic markers in a population of CMV colonizing successive leaves of tobacco host plants, suggesting the persistence of strong bottlenecks all along the infection. Similarly, in animal viruses, Kuss *et al.* [48] monitored the genetics of an artificial population of poliovirus during the colonization of immuno-suppressed mice expressing a poliovirus human receptor. They also observed a constant decrease in the genetic diversity during virus progression, thus postulating that physical barriers imposed bottlenecks at several stages of this process.

Overall, because hints in favor of one or the other scenario can be found both in animals [37,42•] and plant viruses [39], it would not be surprising that a whole range of different situations exists in nature. Complex interactions between the viral rush for host invasion, the onset of host defenses, the availability of susceptible cells and the

inhibition of superinfection might have outcomes alternating between strong and relaxed virus population bottlenecks at various stages of the infection. It is also possible that 'going alone' or 'going together' might represent adaptive viral strategies. More explicitly, virus species with many gene products acting (or complementing) in *trans*, and/or with a high recombination rate, may gain benefits from relaxed bottlenecks whereas other species poorly complementing or recombining may only get the costs of competition. The question of whether such adaptive strategies exist is highly challenging, and will require increased efforts in characterizing the dynamics of within-host colonization for a wide and diverse panel of virus species.

Getting out: the transmissible population

After infection of a compartment, the colonizing population can further evolve differentially, owing to compartment specific conditions, to isolation, or conversely through migration of additional virus genomes (reviewed in [34] for HIV-1). Compartments of particular interest are those from where the transmission to the next host will actually occur. The population contained in such compartments is hereafter called the 'transmissible population'. It is obvious that these compartments differ among virus–host models, but whether they do contain subpopulations specifically adapted to transmission is only a nascent question, despite numerous occurrences of such a phenomenon in other non-viral parasites [49,50]. The advent of high-throughput sequencing technologies has facilitated analysis of viral population genetics, and these tools are currently used to decipher the genetic structure of transmissible-populations for different viruses: HIV-1 in the genital tract and in the blood [41••,42•,51], HCV in the blood [20], rhinoviruses [52] or equine influenza virus [25•] in nasal swabs, Foot-and-mouth disease virus in hooves [53] or West Nile virus in salivary glands of mosquitoes [37] (note that there is no comparable example published from plant viruses thus far). Unfortunately, for obvious practical reasons, most studies cited above have focused on a limited number of infected individuals. The consequence is that, for a given virus, no common feature among transmissible populations in different individual hosts could be detected, although the transmissible populations proved to be distinct from those in other compartments.

All the above-cited reports suggest that transmissible populations contain a relatively elevated genetic diversity, indicating that dramatic genetic bottlenecks often observed after transmission are not owing to homogeneous transmissible populations. One would expect then that the most frequent genotypes in the transmissible population would be most often transmitted. One study [41••], however, seems to uncover an intriguing process. In an analysis comparing the HIV-1 transmissible populations in the genital tract of donors and the actually transmitted populations in recipients, it was clearly demonstrated that the frequent genotypes are not the ones transmitted. This rather unexpected result suggests two potential scenarios: (i) either numerous genotypes are transferred to the recipient and only specific ones (present at low frequencies) are able to initiate infection, or (ii) specific genotypes (though present at low frequencies) are the only ones transferred. Interestingly, a series of papers have shown that founder HIV-1 virus genotypes have distinct features like fewer glycosylated sites in the envelope proteins [54,55,56•,57]. These observations point to the existence of viral 'morphs' specifically adapted to transmission in HIV-1 populations. Transmission morphs have been described for baculoviruses, a viral family infecting insects. Baculoviruses have a life cycle involving two types of virions with specific envelopes, one dedicated to within-host colonization and the other essential for transmission. The generality of the existence of viral transmission morphs, whether they only appear or localize in specific host compartments, and whether their specificity has both a phenotypic and/or a genetic determinism, represent particularly appealing future prospects.

