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The effect of genetic complementation on the fitness and diversity of viruses spreading as collective infectious units

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ABSTRACT

Viruses can spread collectively using different types of structures such as extracellular vesicles, virion aggregates, polyploid capsids, occlusion bodies, and even cells that accumulate virions at their surface, such as bacteria and dendritic cells. Despite the mounting evidence for collective spread, its implications for viral fitness and diversity remain poorly understood. It has been postulated that, by increasing the cellular multiplicity of infection, collective spread could enable mutually beneficial interactions among different viral genetic variants. One such interaction is genetic complementation, whereby deleterious mutations carried by different genomes are compensated. Here, we used simulations to evaluate whether complementation is likely to increase the fitness of viruses spreading collectively. We show that complementation among co-spreading viruses initially buffers the deleterious effects of mutations, but has no positive effect on mean population fitness over the long term, and even promotes error catastrophe at high mutation rates. Additionally, we found that collective spread increases the risk of invasion by social cheaters such as defective interfering particles. We also show that mutation accumulation depends on the type of collective infectious units considered. Co-spreading viral genomes produced in the same cell (e.g. extracellular vesicles, polyploid capsids, occlusion bodies) should exhibit higher genetic relatedness than groups formed extracellularly by viruses released from different cells (aggregates, binding to bacterial or dendritic cell surfaces), and we found that increased relatedness limits the adverse effects of complementation as well cheater invasion risk. Finally, we found that the costs of complementation can be offset by recombination. Based on our results, we suggest that alternative factors promoting collective spread should be considered.

1. Introduction

Over the last years, it has been shown that viral spread often involves groups of co-dispersing infectious particles, in contrast to the more classical mode of dispersal involving independent virions (Sanjuán, 2017; Altan-Bonnet, 2016; Sanjuán, 2018; Mutsafi and Altan-Bonnet, 2018). Such collective infectious units (CIUs) increase the multiplicity of infection (MOI), defined as the average number of viral genomes that are delivered to a cell (Cuevas et al., 2017; Santiana et al., 2018). CIUs can take widely different shapes and have been documented in different types of viruses. In some cases, viral genomes produced in the same cell disperse together, whereas in other cases clustering of virions takes place at the extracellular level, potentially bringing together viral genomes from different cells. This distinction may be relevant, because groups of viral particles from the same cell should exhibit higher genetic relatedness than those originated from different cells.

Examples of en bloc transmission of virions produced in the same cell include encapsulation in extracellular vesicles, baculovirus occlusion bodies (Slack and Arif, 2007), and polyploid viral capsids. Viral spread through extracellular vesicles has been demonstrated in hepatitis A virus (Feng et al., 2013), hepatitis C virus (Ramakrishnaiah et al., 2013), enteroviruses (Robinson et al., 2014b; Bird et al., 2014; Chen et al., 2015), marseilleviruses (Arantes et al., 2016), noroviruses (Santiana et al., 2018), and rotaviruses (Santiana et al., 2018). Virion polyploidy has been shown in bacteriophage f1 (López and Webster, 1983), measles virus (Rager et al., 2002), infectious bursal disease virus (Luque et al., 2009), Ebola virus (Beniac et al., 2012), and infectious pancreatic necrosis virus (Lago et al., 2016). Although it does not produce structurally well-defined multi-virion propagules, the direct transfer of viruses from cell to cell through plasmodesmata, immunological synapses, or neural synapses, as well as through specific

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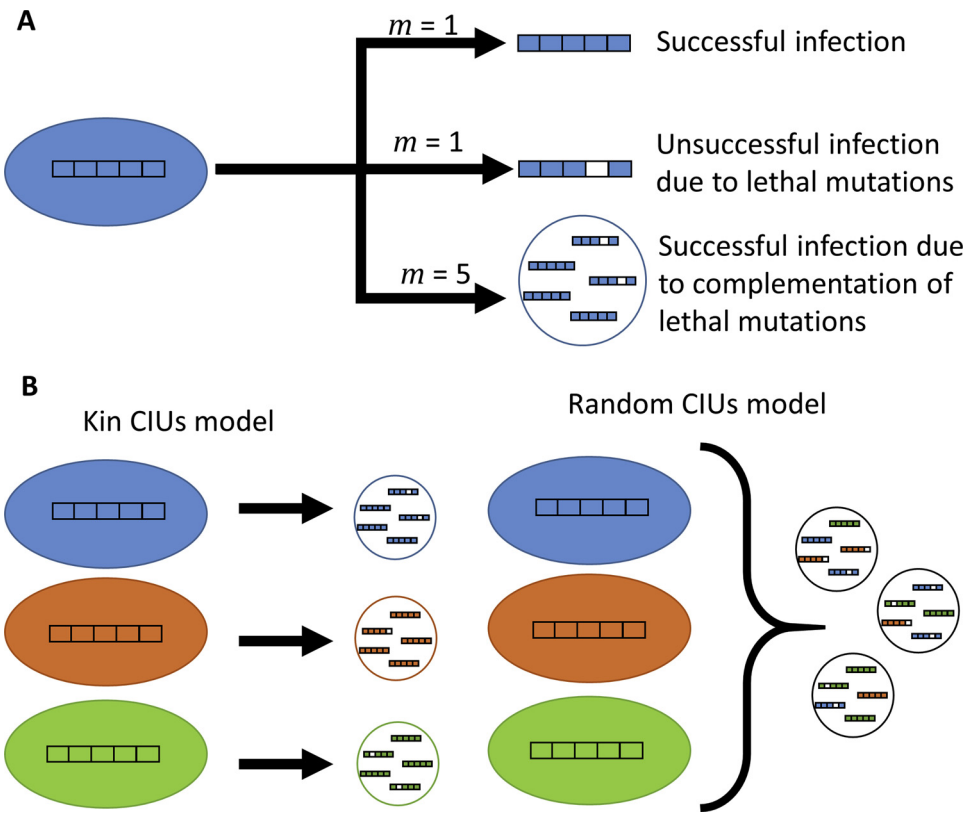

