Why viruses sometimes disperse in groups[†]

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Abstract

Many organisms disperse in groups, yet this process is understudied in viruses. Recent work, however, has uncovered different types of collective infectious units, all of which lead to the joint delivery of multiple viral genome copies to target cells, favoring co-infections. Collective spread of viruses can occur through widely different mechanisms, including virion aggregation driven by specific extracellular components, cloaking inside lipid vesicles, encasement in protein matrices, or binding to cell surfaces. Cell-to-cell viral spread, which allows the transmission of individual virions in a confined environment, is yet another mode of clustered virus dissemination. Nevertheless, the selective advantages of dispersing in groups remain poorly understood in most cases. Collective dispersal might have emerged as a means of sharing efficacious viral transmission vehicles. Alternatively, increasing the cellular multiplicity of infection may confer certain short-term benefits to viruses, such as overwhelming antiviral responses, avoiding early stochastic loss of viral components required for initiating infection, or complementing genetic defects present in different viral genomes. However, increasing infection multiplicity may also entail long-term costs, such as mutation accumulation and the evolution of defective particles or other types of cheater viruses. These costs and benefits, in turn, should depend on the genetic relatedness among collective infectious unit members. Establishing the genetic basis of collective viral dispersal and performing controlled experiments to pinpoint fitness effects at different spatial and temporal scales should help us clarify the implications of these spread modes for viral fitness, pathogenicity, and evolution.

Key words: viral spread; dispersal; collective infectious unit; multiplicity of infection; viral transmission

1. Viruses sometimes travel in groups

The viral particle, or virion, has been traditionally viewed as the structure that mediates viral spread within and between individuals or hosts. Although this is probably be true in many cases, it is also well established that single virions often fail to establish productive infections. Such low infectivity can be explained by different factors, including structural or genetic defects of viral particles resulting from deficient morphogenesis

or from degradation, but also from host factors related to cellular permissivity to the virus (Klasse 2015; Sanjuán 2018). Interestingly, over the last years, several lines of evidence have indicated that viral dissemination can also occur through processes that involve different types of complex multi-virion structures in addition to free individual virions. An expected consequence of these collective infectious units is that they should increase the cellular multiplicity of infection (MOI), defined as the average number of viral genomes that initiate a cell infection. Elevating the MOI locally may contribute to increasing infectivity and may favor co-infections. This might be important to ensure virus propagation in certain tissues or

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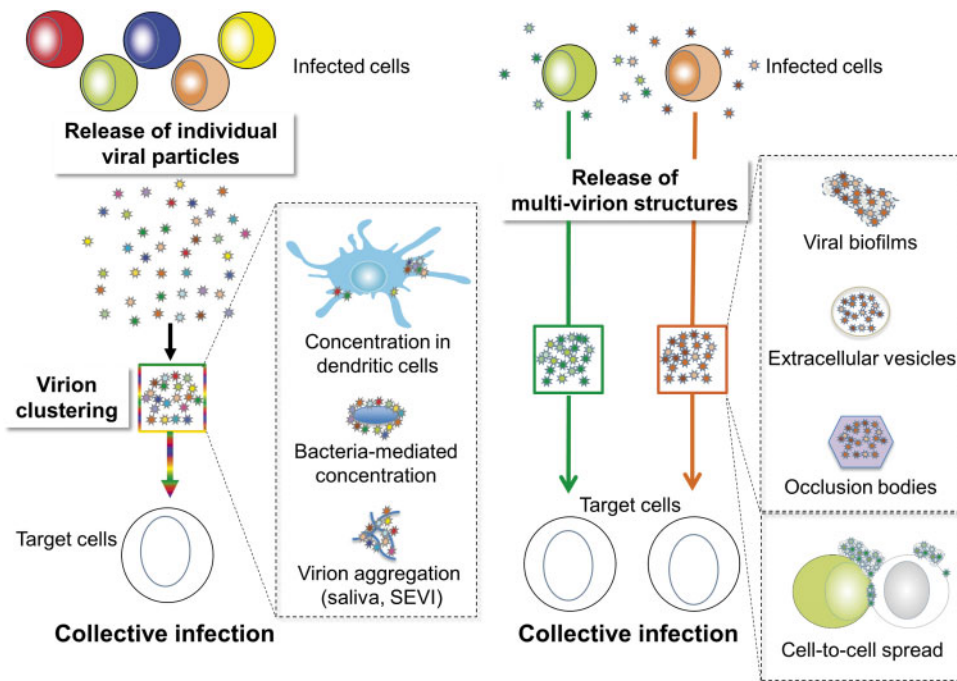
Figure 1. Summary of collective dispersal modes in viruses. In some cases, virions in the extracellular milieu, which can potentially originate from different cells, are subsequently clustered by different mechanisms, including HIV-1 concentration in dendritic cells (DCs) attachment to bacterial cells from the host microbiota (enteroviruses), or virion aggregation (VSV in semen, VSV in saliva). In other cases, pools of virions originating from the same cell undergo co-dispersion. This is the case of viral biofilms (HTLV-1), virions encapsulated in extracellular vesicles (HAV, enteroviruses, marseilleviruses, rotaviruses, and noroviruses), and baculovirus OBs. Cell-to-cell spread is yet another process whereby multiple virions are jointly transferred between cells.

