 doi:10.20944/preprints201906.0106.v1

Cheating in the viral world

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The extent to which cheating occurs in the natural world has proved contentious. We suggest that viruses offer an exceptional opportunity for studying cheats, individuals that exploit the cooperative behaviour of others. In particular, we show that: (1) cheating is common in viruses; (2) there are many different types of viral cheat; (3) viral cheats offer novel problems for social evolution theory; (4) viruses offer excellent empirical opportunities for studying cheating; (5) cheating shows that viral populations experience substantial conflict, changing how we think about how viral infections evolve; (6) evolutionary theory about cheating could help us understand viral evolution; (7) a greater understanding of cheating in viruses could aid viral intervention strategies.

virus evolution | cheat | cooperation | social evolution | defective interfering genome | satellite virus

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Introduction

The existence of cooperation provides an opportunity for cheats to evolve, that spread by exploiting cooperation (West et al., 2007a; Ghoul et al., 2013; Jones et al., 2015). Given that cooperation occurs at all levels of biology, from genes to complex animal societies, then this allows the possibility for cheating at all levels of biology (Maynard Smith & Szathmary, 1995; Bourke, 2011). However, the extent to which cheating occurs in nature has proved contentious, leading some authors to suggest that it is more important in theory than in reality (Ghoul et al., 2013; Jones et al., 2015; Frederickson, 2017).

We suggest that viruses offer an exceptional opportunity for studying cheating in the natural world. Viruses cooperate in a number of ways, and a range of different viral cheats exist, that exploit these different forms of cooperation (Díaz-Muñoz et al., 2017). For example, viruses that exploit gene products produced by others, or viruses that grow unsustainably fast. Furthermore, viral cheats can span different evolutionary timescales. While some viral cheats originate rapidly in new infections but do not persist, others persist over evolutionary timescales and become closely adapted to exploiting others. Finally, viral cheats seem to have particularly devastating effects on the fitness of cooperator viruses, suggesting that there is the potential for strong selection pressures driving coevolution between cooperators and cheats.

Viruses also offer a number of practical advantages for studying cheating. The wealth of publicly available sequencing data means that we can study the evolutionary dynamics of viral cheats in nature (Firth & Lipkin, 2013; Geoghegan & Holmes, 2018). Because cheats arise de novo in many different viruses, we can study the natural dynamics of cheat populations in parallel across a broad range of viruses and hosts. Furthermore, viruses are excellent candidates for experimental evolution studies, since they evolve quickly in the lab and allow a range of experimental manipulations (Elena & Sanjuán, 2007). This potentially allows us to document the full evolutionary process, from genetic basis to population dynamics in the wild (Barrett et al., 2019). Viruses therefore offer an excellent opportunity to test fundamental ideas about the evolution of cheating and cooperation, many of which were originally developed with animal behaviour in mind.

We first define cheating, and provide a detailed example of how this definition can be applied to viruses. We argue that the biology of viruses could lead to cheating being much more common in viruses than in some other taxa, such as multicellular animals. We then use our definition of cheating as a framework to identify and classify different kinds of cheating in the viral world. We cover known examples, and suggest where further cheats may be found. Finally, we discuss how studying cheating in viruses could challenge and extend social evolution theory, and how applying ideas from social evolution could be useful in virology.

What is a cheat?

Cheats are selfish individuals that avoid paying the costs of cooperation but still benefit from the cooperation of other individuals (Ghoul et al., 2013). One example is brood parasites such as the cuckoo, where individuals of one species trick parents of a different species into neglecting their own chicks and instead feeding the brood parasite's chicks (Davies, 2010). Another example is individuals in the bacterial pathogen *Pseudomonas aeruginosa* which do not produce siderophores (iron-scavenging molecules) (Griffin et al., 2004; Cordero et al., 2012; Andersen et al., 2015). The production of siderophores is a costly cooperative behaviour because individuals pay a cost to produce siderophores, but the benefits of siderophores are experienced by the group of bacteria as a whole (a public good). Some cells do not produce siderophores, but retain the ability to uptake siderophores produced by others, and so act as cheats.