Fig. 1. Collective spread and genetic complementation model. A. Each cell (left) produces progeny, which can carry mutations at certain genes (white boxes, right). Co-spread of multiple viral particles can promote genetic complementation, thereby restoring the infectivity of mutated sequences. **B.** We considered two types of CIU-mediated spread. Kin CIUs were defined as groups of sequences originated from the same cell, whereas random CIUs were formed by random sequences sampled from the entire population.

virus-induced structures such as virological synapses, tunneling nanotubes or syncytia also allow for the joint spread of multiple viral genomes to the same recipient cell (Sattentau, 2011; Zhong et al., 2013; Mothes et al., 2010; Graw and Perelson, 2016; Symeonides et al., 2015). Relatedly, viral biofilms formed by virions embedded in extracellular matrix components at the surface of infected cells is another means of exploiting cell-cell contacts for co-transferring pools of virions (Pais-Correia et al., 2010).

Virions originated from different cells can also form CIUs. Virion aggregation in the extracellular milieu was long ago described, but its biological implications have not been investigated in detail until more recently (Sanjuán, 2017). For example, amyloid fibrils formed by the prostatic acidic phosphatase of the seminal fluid bind HIV-1 virions promote virion aggregation in human ejaculates and enhance infectivity in vitro (Munch et al., 2007; Usmani et al., 2014). Vesicular stomatitis virions also aggregate in the presence of human or cow saliva (Cuevas et al., 2017), and aggregates tend to initiate infection more efficiently than equal numbers of non-aggregated virions (Andreu-Moreno and Sanjuán, 2018). Virion aggregation and its possible implications have also been explored recently in phages (Szermer-Olearnik et al., 2017). Another way in which extracellular viral particles can cluster is by binding to the surface of cells that are not infected but, instead, function as transmission vehicles. This is the case of trans-infection mediated by dendritic cells in HIV-1 (McDonald, 2010). Dendritic cells capture HIV-1 particles at their surface and can later deliver these particles en bloc to CD4 lymphocytes during antigen presentation. Analogously, poliovirus and reovirus particles can accumulate at the surface of gut microbial cells, which subsequently facilitates viral attachment to host cells (Jones et al., 2014; Kuss et al., 2011; Robinson et al., 2014a). Such bacterium-driven viral spread has been shown to enable a local increase of the MOI and to promote enterovirus recombination (Erickson et al., 2018).

In contrast to the extensive evidence for collective viral spread, its implications for viral fitness, diversity and evolution remain underexplored. It has been suggested that high cellular MOIs allow for genetic complementation among deleterious mutants co-infecting the same cell, potentially increasing viral population fitness (Vignuzzi et al., 2006; Borderia et al., 2015; Lauring and Andino, 2010; Domingo and Perales, 2018; Villarreal and Witzany, 2015; Andino and Domingo, 2015; Leeks et al., 2018). RNA viruses show extremely high mutation rates and, thus, high population frequencies of deleterious mutants (Sanjuán and Domingo-Calap, 2016). For this reason, genetic complementation is believed to be particularly relevant to RNA viruses (Lauring and Andino, 2010; Domingo and Perales, 2018; Andino and Domingo, 2015). Consequently, collective viral spread has been proposed to increase viral fitness by promoting genetic complementation in different systems, including extracellular enteroviruses vesicles (Chen et al., 2015; Altan-Bonnet and Chen, 2015), poliovirus aggregates (Aguilera et al., 2017), polyploid measles capsids (Shirogane et al., 2012), and baculovirus occlusion bodies (Simon et al., 2013; Clavijo et al., 2010).