The multiplicity of cellular infection plays a seminal role in bottlenecks

We have thus far reviewed bottlenecks during transmission and during organ colonization. However, perhaps the most important level in viral population dynamics is that of individual cells [34]. The population bottleneck during cell infection, that is the number of viral genomes entering and replicating within a cell, is here called the multiplicity of cellular infection (MOI). The impact of MOI on population bottlenecks at higher organization levels can be straightforward or more subtle. When colonizing new organs, the bottleneck results from the addition of the MOI in the initially infected cells. During transmission, genetic bottlenecks depend on both the MOI in donor cell compartments and that in recipient susceptible cells. Through its ruling of the number of genomes entering cells, this key parameter also impacts on the interactions between virus genotypes [58–61], and on their phenotype when sensitive to the gene copy number [29,31,62••].

For viruses, the cell is the main arena where genomes of a given population can meet and interact. These interactions include recombination, competition and complementation, all major phenomena in the evolution and epidemiology of viruses, which can take place or not depending on MOI values. For example, for a MOI of one, recombination will be precluded, complementation of defective interfering particles largely alleviated, while competition for cellular resources will be relaxed. These are commonly discussed implications of MOI in recent available publications [38•,42•,63,64•].

The MOI also defines the gene copy number and, though this parameter can greatly influence the virus biology, it is most often overlooked in the MOI-related literature [30••]. A spectacular phenotype sensitive to gene copy number variation (CNV) has been described for lysogenic bacterial viruses [29,31,62••]. If the MOI is one (gene copy number is one), the phage multiplies and kills the host. If the MOI is greater than one, the phages tend to integrate into the host genome leaving the cell alive. Thus, a change in the MOI can change the fate of the infected cell and define whether transmission is vertical or horizontal. Such dramatic alteration of viral behavior resulting from potentially small changes in the MOI (or, more generally, in gene copy numbers), have been theoretically studied and predicted to be very common [30••]. The important point here is that phenotype switches triggered by CNV do occur even if the co-infecting genomes are strictly identical. Hence, the total number of genomes passing through a bottleneck (the population bottleneck) is the one that is critical here, not the number of distinct genotypes (the genetic bottleneck). CNV-regulated phenotypes have been shown in organisms as different as phages [29,31,62••], bacteria [65,66] and mammals [67,68], so they might have been largely underestimated in the biology of other viruses. The study of the MOI during virus life cycles has even broader perspectives when viewed under this new light.

The values of the viral MOI in nature remain unknown, probably owing to the technical challenge of their estimation. Currently, formal estimations have been reported for a phage, an insect virus, three plant viruses and HIV-1 (see references in [38•] and Table 1). Figures altogether range between 3 and 13 genomes per cell. With such little information, it is impossible to predict what MOI values can be in the diversity of the 'virosphere'. Nevertheless, we believe that the inherent trade-offs associated to the MOI imply its tight regulation. Thus, the MOI values determined might be considered adaptive traits. This hypothesis predicts that different virus species would have evolved a high or a low MOI strategy depending on their biological properties, as proposed above for severe or relaxed bottlenecks. Since the MOI is directly controlled by viral mechanisms of superinfection exclusion (see Introduction), controlling the MOI could be a mean for viruses to implement its above-mentioned strategy of 'going alone' (low MOI) or 'going together' (high MOI).

Some data from plant viruses are consistent with this hypothesis. Numerous reports of spatial segregation suggest that low-MOI scenarios do exist (reviewed in [12]). Indeed, to our view, the spatial segregation shown in Figure 1 can only be explained by a MOI close to one when the virus exits the vasculature (sieve tubes) and enters the first leaf companion cells, inducing the observed individualization of the two fluorescent clones. The subsequent invasion of neighboring cells in the mesophyl creates leaf territories occupied by specific clones, the overlap between territories being probably prevented by inhibition of superinfection. Because spatial segregation is observed all along infection of the host plant (our own observation on TuMV) a MOI close to one seems to be constantly maintained at least in the companion cells through which the virus exits the sap. We have observed a very different scenario during CaMV infection. In plants co-inoculated with two distinct CaMV variants, the proportion of observed co-infected cells rapidly reached 100% in systemically infected leaves [38•]. This is clearly precluding any possibility of spatial segregation as observed with TuMV (Figure 1). In fact, we have demonstrated that CaMV colonizes cells in groups and that group size (i.e. the MOI) is variable and depends on the viral load in the vasculature [38•,43•].