Testing for cheats

To count as a cheat, an individual must exploit a cooperative behaviour (Ghoul et al., 2013; Jones et al., 2015). Given that a viral genome can potentially exploit products encoded by another genome, we consider a genome as an individual, and ask whether viral genomes can cheat (Díaz-Muñoz et al.,

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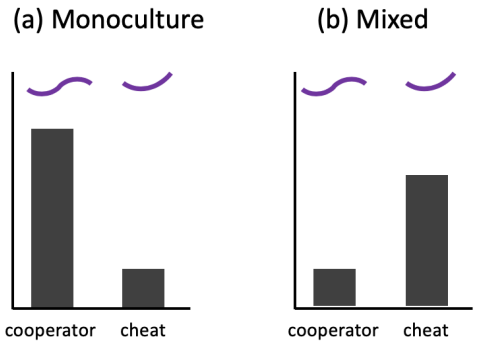

Fig. 1. Testing for cheats. In order for an entity to be classified as a cheat, it must (a) be less fit than the cooperator when each are grown alone; and (b) be more fit than the cooperator when both are grown together in mixture. In many cases, theory predicts that cheats will be most fit when they are rare, and least fit when they are common.

2017). The first step in testing for this is to compare the growth rates of potential cooperators and cheats on their own and in mixture (Fig. 1). For example, genomes that do and do not encode a cooperative trait such as a replicase. If one genome is a cooperator, and another is a cheat, then: (i) the cooperator will have a higher growth rate when grown on its own, but (ii) the cheat will have a higher growth rate when both are grown together (Fig. 1). These results follow from the fact that cooperation provides a benefit at the group level, but can also be exploited by cheats that avoid the costs of cooperating.

When testing for cheats, it is important to rule out alternative explanations for the different growth rates of the two strains. For example, a cooperator's growth rate will be slow if grown in conditions under which cooperation is not favoured. Therefore, it is important to conduct a test for cheating in conditions that reflect the natural environment that cheats and cooperators evolve in as faithfully as possible. In particular, conditions where the cooperative trait being examined is required and beneficial (Ghoul et al., 2013, 2014).

How could viruses cheat?

In viruses, completing an infection cycle inside a host cell can be a cooperative process. This is because successful infection requires viruses to manufacture a number of gene products that benefit of all of the viruses infecting a cell. One example is the replicase, which is the enzyme required to replicate the viral genome. A replicase encoded by one sequence can potentially replicate other sequences inside the same host cell, and so replicases can be a public good. Consequently, a viral sequence could potentially be a cheat if: (i) it lacks the genes for encoding replicase itself; and (ii) it is able to exploit replicases encoded by other sequences.

Another gene product that could be cheated is the capsid protein, which is required to build the viral capsid that transports progeny viral sequences to new cells. Capsid proteins encoded by one viral genome can produce capsids that can potentially contain other viral genomes, and so they can be a public good (Fig. 2). Consequently, a viral sequence could potentially be a cheat if: (i) it lacks the genes for encoding capsid proteins itself; and: (ii) it is able to exploit capsids constructed by proteins encoded by other sequences. A similar line of argument applies to a number of other viral genes, including proteins that suppress host cell defences (Fig. 2). Any viral gene that can be complemented in trans can potentially be cheated.

There are multiple reasons why mutants lacking these functions may gain a replicative advantage over cooperative wild-type viruses, and therefore act as cheats (Fig. 3). The simplest reason is that shorter genome sequences may accumulate more quickly inside a cell because the shorter a sequence is, the more copies can be produced by a replicase in a given amount of time (Spiegelman et al., 1965; Mills et al., 1967). Alternatively, because they don't produce cooperative gene products, they can allocate more time towards replicating copies of themselves (Holland, 1985; Chao & Elena, 2017). Another example is that cheats may replace cooperative genes with sequences that increase their affinity for binding to replicase, allowing them to out-compete the wild-type cooperators for a limited supply of replicases (Lee & Nathans, 1979). Most of these advantages come about by allowing cheats to escape constraints on viral genome sizes. Consequently, cheats may be more likely to gain a large advantage in RNA viruses, which are highly constrained by their small maximum genome size (Holmes, 2003; Belshaw et al., 2008).