physiological compartments. However, research in this topic is still too incipient to draw solid conclusions. Below we review different processes whereby multiple viral genomes are delivered simultaneously to host cells, and we then discuss possible advantages and costs of dispersing in groups for viruses. A summary of different types of collective spread in viruses is provided in Fig. 1. These spread modes have been classified according to whether they mediate the transfer of multiple viral particles originating from the same cell or from different cells.

1.1 Cell-to-cell spread

Probably the best studied process whereby multiple virions are co-transferred between cells is cell-to-cell spread, which is used by many different viruses. Cell-to-cell spread does not actually involve extracellular multi-virion structures, yet it enables the massive transfer of individual virions to the same target cell in a confined environment by exploiting structures that bridge cells. By limiting diffusion of individual viral particles, cell-to cell spread locally increases the MOI and favors entry of multiple virions per cell. Cell–cell contacts can be either pre-existing junctions such as plasmodesmata, immunological synapses and neural synapses, or specific virus-induced structures such as virological synapses, tunneling nanotubes or syncitia resulting from the fusion of infected cells (Mothes et al. 2010; Sattentau 2011; Zhong et al. 2013b; Symeonides et al. 2015; Graw and Perelson 2016). The ability of cell-to-cell spread to promote co-infection was shown for HIV-1 (Del Portillo et al. 2011; Duncan et al. 2014; Law et al. 2016). However, experiments with retroviruses and plant viruses also revealed that a very small fraction of the delivered genomes establish successfully in the new cell (Josefsson et al. 2013; Miyashita et al. 2015). Although cell-to-cell spread is generally local, entire infected cells can also serve as vehicles for

the systemic dissemination of the infection in blood-borne viruses, and this can constitute a relevant mode of transmission among hosts, as shown for HIV-1 (Mothes et al. 2010; Murooka et al. 2012; Real et al. 2018).

1.2 Other collective spread modes involving cell surfaces

A related but slightly different viral spread mode is the accumulation of viral particles in clusters at the surface of the infected cell, as shown for human T-cell leukemia virus (HTLV-1) (Pais-Correia et al. 2010). After egress from the cell, HTLV-1 particles are physically linked together in a virally-induced extracellular matrix network composed by collagen, agrin, and linker proteins including tetherin and galectin-3, forming so-called viral biofilms around infected cells. Pieces of HTLV-1 biofilms containing multiple virions are then transferred upon contact of infected cells with target T lymphocytes, enabling the '*en bloc*' transmission of virions produced by a unique cell.

Another way of using cells as viral dissemination vehicles is trans-infection mediated by dendritic cells (DCs), which has been extensively studied for HIV-1 (McDonald, 2010). During their journey between infected tissues and lymphoid organs, DCs tend to capture viral particles at their surface, which can originate from different infected cells. These particles accumulate in membrane invaginations before being delivered collectively to CD4+ lymphocytes during antigen presentation.

Finally, although mechanistically very different, a spread mode analogous to trans-infection has been described for enteric viruses during transmission between individuals. Similarly to DCs, the surface of some gut microbial cells appears to function as a concentrator of enteric viral particles, which further promotes their attachment to host cells (Kuss et al. 2011; Jones et al. 2014; Robinson, Jesudhasan and Pfeiffer 2014). In the case of poliovirus and reovirus, the 'proviral' effect of gut microbiota

is mediated by the polysaccharide chains exposed at the surface of some specific commensal bacteria (Robinson, Jesudhasan and Pfeiffer 2014). These bacterium-bound viral particles are then delivered to host cells, thus enabling a local increase of the MOI (Erickson et al. 2018).