From these simple observations with TuMV and CaMV infecting the same host species, we can suggest that some viruses colonize cells in groups whereas others go lonely. If one dares to respectively assimilate these two scenarios to contrasted social behaviors, then the rationale for adapting such a distinct way of life in the two species is at this point elusive. Additional exhaustive comparisons between species with different genome structure, replication, and gene expression strategies will be required in order to detect any correlation between a particular viral feature and one or the other type of population dynamics. In addition, it is very likely that a range of intermediate situations exists between the two opposite scenarios sketched here, and this will certainly augment the difficulty in deciphering the parameters driving viruses towards one or the other.

Conclusions

Calling for more quantitative data on bottleneck sizes in different virus models, we now possibly surmise that different viral species might cope differentially with bottlenecks. If our speculation is sound, some viral species do maintain narrow bottlenecks, at least at some specific steps of their cycle, while others appear to maintain them as wide as possible. The enigma of the mechanisms, and the respective benefits and costs, that are hidden behind these different viral strategies represents a very stimulating direction for forthcoming research in this field.

We would like to conclude by highlighting the need for increased consideration of the gene copy number variations in viruses, with regards to bottlenecks and particularly to MOI. As theoretically predicted [30••], and demonstrated experimentally in phages [29,31,62••], the biological properties of a virus can totally change depending on the initial number of genomes within a cell. This uncovers the possibility of complex regulations in the viral cycle through changing MOI in time or in

different host tissues. If so, CNV would certainly be an important unforeseen player in the trade-offs involved in MOI regulation, and more generally in viral population dynamics/genetics.