A clear example of cheating exists in defective interfering genomes of poliovirus. When poliovirus is grown under conditions allowing for frequent multiple infections, defective interfering mutants can emerge (Cole et al., 1971). One such mutant, 'DI PV1' contains a deletion consisting of the entire capsid protein region (Shirogane et al., 2019). When grown on its own, this mutant is unable to package progeny sequences into virions. However, when cooperator and defective interfering genome are grown together, the defective interfering genome can exploit the capsid proteins produced by the cooperator, achieving more than 1,000 times as many genomes inside virions as the cooperator (Shirogane et al., 2019). In this example, the shorter defective interfering "cheat" genome has advantages at two stages of the lifecycle: it is replicated more quickly than the cooperator; and its progeny genomes are more likely to be incorporated into virions. Beyond poliovirus, similar defective interfering genomes can spread as cheats in a wide range of different viruses (Table 1).

For cheats to spread, they must frequently interact with cooperators. Two key factors determine when this happens in natural viral infections: coinfection likelihood, and the degree of mixing between different viral genotypes. Cheats are most likely to spread when coinfection and mixing are both high. In this case, genetically different viral genomes frequently in-

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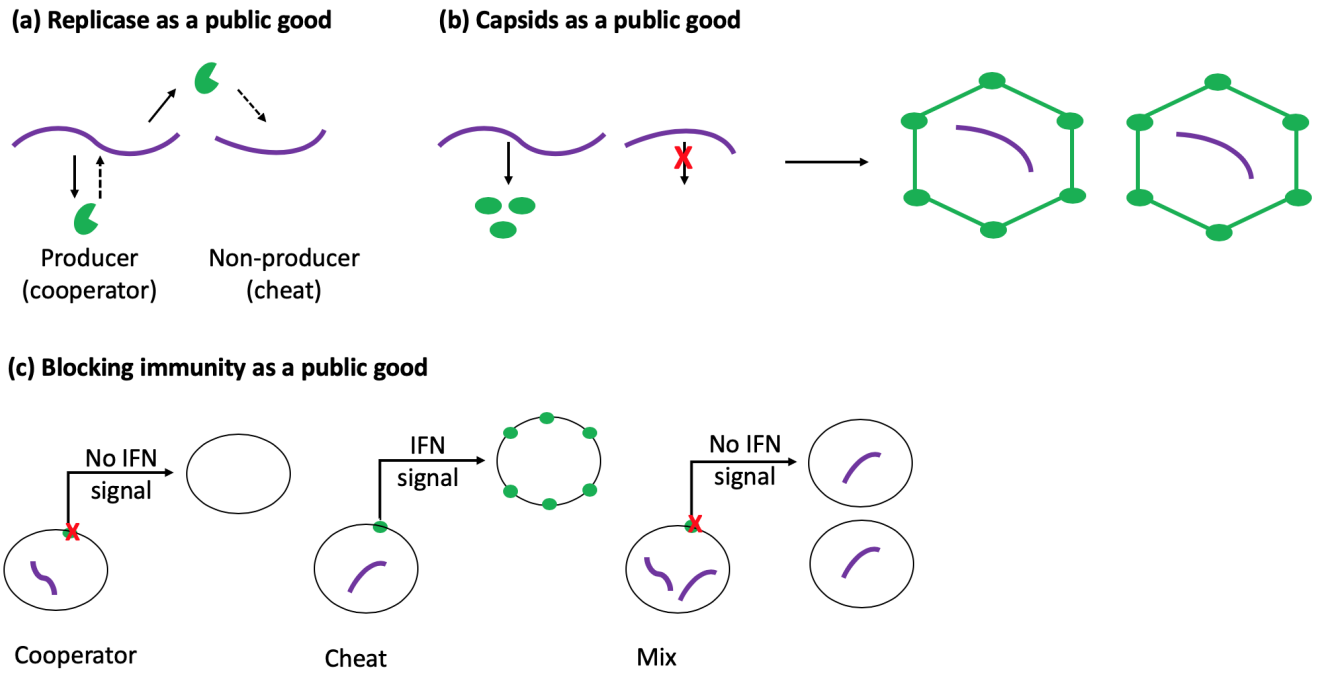

Fig. 2. Viral public goods. In viruses, a range of traits are costly for individuals to produce, but provide benefits to the local group of viruses (public goods). (a) Replicases are required to replicate the viral genome, but replicases encoded by one viral genome can potentially be used by other viral genomes that do not encode their own replicases. (b) Viruses encode proteins that form capsids, which are used to transport viral genome copies to new cells. However, viral genomes can potentially be packaged inside capsids even if they did not produce the proteins required to build the capsid. (c) Viruses which block host immune responses can leave nearby viruses vulnerable to infection, including by other viruses which do not block immune responses.

teract with one another (relatedness is low), allowing cheats to exploit cooperators.