1.3 Extracellular vesicles

In addition to entire cells, viruses can use subcellular structures for the joint spread of multiple viral particles. It has been shown that non-enveloped enteric viruses can be released from cells before lysis as pools of virions cloaked into extracellular vesicles. The enteric hepatitis A virus (HAV) is produced as small pools of virions clustered in exosome-like vesicles that are fully infectious, forming so-called quasi-enveloped virions (Feng et al. 2013). These appear to be the main infectious form of the virus circulating in the blood during acute infection, while individual non-enveloped HAV is mainly shed in feces. Interestingly, the HAV envelope is sensitive to the detergent action of bile acids, strongly suggesting that individual naked HAV found in feces could also derive from enveloped HAV upon stripping of the membrane in the biliary tract (Hirai-Yuki et al. 2016). Whether the infectivity of HAV can be subsequently enhanced by the clustering capacity of enteric commensal bacteria remains to be studied.

In the case of poliovirus, pools of virions are released in autophagosome-derived vesicles and these pools are subsequently delivered *en bloc* to recipient cells (Bird et al. 2014; Robinson et al. 2014; Chen et al. 2015). More recently, variants of this spread mode have been also demonstrated for two other enteric viruses, rotavirus and norovirus (Santiana et al. 2018). Pools of rotaviruses were found to be encapsulated in large vesicles apparently derived from the plasma membrane, whereas vesicles containing smaller groups of noroviruses are probably originated form the exosomal secretory pathway. Vesicle-cloaked rotaviruses were observed in stool from experimentally infected animals and were capable of transiting through the gastrointestinal tract following oral ingestion. Thus, enteric viruses seem to combine two distinct infectious forms: an enveloped form that contains several genomes, and non-enveloped virions containing a single genome, whose infectivity might be enhanced by the clustering capacity of enteric commensal bacteria.

Yet other similar forms of vesicle-encapsulated viral spread has been described for non-enteric viruses, including the enveloped hepatitis C virus (HCV) (Ramakrishnaiah et al. 2013) and the large DNA marseillevirus, which can infect ameba in the form of virion-containing vesicles in addition to individual viral particles. Interestingly, vesicle-encapsulated marseilleviruses use an alternate entry pathway through phagocytosis, while individual free virions follow a canonical endocytosis route from the plasma membrane (Arantes et al. 2016).

1.4 Occlusion bodies

Virion spread in groups has also long been known for baculoviruses. Whereas baculoviruses disseminate systemically in insect larvae in the form of free individual particles (budding virions), groups of tens of virions are encapsulated during late infection stages in a protein matrix made mainly of polyhedrin, forming so-called occlusion bodies (OBs). These OBs serve as vehicles for the co-dispersal of multiple virions at the inter-host level (Slack and Arif 2007). Upon ingestion by larvae, OBs are dissolved under the alkaline pH conditions of the mid gut, releasing occlusion-derived viruses. Hence, OBs do not ensure the joint transmission of virions to the same cells but, instead, to the same host. Yet, baculoviruses present another layer of virion clustering since, in multiple nucleopolyhedroviruses, occlusion-derived viruses are in turn made of small groups of virions wrapped in a common membrane, permitting their joint delivery to cells.

1.5 Virion aggregates

Viral particles can also disperse together if they aggregate in the extracellular milieu following their release as independent particles by infected cells. Early electron microscopy studies noticed a tendency for purified virions to aggregate, a process initially thought to depend primarily on physicochemical conditions (Bald and Briggs 1937; Galasso and Sharp 1962; Galasso 1967; Wallis and Melnick 1967; Floyd 1979). However, the biological relevance of this process was not investigated in much detail. More recent studies have characterized viral particle aggregation in bodily fluids, as well as its implications for infectivity. For instance, the prostatic acidic phosphatase naturally found in seminal fluid forms amyloid fibrils that act as so-called semen-derived enhancers of virus infection (SEVI) for HIV-1. These fibrils attach to HIV-1 virions, favor their clustering and promote their binding to CD4 cells and macrophages, increasing infectivity by several orders of magnitude *in vitro* (Munch et al. 2007). The amyloid fibrils have been imaged directly in human ejaculates, confirming their interaction with the virus and showing that SEVI promote the formation of virion aggregates (Usmani et al., 2014). However, their contribution to HIV-1 dissemination between individuals remains controversial since inhibitors of SEVI-mediated aggregation are poorly efficient when tested *in vivo* for inter-individual transmission (Allen et al. 2015; Van Dis et al. 2016).