Acknowledgements

SB and SG acknowledge support from INRA department SPE and from the grant No. 2010-BLAN-170401 from the French ANR. YM acknowledges support form the CNRS and IRD.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Roossinck MJ: **The good viruses: viral mutualistic symbioses**. *Nature Reviews Microbiology* 2011, **9**:99-108.
2. Charlesworth B: **Fundamental concepts in genetics: effective population size and patterns of molecular evolution and variation**. *Nature Reviews Genetics* 2009, **10**:195-205.
3. Hull R: *Matthews' Plant Virology*. edn 4. Elsevier; 2002.
4. Leigh-Brown AJ: **Analysis of HIV-1 env gene sequences reveals evidence for a low effective number in the viral population**. *Proceedings of the National Academy of Sciences of the United States of America* 1997, **94**:1862-1865.
5. Rouzine IM, Coffin JM: **Linkage disequilibrium test implies a large effective population number for HIV in vivo**. *Proceedings of the National Academy of Sciences of the United States of America* 1999, **96**:10758-10763.
6. Ramratnam B, Bonhoeffer S, Binley J, Hurley A, Zhang L, Mittler JE, Markowitz M, Moore JP, Perelson AS, Ho DD: **Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis**. *Lancet* 1999, **354**:1782-1785.
7. Smith DR, Adams AP, Kenney JL, Wang E, Weaver SC: **Venezuelan equine encephalitis virus in the mosquito vector Aedes taeniorhynchus: infection initiated by a small number of susceptible epithelial cells and a population bottleneck**. *Virology* 2008, **372**:176-186.
8. Huang IC, Li W, Sui J, Marasco W, Choe H, Farzan M: **Influenza A virus neuraminidase limits viral superinfection**. *Journal of Virology* 2008, **82**:4834-4843.
9. Lee YM, Tscherne DM, Yun SI, Frolov I, Rice CM: **Dual mechanisms of pestiviral superinfection exclusion at entry and RNA replication**. *Journal of Virology* 2005, **79**:3231-3242.
10. Lu MJ, Henning U: **Superinfection exclusion by T-even-type coliphages**. *Trends in Microbiology* 1994, **2**:137-139.
11. Nethe M, Berkhout B, van der Kuyl AC: **Retroviral superinfection resistance**. *Retrovirology* 2005, **2**:52.
12. Ziebell H, Carr JP: **Cross-protection: a century of mystery**. *Advances in Virus Research* 2010, **76**:211-264.
13. Elena SF, Agudelo-Romero P, Carrasco P, Codoner FM, Martin S, Torres-Barcelo C, Sanjuan R: **Experimental evolution of plant RNA viruses**. *Heredity* 2008, **100**:478-483.
14. • Moury B, Fabre F, Senoussi R: **Estimation of the number of virus particles transmitted by an insect vector**. *Proceedings of the National Academy of Sciences of the United States of America* 2007, **104**:17891-17896.
This paper describes the first quantitative estimation of a population bottleneck during transmission.
15. Betancourt M, Fereres A, Fraile A, Garcia-Arenal F: **Estimation of the effective number of founders that initiate an infection after aphid transmission of a multipartite plant virus**. *Journal of Virology* 2008, **82**:12416-12421.
16. Takeshita M, Shigemune N, Kikuhara K, Furuya N, Takanami Y: **Spatial analysis for exclusive interactions between subgroups I and II of Cucumber mosaic virus in cowpea**. *Virology* 2004, **328**:45-51.
17. Sacristan S, Diaz M, Fraile A, Garcia-Arenal F: **Contact transmission of Tobacco mosaic virus: a quantitative analysis of parameters relevant for virus evolution**. *Journal of Virology* 2011, **85**:4974-4981.
18. • Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, Sun C, Grayson T, Wang S, Li H *et al.*: **Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection**. *Proceedings of the National Academy of Sciences of the United States of America* 2008, **105**:7552-7557.
Develoment of a phylogenetic approach to infer the number of HIV genotypes initiating infection in single hosts.
19. Gijsbers EF, Schuitemaker H, Kootstra NA: **HIV-1 transmission and viral adaptation to the host**. *Future Virology* 2012, **7**:63-71.
20. Bull RA, Luciani F, McElroy K, Gaudieri S, Pham ST, Chopra A, Cameron B, Maher L, Dore GJ, White PA *et al.*: **Sequential bottlenecks drive viral evolution in early acute hepatitis C virus infection**. *PLoS Pathogens* 2011, **7**:e1002243.
21. Bull RA, Eden JS, Luciani F, McElroy K, Rawlinson WD, White PA: **Contribution of intra- and interhost dynamics to norovirus evolution**. *Journal of Virology* 2012, **86**:3219-3229.
22. Jridi C, Martin JF, Marie-Jeanne V, Labonne G, Blanc S: **Distinct viral populations differentiate and evolve independently in a single perennial host plant**. *Journal of Virology* 2006, **80**:2349-2357.
23. Li H, Roossinck MJ: **Genetic bottlenecks reduce population variation in an experimental RNA virus population**. *Journal of Virology* 2004, **78**:10582-10587.
24. Aaskov J, Buzacott K, Thu HM, Lowry K, Holmes EC: **Long-term transmission of defective RNA viruses in humans and Aedes mosquitoes**. *Science* 2006, **311**:236-238.
25. • Murcia PR, Baillie GJ, Daly J, Elton D, Jervis C, Mumford JA, Newton R, Parrish CR, Hoelzer K, Dougan G *et al.*: **Intra- and interhost evolutionary dynamics of equine influenza virus**. *Journal of Virology* 2010, **84**:6943-6954.
By analyzing natural transmission chains, the authors report an absence of genetic transmission-bottleneck in an air-borne virus, as opposed to strong bottlenecks in other systems.
26. Escriu F, Fraile A, Garcia-Arenal F: **The evolution of virulence in a plant virus**. *Evolution* 2003, **57**:755-765.
27. Dang Q, Chen J, Unutmaz D, Coffin JM, Pathak VK, Powell D, KewalRamani VN, Maldarelli F, Hu WS: **Nonrandom HIV-1 infection and double infection via direct and cell-mediated pathways**. *Proceedings of the National Academy of Sciences of the United States of America* 2004, **101**:632-637.
28. Schrider DR, Hahn MW: **Gene copy-number polymorphism in nature**. *Proceedings of the Royal Society B: Biological Sciences* 2010, **277**:3213-3221.
29. Weitz JS, Mileyko Y, Joh RI, Voit EO: **Collective decision making in bacterial viruses**. *Biophysical Journal* 2008, **95**:2673-2680.
30. •• Mileyko Y, Joh RI, Weitz JS: **Small-scale copy number variation and large-scale changes in gene expression**. *Proceedings of the National Academy of Sciences of the United States of America* 2008, **105**:16659-16664.
Theoretical analysis where the authors show that small changes in the gene copy number (e.g. the MOI for viruses) can have important effects on the outcome of gene expression networks and thus on phenotypes.
31. Kourilsky P, Knapp A: **Lysogenization by bacteriophage lambda. III. Multiplicity dependent phenomena occuring upon infection by lambda**. *Biochimie* 1974, **56**:1517-1523.
32. Cabot B, Martell M, Esteban JI, Sauleda S, Otero T, Esteban R, Guardia J, Gomez J: **Nucleotide and amino acid complexity of hepatitis C virus quasispecies in serum and liver**. *Journal of Virology* 2000, **74**:805-811.