However, the theoretical consistency of the complementation hypothesis has not been explored in detail. Previous models and experiments indicated that, by weakening the effect of deleterious mutations, complementation tends to increase the mutational load of the population, and that complementation can also favor error catastrophe (Froissart et al., 2004; Sardanyés and Elena, 2010; Gao and Feldman, 2009). However, this previous work did not consider collective viral spread. Another important factor to be considered is that high MOIs promote the evolution of defective interfering particles (DIPs) and other types of defective viruses that replicate at the expense of fully functional, “helper” viruses (Marriott and Dimmock, 2010; Rezelj et al., 2018; Manzoni and López, 2018; Brooke, 2017). Because DIPs have shorter sequences and benefit from helper viruses without reciprocating, they can take over the population at high MOIs and reduce viral population fitness dramatically (Turner and Chao, 1999; Díaz-Munoz et al., 2017; Chao and Elena, 2017; Grande-Pérez et al., 2005). Hence, the benefits of complementation, if they exist, might be outweighed by the costs of promoting DIP emergence.

2. Model

We developed a discrete and constrained model with a constant number of infected cells, N. Each cell could only receive one infectious unit, and each infectious unit contained m number of sequences. Hence, m is both the size of the CIU and the cellular MOI. We ignored demography, meaning that populations could not go extinct except if all N viruses became non-infectious. Each viral sequence contained g essential genes and each gene could be functional ('1') or non-functional as a result of mutation ('0'). Thus, each infected cell was represented by a $m \times g$ matrix (Fig. 1A).

We assumed that a single copy of each viral gene was necessary and sufficient for a successful cellular infection, and that the number of functional gene copies had no effect on progeny production, meaning that all successfully infected cells produced the same amount of progeny sequences irrespective of m. This may not be completely accurate in certain cases. For instance, we have recently shown that the cellular MOI determines vesicular stomatitis virus early progeny production (Andreu-Moreno and Sanjuán, 2018). However, our purpose here was to investigate whether genetic complementation produces a positive correlation between the MOI and viral fitness. Adding an intrinsic, complementation-independent positive effect of the MOI on fitness would thus merely introduce a confounder in the interpretation of the results.

The system was iterated by sampling $N \times m$ sequences among productive infections to initiate the following generation. In each generation, each gene mutated with probability α. For simplicity, we ignored back mutation. In the simplest case in which all mutations considered were lethal, since genes had only two possible states and viral output was independent on the number of functional gene copies, mean population fitness was equal to the fraction of infected cells experiencing a productive infection. If $m = 1$, mean population fitness should simply equal:

$$f = (1 - \alpha)^g \quad (1)$$

Also, since mutations were lethal in the absence of complementation, for $m = 1$ their abundance should be directly given by mutation probabilities. In contrast, for $m > 1$, mutated genes were able to leave progeny if complemented and, hence, fitness should deviate from this quantity.

We considered two types of CIUs. In the first type, all m sequences in a CIU originated from the same infected cell. This created families of sequences that were jointly transmitted across cells. We therefore used the term "kin CIUs" to refer to this spread mode (Fig. 1B). In the second type, CIUs were random groups of sequences sampled from the overall population and, hence, did not necessarily originate from the same cell. These were called random CIUs. To a rough approximation, virion-containing extracellular vesicles, polyploid capsids and occlusion bodies may be viewed as kin CIUs, whereas random CIUs represent the extreme case in which virion clustering takes place extracellularly with complete mixing.

To allow for deleterious but non-lethal mutations, we used a model in which fitness decreased proportionally to Hamming distance. Specifically, $f_n = (g - n)/g$, being 'g' the number of genes and 'n' the number of mutations carried by a given sequence. Complementation was implemented as above such that, for each gene, function was fully restored if at least one of the m sequences was non-mutated.

To include recombination, in cells infected with $m > 1$ sequences we assigned a probability α_r that each new sequence was generated as a random reassortment of two parental sequences.

To consider DIPs, we added two new columns to the $m \times g$ matrix. The first column indicated whether that sequence was DIP, whereas the second column indicated the intracellular competitive ability of the DIP relative to helper (normal) sequences. We assigned '1' to every normal sequence ($f_W = 1$) and some higher value to DIPs ($f_D > 1$). Every generation, sequences had a probability α_D of mutating to a DIP. For each viral sequence within a cell, the probability of infecting a cell in the next generation was $\frac{m}{N} \times \frac{f_i}{\sum_{i=1}^{m} f_i}$ provided that the infection was successful. This way, f did not affect the probability of one cell to infect another, but only the probability of sequences to be selected for the next generation.

Throughout the text, we show results for $g = 5$, $N = 1000$ infected cells, and $1000 \times m$ viral sequences, except when including recombination. Simulations were initiated with a single and non-mutated infected cell, and iterations (i.e. generations) were performed until an equilibrium was reached (typically within 500 iterations). Because our model was not demographical, changing N merely modified the variance of the results by changing sampling errors, but did not alter the conclusions. Higher g values rendered the population more sensitive to the effects of mutation and increased the adverse effects of complementation on mean population fitness at equilibrium (not shown).