Virion aggregation has also been demonstrated for vesicular stomatitis virus (VSV) incubated with human or cow saliva (Cuevas, Durán-Moreno, and Sanjuán 2017). Co-fluorescence analysis with VSV constructs encoding GFP or mCherry showed that virion aggregation strongly increases the chances that multiple viral genomes are jointly delivered to the same target cells. Because the oral cavity is a major infection site for this virus, infected animals may shed virion aggregates through saliva, with potential implications for VSV transmission. Putatively, this process might also take place in the related, saliva-transmitted, rabies virus.

2. Possible advantages and costs of dispersing as groups

Everything else being equal, the clustered dispersal of viral particles should incur a fitness cost. Indeed, a cluster (aggregate, vesicle, OB, etc.) of K viral particles will infect a single target (cell or host) whereas K targets could potentially be infected by these same particles if they dispersed independently. This cost should be significant if most targets receive a single infectious unit, and is expected even if a very small fraction of all particles produced by a host undergo transmission. To see this, imagine that a virus produces N progeny virions in aggregates of size K, hence producing N/K infectious units, and that a very low fraction f of the total particles present in a host will be transmitted, such that $Nf < 1$. Then, the number of successful transmissions per host should follow a Poisson distribution of mean and variance equal to Nf/K, meaning that increasing K should reduce transmissibility. Assuming that K is an evolvable trait, for $K > 1$

to be maintained the transmissibility cost should be offset by some other fitness advantages. Some suggested benefits are discussed below.

2.1 Selection for virion stability

In some cases, virion clustering could emerge as an indirect effect of selection to prolong the infectivity of virions in the extracellular milieu. Baculovirus OBs constitute an interesting example. Baculoviruses experience long periods of time in the environment. Hence, structures that increase resistance to UV radiation, desiccation, and other factors promoting virion degradation should be strongly selected. OBs provide a protein shield to viral particles, which contains polyhedron envelope protein (PEP) among other components, and it was found that the thickness of the PEP layer correlates with the infectivity of occlusion-derived viruses (Sajjan and Hinchigeri 2016). Occlusion has evolved multiple times in different families of insect viruses, such as in entomopoxviruses and cytoplasmic polyhedroviruses (*Reoviridae*) (Slack and Arif 2007) in addition to baculoviruses. However, baculovirus OBs are unique in harboring multiple virions. Furthermore, this form of virion aggregation is probably not a structurally mandatory feature of baculovirus OBs, since the granulovirus genus in the family *Baculoviridae* forms single-virion OBs. Therefore, although the advantages of environmental stability may have driven the evolution of occlusion, this does not explain why most baculoviruses transmit from host-to-host in an aggregated manner. One possibility is that sharing the OBs reduces the costs of producing these inter-host transmission structures. Alternatively, dispersing in groups might per se have a direct fitness effect.

Increased virion stability has also been demonstrated in enteroviruses bound to polysaccharides at the surface of gut bacteria, as shown by evaluating resistance to heat and chlorine (Robinson, Jesudhasan, and Pfeiffer 2014). Yet, again, this does not show or disprove that attachment to bacteria evolved as a means of increasing stability. Vesicle-cloaked rotaviruses were found to be more infectious in mouse pups than equal numbers of free virions derived from broken vesicles (Santiana et al. 2018). Although the reason for this higher infectivity is still unclear, it was suggested that encapsulation in vesicles may protect virions from degradation by proteases and/or bile acids present in the gastrointestinal tract. In the case of HAV, membrane-wrapped viral particles were indeed shown to be sensitive to the action of bile acids, leading to the release of fully infectious naked virions that are more stable in the environment and could be optimized for transmission between hosts (Hirai-Yuki et al. 2016).