Why might cheating be common in viruses?

Cheats may arise relatively easily in viruses compared to in other organisms. Firstly, a range of different cheats are possible, in many different kinds of virus, because many different viral gene products can potentially act as cooperative public goods (Fig. 2). These include essential gene products found in all viruses, such as replicases and capsid proteins, as well as specific gene products that are found in particular viral species Secondly, viral genomes may commonly lose cooperative functions, since viral replication and recombination are error-prone and frequently result in lack-of-function or deletion mutants, that lack cooperative traits (Holmes, 2003; Sanjuán et al., 2010). Finally, as outlined in the previous section, there are multiple generic mechanisms by which non-cooperative viruses are likely to gain a replication advantage over cooperative wild-types, and therefore be able to act as cheats (Fig. 3).

However, just because cheats arise easily, does not necessarily mean that we will find them easily (Ghoul et al., 2013). If cheats are not able to sufficiently exploit cooperators, then we would expect them to be outcompeted, and so not maintained in the population. In contrast, if cheats can sufficiently exploit cooperators, then they could increase in frequency, eliminating cooperation from the population. In this case, we wouldn't be able to observe cheating because the cooperation would not be there. We can only observe cheating when some mechanism allows both cooperators and cheats to be maintained in the population.

There are several reasons why in viruses, we could expect both cooperators and cheats to coexist in the same population, rather than one driving the other extinct. Coexistence requires that the fitness of viral cheats is higher when they are rarer (negative frequency dependence) (Ross-Gillespie et al., 2007). One factor that could lead to negative frequency dependence is that viral populations with a higher frequency of cheats usually grow slower, meaning that as cheats become more common, growth for all genotypes becomes more difficult and so cheats cannot spread at the expense of cooperators (Domingo-Calap et al., 2019). Another reason is that many viruses grow in spatially structured environments, meaning that cheats are disproportionately more likely to interact with other cheats. In this case, when cheats are common, the lower degree of cooperation disproportionately affects other cheats and so cheat growth is slowed more than cooperator growth (Ross-Gillespie et al., 2007). Both of these conditions seem to be common in natural viral infections. Therefore, we expect that viral cheats may both arise commonly, and stick around, making them relatively easy to find.

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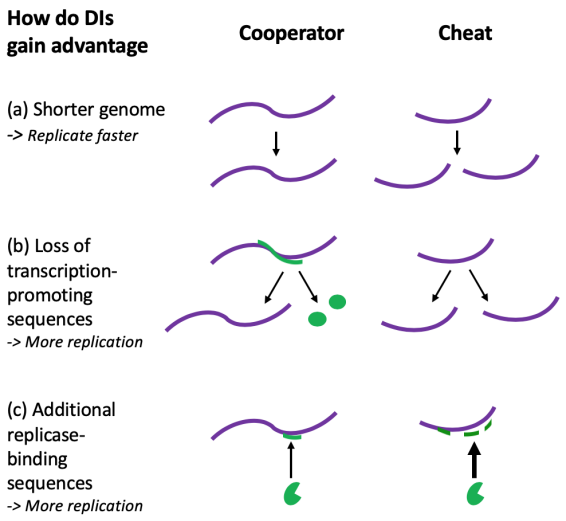

Fig. 3. Mechanisms by which defective interfering genomes can gain advantages over the cooperative wild-type genomes they are derived from. (a) Shorter genomes can be replicated faster, allowing for more copies to be produced in the same amount of time. (b) Genomes can lose regions that promote the production of cooperative gene products, potentially leading to more genome copies being produced if there is a trade-off between genome replication and gene product transcription. (c) Genomes can insert additional sequences that promote the binding of viral replicase.

Where are viral cheats found?

We suggest that cheats are common throughout the viral world (Table 1). We use our definition of cheating as a framework to identify and classify different kinds of viral cheats. Our suggestions are based on both indirect and observational evidence, but in some cases direct experimental evidence for cheating is still required (Table 1).