All simulations were performed with MATLAB R2018b. Scripts and instructions for reproducing our results are available upon request.

3. Results

3.1. Complementation favors error catastrophe

We analyzed how dispersing collectively in groups of size m modified the average fitness of the viral population at equilibrium. In our system, because mutations were lethal in the absence of complementation and per-cell viral yield was independent on m, the average fitness of the population was simply equivalent to the fraction of successful infections. For the simplest case $m = 1$, the simulated fitness matched exactly the fraction of functional sequences after mutation, as described by Eq. (1) (Fig. 2A). We found that, for low mutation probabilities (α), complementation had no effect on the mean fitness of the population. This was expected since, according to the Haldane-Muller principle, mean population fitness at mutation-selection balance depends on mutation rate but not on the fitness effects of mutations. Hence, although genetic complementation among CIU members reduced selection against deleterious mutations, this did not change equilibrium fitness. In contrast, at high mutation probabilities, we found that collective spread had a negative impact on mean population fitness. Compared to singly spreading particles, collective spread led to relatively abrupt drops in mean population fitness beyond certain α values. When we inspected the composition of populations at these mutation probabilities, we found that these values corresponded to error thresholds in which the non-mutated sequence became equally frequent as mutants (Fig. 2B). We also observed subsequent thresholds in which mutants carrying all but one functional gene were invaded by higher order mutants, and so on. Hence, complementation rendered the population more susceptible to error catastrophe.

3.2. Preferential interactions among kin partially alleviate the effects of complementation

As detailed above, CIUs can be groups of viral genomes produced in the same cell or groups of viral particles formed extracellularly. The results shown above correspond to CIUs originated from the same cell, such that all group members shared a recent ancestry (kin CIUs). In contrast, when CIUs are assembled extracellularly, they can contain genealogically unrelated particles produced in different cells. To model this, we repeated the simulations with groups of size m sampled randomly from the entire population (random CIUs), instead of from each producer cell. We found some quantitative differences between kin and random CIUs (Fig. 3). Specifically, spreading as random CIUs made the population more sensitive to error catastrophe than spreading as kin CIUs, and the fraction of mutated sequences at equilibrium was higher for random CIUs. Mixing of unrelated viral sequences made complementation more efficient and hence reduced the efficacy of selection.

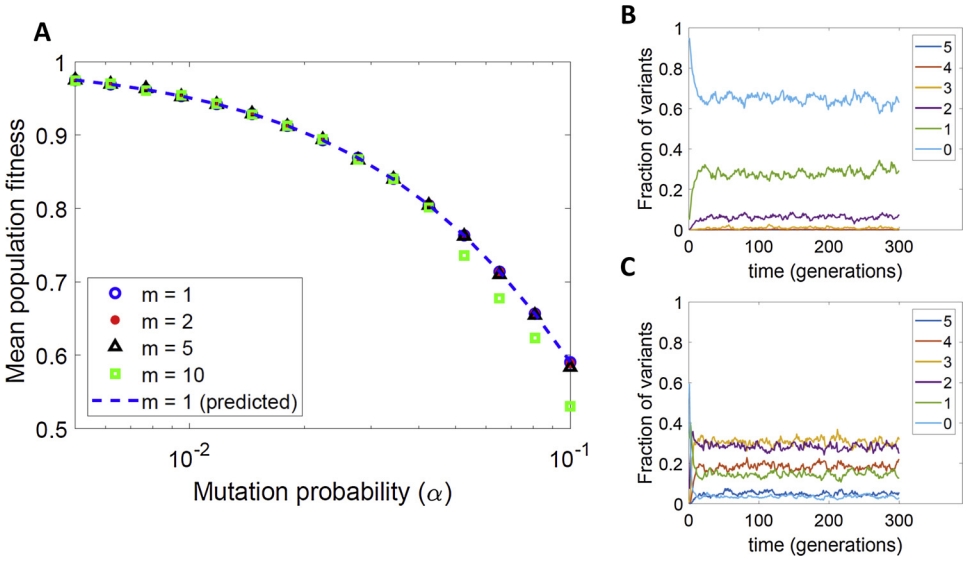

Fig. 2. Effect of mutation and complementation on the fitness and diversity of viruses spreading as kin CIUs. A. Mean population fitness at equilibrium as a function of mutation probability. **B-C:** Dynamics of the distribution of sequences with the indicated number of mutations in a single simulation. We show an example of a stable equilibrium between the non-mutated sequence and mutants (B) and another in which the non-mutated sequence is invaded by mutants (C). All simulations where performed with $N = 1000$ and $g = 5$. In B, $\alpha = 0.01$ while in C, $\alpha = 0.1$, with $m = 5$ in both cases.