2.2 Avoidance of circulating antibodies

Antibody neutralization contributes to reducing viral loads after the acute phase of the infection and hence imposes a selective pressure to the external viral proteins, as shown amply in well-studied viruses such as HIV-1 (Wei et al. 2003) and HCV (Dowd et al. 2009), among others. The role played by cell-to-cell spread in HIV-1 neutralization has been studied in detail and, albeit it allows the virus to avoid neutralization in many cases (Abela et al. 2012), some antibodies remain efficient at blocking cell-to-cell spread (Zhong et al. 2013a; Agosto, Uchil, and Mothes 2015; Reh et al. 2015).

In principle, some of the mechanisms mediating viral dispersal in the form of clusters, such as viral biofilms or encapsulation in vesicles, may also hinder virions from antibodies. This has been proposed, for instance, for HTLV-1 viral biofilms (Thoulouze and Alcover 2011). In the case of HAV and HCV, membrane-wrapped viral clusters circulating in blood during acute infection were shown to be more resistant to neutralizing antibodies, likely facilitating HAV dissemination within the host. Cloaking of virions inside vesicles should also protect other enteric viruses (poliovirus, rotaviruses, and noroviruses) from neutralization, but this remains to be conclusively shown because viral particle neutralization might still be possible if vesicles become transitorily permeable, for instance during cell entry. Furthermore, antibody pressure would not explain why vesicles contain multiple virions instead of single virions. Finally, virion aggregates may hinder antibody neutralization, but aggregation may also promote neutralization if antibody sensitivity is dominant over resistance in the clusters, as reported for VSV (Cuevas, Durán-Moreno and Sanjuán 2017). The causal associations between collective dispersal and antibody-mediated neutralization thus remain uncertain.

2.3 Increase infectivity through a 'mass effect'

The specific infectivity of a given virus, defined as the probability that a viral particle initiates a productive infection, shows ample variation among cell types. Typically, tumoral cells are more permissive to infection than normal cells, either because they are metabolically more active and hence provide more resources to viruses, or because they suffer from innate immunity defects. One possibility is that the infection barriers encountered in less permissive cells are more easily overcome by the virus if the cell receives multiple viral particles simultaneously. For instance, HIV-1 showed similar infectivity by the free virus and the cell-to-cell routes in highly permissive cells such as HEK293 or MT4 leukemia T cells, but infectivity was superior by the cell-to-cell route in primary CD4 cells (Zhong et al. 2013a). Cell-to-cell spread was also found to aid infection under adverse conditions such as antiretroviral therapy (Sigal et al. 2011; Agosto, Uchil, and Mothes 2015). Cell-to-cell spread accelerates HIV-1 gene expression, and this effect is more marked in primary peripheral blood mononuclear cells than in a leukemia-derived cell line (Boulle et al. 2016). This acceleration was recapitulated by inoculating cells with free virions at high MOI, suggesting that the advantage of cell-to-cell spread indeed resided in infecting cells with multiple viral genome copies at once. In the case of marseillevirus, infection by vesicle-encapsulated viruses was also shown to accelerate initiation of the viral replication in ameba (Arantes et al. 2016). Whether this results from the entry of multiple genomes per cell or from the alternative entry route used for collective infection remains unclear.

However, these studies did not assess whether a given number of virions spreading from cell-to-cell were overall more infectious than the same number of free-dispersing virions, which is the relevant question for addressing whether spreading in groups is selectively advantageous. This was more directly addressed in another study, which used microfluidics to place single vaccinia virions at the surface of HeLa cells and found that most cells receiving single viral particles remained uninfected, whereas infection probability increased disproportionately with the number of virions deposited per cell (Stiefel et al. 2012). HeLa are highly permissive cells, and this effect might thus even be more marked in other cell types. A similar result has been obtained recently using VSV by comparing equal numbers of free versus saliva-aggregated virions (Andreu-Moreno and Sanjuán 2018). It was found that virion aggregation

tended to accelerate the infection cycle in a cell type-dependent manner, the effect being stronger in less permissive cells.