We distinguish between cheats that exist within a host but don't spread between hosts ('short-sighted'), and cheats that spread both within and between hosts ('long-sighted') (Lythgoe et al., 2017). This distinction matters for at least two reasons. Firstly, long-sighted and short-sighted cheats are likely to experience different selection pressures, because short-sighted cheats will be selected just to spread within hosts, whereas long-sighted cheats will be selected both to spread within and transmit between hosts. Secondly, long-sighted cheats potentially exist over much longer timescales than short-sighted cheats, and so they may display more complex adaptations for cheating that reflect a longer period of cooperator-cheat coevolution. Long-sighted cheats can be seen as adaptive cheats, as defined by Ghoul et al (2013), whereas short-sighted cheats are transient cheating that natural selection will weed out eventually. Short-sighted cheats are most likely to be found if mutations to cheating are common and if cheats can increase in frequency locally, such as within a host.

Short-sighted cheats

Defective interfering genomes. Viral replication is error prone, often producing mutants that are 'defective' because they lack entire genes or sections of genes. These mutants can only spread if: (i) they lack a gene in a potentially cooperative trait (a trait that can be complemented in trans), such as replicase or capsid proteins; and (ii) they can coinfect cells alongside a complete wild-type genome. When both conditions are met, these mutants can exploit the cooperative trait encoded by the wild type, and often out-compete the wild type within cells due to their shorter genome length (Fig. 3). These mutants are both defective and interfering ('defective interfering') and they can spread as cheats through viral populations (Huang & Baltimore, 1970).

Defective interfering genomes have been known about since the earliest days of virology and are known to affect a wide range of viruses when grown in tissue culture (von Magnus, 1947; Vignuzzi & López, 2019). They have usually been found in natural isolates when they have been looked for, they interfere with viral stocks grown for vaccine production, and they have inspired new kinds of antiviral therapeutic (Li et al., 2011; Metzger et al., 2011; Saira et al., 2013; Dimmock & Easton, 2014). Consequently, a substantial body of theory has developed around predicting the dynamics of defective interfering genomes, with many specific models that are consistent with general predictions from social evolution theory (Szathmáry, 1993; Kirkwood & Bangham, 1994; Frank, 2000; Brown, 2001; Rüdiger et al., 2019).

Loss-of-function mutants. As well as producing deletion and rearrangement mutants, viruses frequently spawn loss-of-function mutants, which have small deletions or point mutations that impair the function of particular genes. These mutants can be cheats if they have lost costly cooperative traits. For example, if they avoid performing a time-intensive cooperative function such as blocking an immune response but can still benefit from the immune blocking of others.

The synthetically-generated D51 mutant of vesicular stomatitis virus (VSV) has a partially defective protein that does not block the release of interferon, a key component of mammalian innate immunity (Stojdl et al., 2003). By not blocking interferon release, D51 mutants have a faster growth rate inside a host cell, and so they out-compete the cooperative wild-type VSV in mixed conditions (Domingo-Calap et al., 2019). However, the release of interferon from D51-infected cells comes at a cost to the local population of viruses, because interferon spreads between host cells, causing them to activate their antiviral defences and become resistant to viral infection. Consequently, D51 does very poorly relative to the cooperative wild-type when each is grown on their own (a high relatedness condition). D51 is therefore a cheat that exploits the costly cooperative trait of blocking interferon release.

Loss-of-function mutants are extremely common in most viruses due to the high error rate of virus-encoded replicases.

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For example, in influenza, between 70% and 99% of genomes can be defective in at least one gene (Diefenbacher et al., 2018). However, only a small fraction of these mutants are likely to be cheats, since this requires a gene that controls a costly cooperative trait to be knocked out. Furthermore, unlike defective genomes, loss-of-function mutants are a similar length to the wild-type cooperator, and so they are unlikely to gain a generic replication advantage through being shorter, and instead will only be favoured if they have lost a trait that is costly to express.