33. Okamoto H, Nishizawa T, Takahashi M, Asabe S, Tsuda F, Yoshikawa A: **Heterogeneous distribution of TT virus of distinct genotypes in multiple tissues from infected humans**. *Virology* 2001, **288**:358-368.

34. Pybus OG, Rambaut A: **Evolutionary analysis of the dynamics of viral infectious disease**. *Nature Reviews Genetics* 2009, **10**:540-550.

35. Frost SD, Dumaurier MJ, Wain-Hobson S, Brown AJ: **Genetic drift and within-host metapopulation dynamics of HIV-1 infection**. *Proceedings of the National Academy of Sciences of the United States of America* 2001, **98**:6975-6980.

36. Levins R: **Some demographic and genetic consequences of environmental heterogeneity for biological control**. *Bulletin of the Entomological Society of America* 1969, **15**:237-240.

37. Brackney DE, Pesko KN, Brown IK, Deardorff ER, Kawatachi J, Ebel GD: **West Nile Virus genetic diversity is maintained during transmission by culex pipiens quinquefasciatus mosquitoes**. *PLoS ONE* 2011, **6**:e24466.

38. • Gutierrez S, Yvon M, Thebaud G, Monsion B, Michalakis Y, Blanc S: **Dynamics of the multiplicity of cellular infection in a plant virus**. *PLoS Pathogens* 2010, **6**:e1001113.
Monitoring the viral MOI along the whole within-host infection cycle shows important changes in different organs and suggests that these changes are linked to the dynamics of the viral load in the vasculature.

39. Monsion B, Froissart R, Michalakis Y, Blanc S: **Large bottleneck size in Cauliflower Mosaic Virus populations during host plant colonization**. *PLoS Pathogens* 2008, **4**:e1000174.

40. Lim SG, Cheng Y, Guindon S, Seet BL, Lee LY, Hu P, Wasser S, Peter FJ, Tan T, Goode M *et al.*: **Viral quasi-species evolution during hepatitis Be antigen seroconversion**. *Gastroenterology* 2007, **133**:951-958.

41. •• Boeras DI, Hraber PT, Hurlston M, Evans-Strickfaden T, Bhattacharya T, Giorgi EE, Mulenga J, Karita E, Korber BT, Allen S *et al.*: **Role of donor genital tract HIV-1 diversity in the transmission bottleneck**. *Proceedings of the National Academy of Sciences of the United States of America* 2011, **108**:E1156-E1163.
The analysis of eight heterosexual transmission chains suggests the existence of HIV-1 genotypes specialized for transmission which are not necessarily the most frequent in the donor host.