This mass effect is akin to the Allee effect in ecology, which is defined as a positive relationship between population size and per-capita growth rate. The Allee effect restricts the ability of a small founder populations to become established (invasion probability) and is relevant to other processes such as extinction (Taylor and Hastings 2005; Kramer et al. 2009). Allee effects have been documented in many species including microorganisms (Kaul et al. 2016), but remained largely unexplored in viruses until recently (Andreu-Moreno and Sanjuán 2018). In the context of a mass effect acting on infectivity, the relevant scale at which the virus founder population size should be evaluated is the individual cell (i.e. the cellular MOI). Similar to the Allee effect in animals, which can result from cooperative breeding, cooperative hunting or predation avoidance, among other processes, the increased infectivity of groups of virions could result from various mechanisms. For instance, by accelerating infection, the virus might be better able to cope with antiviral barriers such as CRISPR immunity in bacteria, interferon-signaled innate immune responses and other restriction factors. Alternatively, by initiating the infection with multiple genome copies, the virus might reduce the changes of early stochastic loss, which may occur if some essential and limiting factors (e.g. the polymerase) are degraded, diluted, sequestered, or expressed at insufficient levels at the earliest stages of cellular infection. Presumably, all these stochastic processes should be less likely to curtail the infection cycle if the number of copies of each viral product is higher.

2.4 Diversity-based benefits

Viruses, particularly RNA viruses and viroids, display higher mutation rates per base than any other biological entity (Sanjuán and Domingo-Calap 2016), meaning that a large fraction of RNA virus genomes contain mutations, most of which are deleterious. This has substantiated the notion that high MOIs may facilitate trans-complementation of viral genetic defects, allowing individually non-infectious virions to be 'reactivated' at high MOIs (Andino and Domingo 2015). Another possible reason for combining different genomes in a cell, potentially beneficial for the virus, is related to the fact that many viruses have compact genomes encoding multifunctional proteins, resulting in fitness tradeoffs or pleiotropic effects that hamper evolutionary optimization (Belshaw et al. 2008). In such compact genomes, beneficial mutations tend to display negative epistasis that is, genomes combining different beneficial mutations tend to be less fit than expected from single-mutation effects (Sanjuán and Elena 2006). As suggested previously, these limits to adaptability could be overcome if beneficial mutations in a particular gene are provided from different copies of genomes co-infecting the same cell (Bordería et al. 2015; Sanjuán 2017).

There is some empirical evidence supporting diversity-based benefits associated to high MOIs. In the case of segmented viruses such as influenza and Rift Valley viruses, a likely reason why individual virions are often non-infectious is that they miss one essential genome segment (Brooke et al. 2013; Wichgers Schreur, and Kortekaas 2016). These virions, named 'semi-infectious particles' for influenza viruses, are biologically active and can regain infectivity at high MOIs through complementation upon co-infection (Brooke 2017). Multipartite viruses constitute the most extreme case, because their segments are encapsidated separately and therefore high MOIs are

critical for infectivity, as discussed previously (Sicard et al. 2016; Lucía-Sanz and Manrubia 2017). Different influenza virus strains or isolates can also interact synergistically. This was shown using one variant carrying a mutation in the variant encoding for a neuraminidase that changed viral particle release from infected cells (Xue 2016). However, it remains to be clarified whether genetic complementation of influenza virus simply relies on random co-infection by free virions or is promoted by some other form of co-dispersal. In measles virus, this is achieved by capsids that accommodate more than one copy of the genome. Co-encapsidation of viral genomes can promote genetic complementation, but also the emergence of new phenotypes resulting from the combination of different genetic variants in the same cell (Shirogane, Watanabe, and Yanagi 2012). A similar strategy could be used by the respiratory syncytial virus (RSV), which forms long filamentous particles containing several viral genomes (Liljeroos et al. 2013). These were recently shown to be highly infectious forms of RSV (Ke et al. 2018). The diversity-based tenet has also been put forward for baculovirus OBs. Despite being large DNA viruses with presumably low mutation rates, baculoviruses exhibit high population mutation frequencies, including large deletions that should abolish infectivity (Chateigner et al. 2015). OBs may promote genetic complementation among these mutants and hence contribute to the maintenance of diversity (Clavijo et al. 2010; Simon et al. 2013).