Growth mutants. So far, we have considered cheats that gain an advantage through not producing something. However, cooperation can take forms other than producing some factor that benefits the local population. Slow growth can be seen as form of cooperation, because it avoids hosts being overexploited, allowing for more transmission opportunities in the long run (Frank, 1996). 'Rapacious' phages are phages which burst their bacterial hosts especially quickly, and they have been described in a number of different phage species (Kerr et al., 2006; Wild et al., 2009; Berngruber et al., 2015). When grown on their own, rapacious phages quickly exhaust the supply of bacterial hosts and go extinct, whereas wild-type phages, with a more conservative growth rate, can maintain the infection for longer. However, when rapacious phages and wild-type phages are grown in mixture, rapacious phages out-compete wild-type phages. Consequently, rapacious phages are a kind of cheat, exploiting the conservative growth rate of wild-type phages in order to spread in the short term.

Long-sighted cheats

Satellites. Satellites are defective forms of a virus that encode fewer genes than the complete wild-type virus does (Hull, 2009). Because they do not encode all of the genes required for successful infection, satellites need to coinfect a cell alongside a complete wild-type virus, in a similar way to defective interfering genomes. However, while defective interfering genomes emerge spontaneously but are generally transient, satellites have more mysterious origins, sharing little sequence homology with the wild-type viruses that they exploit, and persisting over long evolutionary timescales (Vogt & Jackson, 1999; Simon et al., 2004). They are therefore more analogous to when cheating is across species, as when cuckoos parasitise the nests of other species (Davies, 2010; Ghoul et al., 2013).

In many cases, satellites are cheats for the same reasons that defective interfering genomes are, since they rely on exploiting cooperative traits encoded by the wild-type, such as replicases and capsid proteins. However, in some cases satellites may contribute to more successful viral infections, for example by coding for gene products not found in the wild-type viral genome (Simon et al., 2004). In these cases, satellites may be better viewed as mutualists. Satellites are mostly found in plant viruses, although they can be found in animals, and at least one infects humans (Hepatitis Delta Virus is a satellite of Hepatitis B Virus) (Wille et al., 2018; Chang et al., 2019).

Why are some cheats short-sighted and others long-sighted?

We suggest that the extent to which viral bottlenecks occur between hosts plays a key role in determining whether cheats are short- or long-sighted. As most cheats need to coinfect a host cell alongside a wild-type cooperator, they can generally only spread between hosts when both cooperator and cheat genomes are transmitted simultaneously. However, viral transmission between hosts often involves strong bottlenecks, which result in only a small number of viral particles successfully transmitting (Zwart & Elena, 2015; McCrone & Lauring, 2018; Xue & Bloom, 2019). These bottlenecking events may be a key reason why defective interfering genomes and other 'short-sighted' viral cheats appear to only rarely transmit between hosts (Li et al., 2011; Saira et al., 2013).

In contrast, less extreme bottlenecking would allow viral cheats to spread between hosts, and hence become 'long-sighted'. For example, in plant viruses, which more commonly have long-sighted cheats such as satellites, very large numbers of viral genomes can sometimes be transmitted between hosts by aphid vectors (Monsion et al., 2008). Similarly, long-sighted cheats may be facilitated by 'collective infectious units', which are viral structures that allow for the transmission of multiple genomes simultaneously to a new host (Sanjuán, 2017; Leeks et al., 2019; Segredo-Otero & Sanjuán, 2019). Consistent with this idea, deletion mutants can spread between hosts in natural populations of baculoviruses, in which viruses transmit between hosts inside large collective infectious units (Simón et al., 2006).

Where else could cheating happen in viruses?

Since cheating can in principle evolve wherever cooperation exists, we can look at other examples of viral cooperation to see where further cheats may be found. We would be most likely to find cheats when cooperative traits are costly, and when there is appreciable population mixing, allowing cheats to exploit cooperators. One example where cheats could exist but have not yet been found is in the recently-discovered 'Arbitrium' quorum-sensing system in phages of Bacillus bacteria, where phages could cooperate by signalling beneficial information about the density of infected cells to each other (Erez et al., 2017). On the other hand, some well-studied groups of viruses appear not to have any cheats, despite theoretical reasons why they might, such as the lentiviruses, the group that includes HIV (Nee, 2016). This suggests that in some viral groups, there are currently unknown factors that make it difficult for cheats to form or spread.