42. • Josefsson L, King MS, Makitalo B, Brannstrom J, Shao W, Maldarelli F, Kearney MF, Hu WS, Chen J, Gaines H *et al.*: **Majority of CD4+ T cells from peripheral blood of HIV-1-infected individuals contain only one HIV DNA molecule**. *Proceedings of the National Academy of Sciences of the United States of America* 2011, **108**:11199-11204.
This study demonstrates a MOI of 1 in some cell types of patients infected by HIV-1 (CD4+), as opposed to a reported MOI of 3 in spleen cells.

43. • Gutierrez S, Yvon M, Pirolles E, Garzo E, Fereres A, Michalakis Y, Blanc S: **Circulating virus load determines the size of bottlenecks in viral populations progressing within a host**. *PLoS Pathogens* 2012, in press.
This study demonstrates a direct link between the dynamics of the viral load in the vascular system of a host and the size of the viral populations colonizing organs.

44. • Zwart MP, Daros JA, Elena SF: **One is enough: in vivo effective population size is dose-dependent for a plant RNA virus**. *PLoS Pathogens* 2011, **7**:e1002122.
First demonstration of a dose effect on population bottlenecks during systemic infection of plants.

45. Fabre F, Montarry J, Coville J, Senoussi R, Simon V, Moury B: **Modelling the evolutionary dynamics of viruses within their hosts: a case study using high-throughput sequencing**. *PLoS Pathogens* 2012, **8**:e1002654.

46. Sacristan S, Malpica JM, Fraile A, Garcia-Arenal F: **Estimation of population bottlenecks during systemic movement of tobacco mosaic virus in tobacco plants**. *Journal of Virology* 2003, **77**:9906-9911.

47. French R, Stenger DC: **Evolution of Wheat streak mosaic virus: dynamics of population growth within plants may explain limited variation**. *Annual Review of Phytopathology* 2003, **41**:199-214.

48. Kuss SK, Etheredge CA, Pfeiffer JK: **Multiple host barriers restrict poliovirus trafficking in mice**. *PLoS Pathogens* 2008, **4**:e1000082.

49. Blanc S, Uzest M, Drucker M: **New research horizons in vector-transmission of plant viruses**. *Current Opinion in Microbiology* 2011, **14**:483-491.

50. Matthews KR: **Controlling and coordinating development in vector-transmitted parasites**. *Science* 2011, **331**:1149-1153.

51. Kemal KS, Foley B, Burger H, Anastos K, Minkoff H, Kitchen C, Philpott SM, Gao W, Robison E, Holman S *et al.*: **HIV-1 in genital tract and plasma of women: compartmentalization of viral sequences, coreceptor usage, and glycosylation**. *Proceedings of the National Academy of Sciences of the United States of America* 2003, **100**:12972-12977.

52. Tapparel C, Cordey S, Junier T, Farinelli L, Van Belle S, Soccal PM, Aubert JD, Zdobnov E, Kaiser L: **Rhinovirus genome variation during chronic upper and lower respiratory tract infections**. *PLoS ONE* 2011, **6**:e21163.

53. Wright CF, Morelli MJ, Thebaud G, Knowles NJ, Herzyk P, Paton DJ, Haydon DT, King DP: **Beyond the consensus: dissecting within-host viral population diversity of foot-and-mouth disease virus by using next-generation genome sequencing**. *Journal of Virology* 2011, **85**:2266-2275.

54. Chohan B, Lang D, Sagar M, Korber B, Lavreys L, Richardson B, Overbaugh J: **Selection for human immunodeficiency virus type 1 envelope glycosylation variants with shorter V1–V2 loop sequences occurs during transmission of certain genetic subtypes and may impact viral RNA levels**. *Journal of Virology* 2005, **79**:6528-6531.