If the sequences coinfecting a cell share a recent ancestry, they also tend to share mutations, making it more likely that a given gene lacks any functional copy and that this combination is selected against and removed from the population.

3.3. CIUs transiently increase robustness against deleterious mutations

In the simplest case $m = 1$, since we only considered lethal mutations, the mutation-selection balance was reached instantaneously and mean population fitness was thus constant and equal to the probability of not mutating. In contrast, for $m > 1$, deleterious mutations were initially buffered efficiently by genetic complementation, increasing mean population fitness transitorily relative to the case in which $m = 1$ (Fig. 4). Also, random CIUs were able to buffer the deleterious effects of mutations for longer periods than kin CIUs before reaching the mutation-selection balance and, consequently, random CIUs showed higher mean fitness than kin CIU in the short term. We also found that, for an equal mutation probability and similar mean population fitness, random CIUs were substantially more diverse than kin CIUs.

3.4. Sublethal mutations

To consider the more realistic scenario in which mutations can be have deleterious but non-lethal effects, we used a model in which fitness dropped proportionally to the number of mutations carried by a sequence. We found no major differences in the equilibrium fitness of the population between this model and the all-lethal model (Fig. 5). Yet, error catastrophe occurred at lower mutation probabilities. Hence, the adverse effects of complementation on mean population fitness at the mutation-selection balance were exacerbated by non-lethality. We notice that, for this model, the error catastrophe was also possible for $m = 1$, since mutants replicated and, hence, could accumulate in the population and outnumber the non-mutated sequence at sufficiently high mutation probabilities.

3.5. Recombination

Viral recombination occurs when cells are coinfected with different parental sequences and, thus, CIUs can promote recombination. Hence, we set out to address how the effects of collective dispersal on mean population fitness and diversity were modified by recombination. We found that the effects of CIUs on mean population fitness were offset by

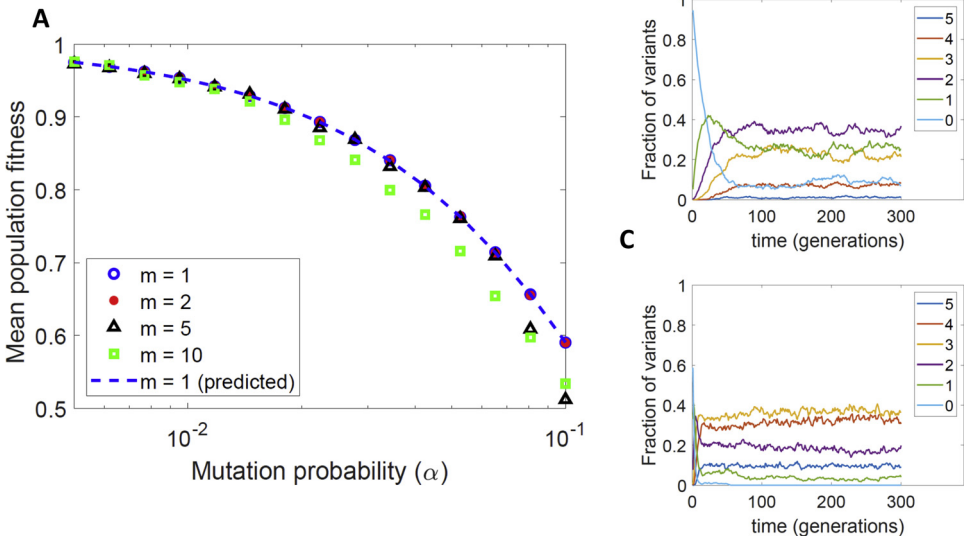

Fig. 3. Effect of mutation and complementation on the fitness and diversity of viruses spreading as random CIUs. A. Mean population fitness at equilibrium as a function of the mutation probability. **B-C:** Dynamics of the distribution of sequences with the indicated number of mutations in a single simulation. We show an example of a stable mutation-selection balance between the non-mutated sequence and mutants (B) and another in which the non-mutated sequence is overcome by mutants (C). All simulations where performed with $N = 1000$ and $g = 5$. In B, $\alpha = 0.01$ while in C, $\alpha = 0.1$, with $m = 5$ in both cases.