An important consideration, though, is that genetic complementation is only one of several possible types of interactions that could take place between genetically diverse, co-infecting viruses. Another such interaction is negative dominance, which takes place when the fitness of one virus variant is diminished in cells co-infected by another, deleterious variant, while the deleterious variant does not benefit from the fitter virus. In cases where there is no mutual benefit, co-dispersal reduces the ability of the fittest variant to be favored by natural selection (Miyashita and Kishino 2010). Negative dominance is particularly likely in oligomeric structures such as viral capsids with the ability to form chimera, but can also occur in monomeric structures if the mutant product is toxic (Crowder and Kirkegaard 2005). A compelling demonstration of negative dominance and its evolutionarily implications was made using a drug that reduces the flexibility of poliovirus capsids and prevents uncoating (Tanner et al. 2014). A newly arisen resistance mutation against this drug will tend to pseudotype with wild-type, drug-sensitive capsids, or to form chimeric capsids that are sensitive to the drug. Hence, drug-resistant viruses were suppressed by drug-sensitive viruses infecting the same cell, retarding the evolution of resistance. Whether the drug-resistant variant will ultimately evolve depends on the MOI because high rates of co-infection increase the chances that negative dominance prevents the selection of resistant strains. Therefore, in this case, dispersal in groups should have a negative impact on viral fitness.

3. Collective dispersal and cooperation

For clustered dispersal to be considered a cooperative trait, it should have evolved in response to the benefits of sharing the dispersal vehicle or the recipient cell with other members of the viral population. A basic question to be addressed here is whether the mode of dispersal at play has a genetic basis. This is unknown in most cases, including viral aggregation in saliva or semen, enteric virus vesicle formation, production of viral biofilms, most types of cell-to-cell spread, and bacterial binding. The baculovirus genes involved in OB formation are known (the

polyhedrin gene), but the genetic basis for the formation of multiple versus simple occlusion-derived bodies is not (Rohrmann 2014). In addition to establishing the heritability of virion clustering, the benefits of dispersing in groups should be demonstrated and could in principle stem from any of the above-discussed processes, i.e. increased stability, immune avoidance, increased infectivity due to mass effects, or positive interactions among genetic variants.

Another important aspect to be considered is that every cooperative trait risks invasion by cheaters, which are in principle favored by selection because they benefit from cooperators without reciprocating. A clear example of cheating in viruses is provided by defective interfering particles (DIPs), which carry large deletions and can only replicate in the presence of another, viable copy of the virus, sometimes called 'helper' (Marriott and Dimmock 2010; Díaz-Munoz, Sanjuan and West 2017). Classical work established that passaging a virus repeatedly at a high ratio of total infectious particles to cells (i.e. high viral density) increases the cellular MOI and tends to select for DIPs, which take over the population because their shorter genomes are replicated faster (Marriott and Dimmock 2010). Inside the cell, the machinery required for viral replication, gene expression, and encapsidation is, at least to some extent, a public good and, at high MOIs, DIPs, or other types of cheater viruses benefit from 'helper' viruses without contributing to the public goods (Chao and Elena 2017).

Therefore, by increasing the cellular MOI, viral spread in groups should in principle favor cheaters, in turn selecting against collective spread and cooperation. However, the structures used for collective spread may not produce an effect on the MOI equivalent to that of passaging viruses at high density. First, dispersing in groups does not necessarily imply that there is unrestricted mixing between individuals. Upon cell-to-cell spread, viral biofilm formation, OB production, or spread in vesicles, group members are derived from the same donor cell and hence are highly related genetically. As shown in the social evolution literature, genetic relatedness is a key determinant of the evolution of cooperation and diminishes the likelihood of cheater invasion (West et al. 2006; Gardner, West, and Wild 2011; Díaz-Munoz, Sanjuan and West 2017). More generally, cheaters should not evolve if the population is assorted in a way that cooperators interact preferentially with other cooperators (Nowak 2006; Gardner, West, and Wild 2011).

However, if the maintenance of cooperation relies on excluding genetically unrelated individuals, the evolution of diversity-based cooperation seems difficult a priori. We thus may expect that the benefits of collective dispersal modes have to do with increasing stability in the extracellular milieu, avoiding circulating antibodies, or increasing infectivity through a mass effect, rather than with genetic complementation or other diversity-based types of cooperation. The later would require mechanisms for discriminating among cooperator and non-cooperator variants, or that diversity-based cooperation is so critical for survival that the presence of cheaters is less detrimental for fitness than failure to complement. Hence, although instances of genetic complementation and other types of diversity-associated benefits have been described in viruses (see above), we currently lack solid evolutionary models that help us interpret such benefits in terms of viral cooperation.