55. Derdeyn CA, Decker JM, Bibollet-Ruche F, Mokili JL, Muldoon M, Denham SA, Heil ML, Kasolo F, Musonda R, Hahn BH *et al.*: **Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission**. *Science* 2004, **303**:2019-2022.

56. • Go EP, Hewawasam G, Liao HX, Chen H, Ping LH, Anderson JA, Hua DC, Haynes BF, Desaire H: **Characterization of glycosylation profiles of HIV-1 transmitted/founder envelopes by mass spectrometry**. *Journal of Virology* 2011, **85**:8270-8284.
Proteomic approach showing that the glycosylation patterns in transmitted genotypes initiating acute infection are different from those of genotypes during chronic HIV-1 infection.

57. Liu Y, Curlin ME, Diem K, Zhao H, Ghosh AK, Zhu H, Woodward AS, Maenza J, Stevens CE, Stekler J *et al.*: **Env length and N-linked glycosylation following transmission of human immunodeficiency virus Type 1 subtype B viruses**. *Virology* 2008, **374**:229-233.

58. Cicin-Sain L, Podlech J, Messerle M, Reddehase MJ, Koszinowski UH: **Frequent coinfection of cells explains functional in vivo complementation between cytomegalovirus variants in the multiply infected host**. *Journal of Virology* 2005, **79**:9492-9502.

59. Froissart R, Roze D, Uzest M, Galibert L, Blanc S, Michalakis Y: **Recombination every day: abundant recombination in a virus during a single multi-cellular host infection**. *PLoS Biology* 2005, **3**:e89.

60. Levy DN, Aldrovandi GM, Kutsch O, Shaw GM: **Dynamics of HIV-1 recombination in its natural target cells**. *Proceedings of the National Academy of Sciences of the United States of America* 2004, **101**:4204-4209.

61. Lopez-Ferber M, Simon O, Williams T, Caballero P: **Defective or effective? Mutualistic interactions between virus genotypes**. *Proceedings of the Royal Society B: Biological Sciences* 2003, **270**:2249-2255.

62. •• Zeng L, Skinner SO, Zong C, Sippy J, Feiss M, Golding I: **Decision making at a subcellular level determines the outcome of bacteriophage infection**. *Cell* 2010, **141**:682-691.
Demonstration that the initial viral gene copy number entering a cell (MOI) changes cell fate and viral life cycle.

63. Gonzalez-Jara P, Fraile A, Canto T, Garcia-Arenal F: **The multiplicity of infection of a plant virus varies during colonization of its eukaryotic host**. *Journal of Virology* 2009, **83**:7487-7494.

64. Miyashita S, Kishino H: **Estimation of the size of genetic**
• **bottlenecks in cell-to-cell movement of soil-borne wheat mosaic virus and the possible role of the bottlenecks in speeding up selection of variations in trans-acting genes or elements**. *Journal of Virology* 2010, **84**:1828-1837.
The authors have developed an imaginative approach to estimate the MOI in plant viruses overcoming the confounding problem of spatial segregation.

65. Klappenbach JA, Dunbar JM, Schmidt TM: **rRNA operon copy number reflects ecological strategies of bacteria**. *Applied and Environment Microbiology* 2000, **66**:1328-1333.

66. Stevenson BS, Schmidt TM: **Life history implications of rRNA gene copy number in *Escherichia coli***. *Applied and Environment Microbiology* 2004, **70**:6670-6677.

67. DeLuna A, Vetsigian K, Shoresh N, Hegreness M, Colon-Gonzalez M, Chao S, Kishony R: **Exposing the fitness contribution of duplicated genes**. *Nature Genetics* 2008, **40**:676-681.

68. Perry GH, Dominy NJ, Claw KG, Lee AS, Fiegler H, Redon R, Werner J, Villanea FA, Mountain JL, Misra R *et al.*: **Diet and the evolution of human amylase gene copy number variation**. *Nature Genetics* 2007, **39**:1256-1260.