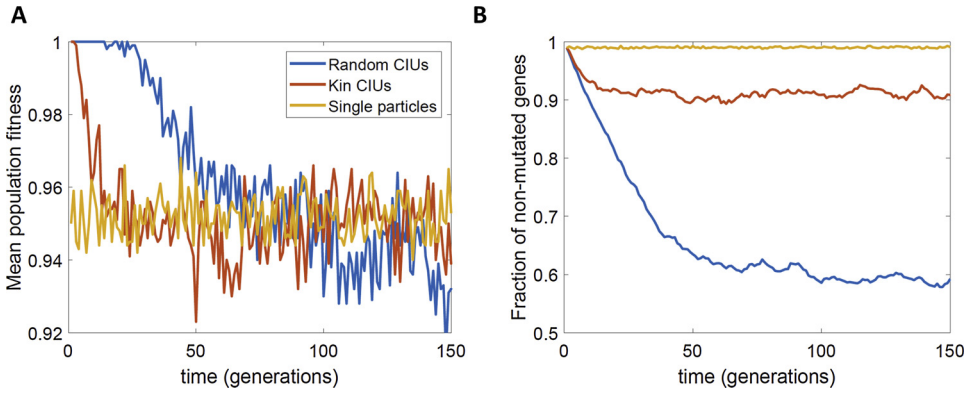

Fig. 4. Fitness and mutation accumulation for single particles, kin CIUs, and random CIUs. Dynamics of mean population fitness (A) and of the fraction of non-mutated genes (B) with $g = 5$, $m = 5$, $\alpha = 0.01$ and $N = 1000$. A single representative simulation is shown.

recombination (Fig. 6). Thus, at high mutation probabilities, recombination had a positive effect on mean population fitness, although high recombination rates were needed to reach this effect. This result was expected because recombination makes it possible to recover mutation-free sequences and, hence, increases the ability of selection to maintain the non-mutated sequence in the population.

3.6. DIPs

Whereas genetic complementation allows for the mutual compensation of defective sequences, DIPs are social cheaters that do not provide any advantage to other coinfecting sequences and, yet, benefit from an intracellular competitive advantage. We found that collective spread promoted the emergence of DIPs, but that random CIUs were more easily invaded by DIPs than kin CIUs (Fig. 7). For kin CIUs, DIP invasion depended on the intracellular competitive advantage of DIPs relative to normal sequences (f_D), but also on the probability of DIP appearance (α_D). For random CIUs, in contrast, α_D played a relatively minor role compared to f_D. This can be explained by the fact that, for kin CIUs, DIPs had to independently appear and invade different genealogies of sequences, whereas for random CIUs a single DIP mutational event was in principle sufficient for invading the entire population. Interestingly, DIP invasion was concomitant with a marked drop in sequence diversity, because as more CIU members became DIPs, the chances for genetic complementation became reduced. This effect was more marked for random CIUs than for kin CIUs.

4. Discussion

In line with previous work, our simulations indicated that, when mutation rates are low, genetic complementation has essentially no effect on mean population fitness at mutation-selection balance whereas, at high mutation rates, complementation favors error catastrophe (Sardanyes and Elena, 2010, Gao and Feldman, 2009). Error catastrophe is a situation in which selection fails to maintain the non-mutated sequence at frequencies higher than those of low-fitness mutated sequences (Bull et al., 2005; Domingo et al., 2005, Sardanyes and Elena, 2010, Eigen, 1971). We found that, although complementation had no positive effect on population fitness at equilibrium, it nevertheless increased robustness against deleterious mutations and mean fitness transiently. Hence, spreading as CIUs may provide a fitness advantage to populations that have not yet reached the mutation-selection balance. This should occur in several situations. For instance, host-to-host transmission and intra-host dissemination typically involve severe population bottlenecks (Zwart and Elena, 2015; Gutiérrez et al., 2012; McCrone and Lauring, 2018; Richard et al., 2017; Salemi and Rife, 2016). These bottlenecks increase the strength of random genetic drift relative to selection and, hence, move populations away from mutation-selection balance. Adaptation to novel selective pressures is another non-equilibrium scenario which should occur frequently in viruses, such as for instance during a host shift or during immune escape. By increasing cryptic genetic variation, that is, genetic variation without a phenotypic effect in the current environment, complementation could