A second reason why dispersal in groups is not evolutionarily equivalent to high viral population densities is that these two processes may increase the cellular MOI at different stages of infection. For instance, virion aggregation in extracellular fluids such as saliva in VSV or semen in HIV-1 should act only at the level of inter-host transmission, as well as baculovirus clustering in OBs or bacteria-mediated clustering of enteroviruses. In contrast, high viral densities require extensive viral replication and hence should occur at later infection stages. The selective pressures acting during inter-host transmission or in the first cellular infection cycles following transmission might differ from those acting later on. For instance, increasing particle stability is expected to be particularly important for successful inter-host transmission in viruses that stay in the environment for long periods. Alternatively, during the first infection stages of a new host, it may be critical to boost infectivity or accelerate the infection cycle to overcome early infection barriers, as discussed above. Finally, it is also possible that diversity-driven fitness benefits could be evolutionarily stable if virion clustering is episodic, since this may limit the opportunities for cheater invasion.

4. Conclusions

Dispersal in groups has been now documented in widely different viruses including enveloped and non-enveloped viruses, segmented and non-segmented viruses, and viruses with different types of genome, such as (+)ssRNA, (−)ssRNA, dsRNA, and dsDNA viruses, as well as retroviruses. The vehicles mediating collective spread also vary widely and include lipid vesicles, protein matrices, different forms of aggregation, and binding to the surface of host or non-host cells. Furthermore, these can act at the level of intra or interhost spread/transmission. The properties of different modes of viral dispersal in groups are summarized in Table 1.

A major open question is why collective dispersal is used by viruses. The answer may vary depending on the virus, the scale of transmission (intra- or interhost), the transmission vehicle used, the nature and architecture of the physiological compartment involved, and so on. Interestingly, collective dispersal always coexists with the standard free virion spread mode. In some cases, virion clustering occurs only episodically, such as for instance during aggregation of HIV-1 in semen amyloid fibrils. In other cases, collective and individual spread occur nearly simultaneously, such as for instance upon release of enteroviruses from cells inside vesicles versus free virions. Overall, viral cycles appear to combine individual virions and clusters, enveloped and non-enveloped forms, the shift from one spread mode to another depending on the biology of the virus. This has been partially illustrated by recent literature on enteric viruses and opens up new avenues of research.

Reasonably, dispersal in groups or the combination of free virions and collective spread should provide a fitness advantage to viruses, but very few studies have addressed this question directly. Alternatively, dispersal in groups might be a by-product of other processes, such as virus association with cellular membranes, for instance. To clarify this, it would be helpful to establish whether collective viral spread has a genetic basis by identifying mutants that modify this trait. Related to this, we may consider two possible scenarios: first, certain spread mechanisms could confer a gain in fitness themselves, collective infection being a consequence of such spread modes. Increasing environmental stability or avoiding antibodies would fall in the first category. However, one should show why such protective structures are shared by multiple virions. Second, the fitness advantage could reside specifically in infecting cells with multiple genomes. Mass effects and genetic complementation would fall in this second category. If so, such benefits should, in principle, be recapitulated by free virions infecting cells at high MOI.

Table 1. Types of collective dispersal in viruses and some of their features.

	Vehicle	Scale of viral spread	Scale of mixing	Possible advantages
Many viruses	Cell-to-cell junctions	Mainly intrahost	Unique cell	Immunity avoidance, promote spread by preventing dilution, other
HTLV-1	Extracellular matrix	Intrahost, blood	Unique cell	Immunity avoidance, promote spread by preventing dilution
HIV-1	DC surface	Intrahost, infected tissues	Multiple cells	Promote spread by avoiding dilution
Enteroviruses	Bacterial surface	Interhost	Multiple cells	Increase stability, enhance cell attachment
HAV	Extracellular vesicles	Intrahost, blood	Unique cell	Immunity avoidance
Enteroviruses	Extracellular vesicles	Intra/inter host	Unique cell	Increase infectivity
Rotaviruses	Extracellular vesicles	Interhost, stool	Unique cell	Protect from degradation, increase infectivity
Noroviruses	Extracellular vesicles	Interhost, stool	Unique cell	Protect from degradation, increase infectivity
Marseilleviruses	Extracellular vesicles	Interhost	Unique cell	Increase temperature stability, increase infectivity by promoting entry
Baculoviruses	OBs	Interhost, larva cadavers	Unique cell	Environmental stability, genetic complementation
HIV-1	SEVI	Interhost, semen	Multiple cells	Increased infectivity
VSV	Saliva compounds	Interhost, saliva	Multiple cells	Increased infectivity in poorly permissive cells

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