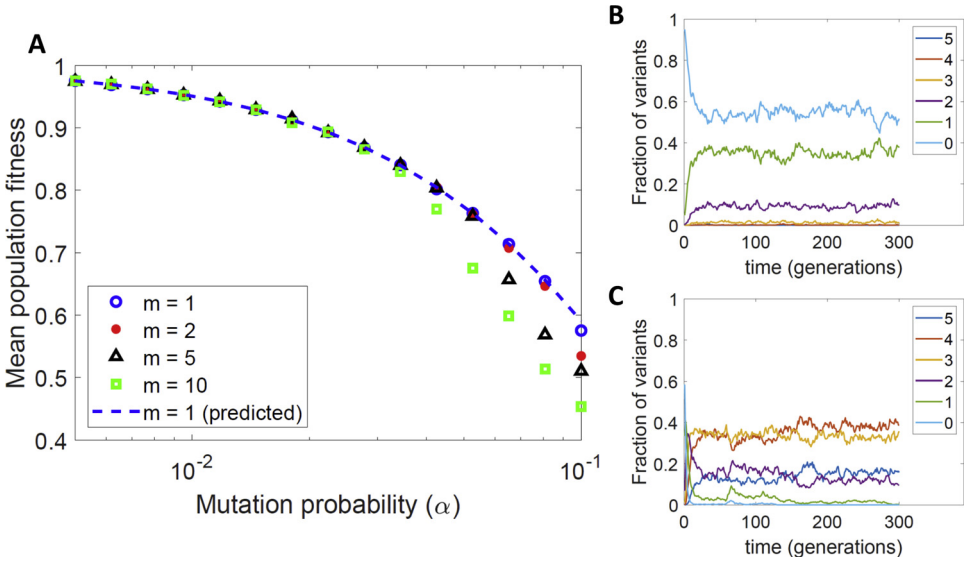

Fig. 5. Effect of mutation and complementation on the fitness and diversity of viruses spreading as kin CIUs for a model allowing for non-lethal mutations. A. Mean population fitness at equilibrium as a function of the mutation probability. B-C: Dynamics of the distribution of sequences with the indicated number of mutations in a single simulation. We show an example of a stable mutation-selection balance between the non-mutated sequence and mutants (B) and another in which the non-mutated sequence is overcome by mutants (C). All simulations where performed with $N = 1000$ and $g = 5$. In B, $\alpha = 0.01$ while in C, $\alpha = 0.1$, with $m = 5$ in both cases.

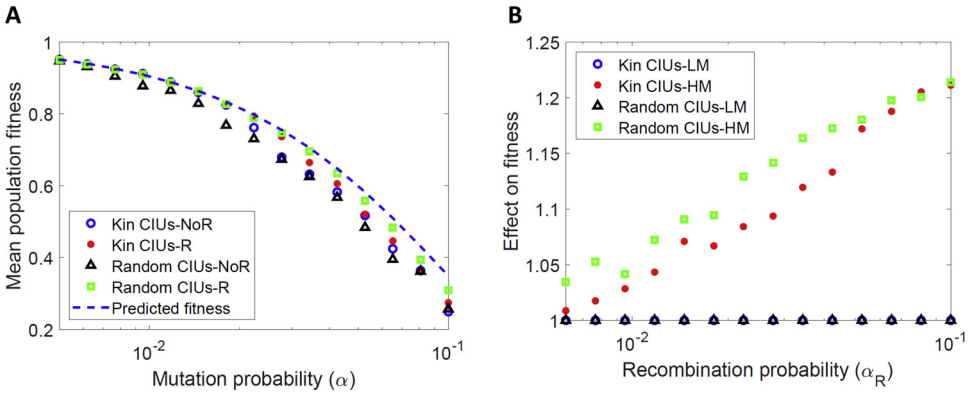

Fig. 6. Effect of recombination on mean population fitness. A. Mean population fitness at equilibrium as a function of mutation probability with or without recombination for viruses spreading as kin or random CIUs. Simulations where performed with $g = 10$, $m = 10$, $N = 1000$, and $\alpha_r = 0.1$. **B.** Normalized effect of recombination on equilibrium fitness for kin and random CIUs at high (HM) and low mutation (LM) probabilities. The mean fitness ratio between recombining and non-recombining populations is shown. Simulations where performed with $g = 10$, $m = 10$, $N = 1000$, and $\alpha = 0.005$ or $\alpha = 0.1$.

increase adaptability to future environments (Payne and Wagner, 2019).

Our model did not take demography into consideration. Demography would introduce yet another cost to spreading as CIUs. Specifically, everything else being equal, spreading as CIUs reduces the number of secondary infections by a factor m relative to spreading as individual particles. As opposed to the error threshold, which refers the relative abundance of different sequences, the extinction threshold is a demographical collapse caused by the accumulation of deleterious mutations (Bull et al., 2007). We expect CIUs to increase the chances of this type of extinction to occur. Another scenario that we did not model was a direct competition between viruses forming CIUs and viruses spreading as individual particles. We expect CIUs to be rapidly outcompeted as a result of the cost of producing m times fewer infectious units.

Together with the above considerations, our results suggest that genetic complementation *per se* may not provide a sufficient explanation the evolution of collective spread, and that additional processes should be considered. Our recent results indicate that aggregates of vesicular stomatitis virus produce more progeny in the short term than equal numbers of non-aggregated particles, offsetting the m-fold cost of CIUs (Andreu-Moreno and Sanjuán, 2018). This work revealed an Allee effect at the cellular level, defined as a positive relationship between the founder population size (here, the MOI) and per-capita growth rate, which could restrict the establishment of new, small populations (Taylor, 2005). This effect was cell-type dependent, suggesting that aggregation may allow the virus to better overcome certain early infection barriers. Such benefits of aggregation were apparently unrelated to genetic complementation, lending support to diversity-independent cooperation processes among viral genomes within cells. For instance, initiating the infection with multiple genome copies could accelerate the infection cycle and help the virus stay ahead of antiviral cellular responses. An association between the cellular MOI and infection speed has also been established for HIV-1 (Boulle et al., 2016). Higher cellular MOIs could also increase infectivity by reducing the chances of early abortive infection. For instance, in negative-strand RNA viruses, viral gene expression is initiated by the viral RNA-dependent RNA polymerase carried in the virion. If the polymerase is missing or inactive, the infection cycle will not proceed. By increasing the cellular MOI, more initial copies of this or other critical factors can be provided, potentially reducing such risks of early failures.

Alternatively, the fitness benefits of CIUs might be unrelated to the MOI. For instance, some of the structures used for spreading in groups, such as occlusion bodies or vesicles, could render viral particles more resistant to degradation in the extracellular environment due to heat, radiation, or the action of host enzymes (e.g. proteases), as well as to antibody-mediated neutralization. Under this scenario, collective spread could be favored if sharing the transmission vehicle (e.g. vesicles) is more efficient than producing one vehicle per virion (Leeks et al., 2019).

Interestingly, our results suggest that the effects of complementation on viral fitness vary depending on the type of CIU involved. By providing a genealogical structure associated to viral spread, CIUs that originate from the same cell, such as virion-containing extracellular vesicles, polyploid capsids, and occlusion bodies, might exhibit lower levels of genetic complementation than CIUs produced in the extracellular milieu. This would limit the adverse effects of complementation in terms of error catastrophe and DIP invasion risks. We notice, though, that virion clustering in the extracellular milieu may not be accurately described by the random CIU model, since spatial structure could nevertheless produce CIUs sharing higher levels of genetic relatedness than expected by purely random mixing. Indeed, in the absence of

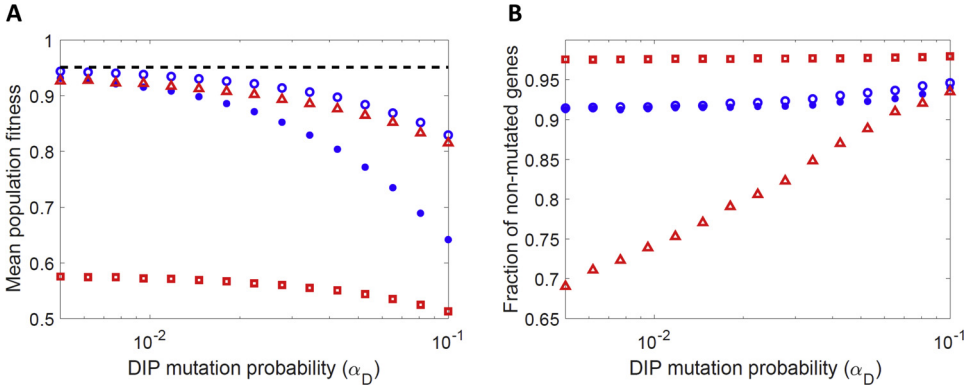

Fig. 7. Effect of DIPs on mean population fitness and diversity. A. Mean population fitness as a function of the probability of mutating from a normal sequence to a DIP for kin and random CIUs and for two different values of the intracellular competitive advantage of DIPs (f_D). **B.** Fraction of non-mutated genes. All simulations were performed with $g = 5$, $m = 5$, $N = 1000$, and $\alpha = 0.01$. Blue: kin CIUs (circles: $f_D = 1.1$; dots: $f_D = 2.0$). Red: random CIUs (triangles: $f_D = 1.1$; squares: $f_D = 2.0$). Dotted line: no DIPs.

spatial structure and demography, our random CIUs model is equivalent to a classical high MOI regime in a well-mixed population. Despite these limitations, our results suggest that a population in which extracellular virion clustering occurred in every generation would be prone to the accumulation of deleterious mutants, including DIPs. It is noteworthy that this type of CIUs have been mainly associated with host-to-host transmission events, such as aggregation in semen for HIV-1 (Munch et al., 2007; Usmani et al., 2014) and in saliva for vesicular stomatitis virus (Cuevas et al., 2017), as well as binding of poliovirus and reovirus particles to gut microbial cells (Jones et al., 2014; Kuss et al., 2011; Robinson et al., 2014a). This might not hold for HIV-1 trans-infection, though, but HIV-1 is a fast-recombining virus and, as shown, recombination can offset the costs of complementation.

Additional theoretical and experimental work is required to better understand the benefits associated to collective spread in viruses. Although there is empirical evidence supporting a role for the cellular MOI in some cases, MOI-independent benefits should not be discarded. Furthermore, there is also strong evidence indicating that a sustained high MOI reduces viral fitness. Hence, CIUs may provide a fitness advantage only in certain stages of the viral infection cycle, such as for instance during inter-host transmission. In turn, these advantages may be diversity-dependent, for instance by allowing genetic complementation, or diversity-independent, for instance by helping the virus overcome Allee effects.

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