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Potential cheat	Virus	Trait Exploited	Loses in Mono?	Wins in Mixed?	Cheat?	Long- or Short-sighted?	Observed in nature?	References
'DI-like' cheats and relatives								
PV1 mutant	Poliovirus	Capsid	Yes	Yes	Yes	Short	Yes	(Cole & Baltimore, 1973; Shirogane *et al.*, 2019)
Multiple deletion mutant	Flock House virus	Replicase	Yes	Yes	Yes	Short	No	(Jaworski & Routh, 2017)
DI-H	Sendai virus	Capsid and replicase	Yes	Yes	Yes	Short	No	(Horikami *et al.*, 1992; Calain & Roux, 1993)
Therapeutic interfering particle	Multiple	Various	Yes	Yes	Yes	Either	No	(Metzger *et al.*, 2011)
'few polyhedra' phenotype	Nucleopolyhedrovirus	Collective infectious unit	Yes	Yes	Maybe*	Long	Yes	(Simón *et al.*, 2006)
E248 stop codon lineage	Dengue virus	Unclear	-	-	Maybe*	Long	Yes	(Aaskov *et al.*, 2006; Ke *et al.*, 2013)
Immune-stimulating DVGs	Many	None	Yes	No	No	Short	Yes	(Manzoni & López, 2018)
'loss-of-function' cheats and relatives								
D51	VSV	Interferon suppression	Yes	Yes	Yes	Short	No**	(Domingo-Calap *et al.*, 2019)
PhiH2	Phi6 phage of *Pseudomonas* spp.	Various	Yes	Yes	Yes	Short	No	(Turner & Chao, 1999)
Semi-Infectious Particles	Influenza	Various	Yes	No	No	Short	Yes	(Diefenbacher *et al.*, 2018)
Acr-negative mutant	DMS3vir phage of *Pseudomonas aeruginosa*	Inhibition of host CRISPR-Cas immunity	Yes	No	No	Short	No	(Chevallereau *et al.*, 2019)
Growth mutants								
Rapacious mutant	T4 coliphage of *Escherichia coli*	Restrained growth	Yes	Yes	Yes	Short	No	(Kerr *et al.*, 2006)
Satellites								
satRNA	Cucumber mosaic virus	Various	Yes	Yes	Yes	Long	Yes	(García-Arenal & Palukaitis, 1999)
SPMV	Panicum mosaic virus	Various	Yes	No	No	Long	Yes	(Qiu & Scholthof, 2001)

Fig. 4. Table 1. Some illustrative examples of viral cheats and cheat-like entities. Many more examples of viral cheats exist, but we have tried to use examples where the mechanisms of cheating are known. Because of frequency dependent selection, some of the cheats that we classify as winning in mixed infection only have an advantage when rare, and do not drive the cooperator to extinction.

* In these cases, the infection does better with both the wild type and cheat-like entity, suggesting the cheat-like entity is better thought of as a mutualist.

** D51 is a synthetic mutant, but mutants with similar phenotypes (not suppressing interferon) are commonly found in wild isolates of VSV and other viruses, and these could reflect natural cheats.

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Multipartite viruses. Multipartite viruses are viruses that have their genome split into multiple segments, and in which each segment transmits independently to new cells inside a separate virion (Hull, 2009). Multipartite viruses are common in plants and fungi, representing up to 40% of described viral genera, and have been found in at least two insect species (Hull, 2014; Hu et al., 2016; Ladner et al., 2016). The wide prevalence of multipartite viruses is surprising because the lifestyle involves a significant cost, as all segments need to be present inside the same host (Sicard et al., 2016; Lucía-Sanz & Manrubia, 2017). Previous work has discussed potential advantages that could outweigh this cost (Nee, 1987; Nee & Maynard Smith, 1990; Iranzo & Manrubia, 2012; Valdano et al., 2019). One possibility is that there is a population-level advantage to multipartitism, allowing multipartite viruses to out-compete their monopartite ancestors. Alternatively, the evolution of multipartite viruses could have been driven by selfish selection, where individual viral sequences gain an advantage through becoming shorter, rather than selection for a mode that is mutually beneficial to all genome segments. This latter case suggests that cheating could play a role in the origins of multipartite viruses. One reason why multipartite viruses are more common in plants and fungi could be that in these hosts, viral gene products can be shared between cells, allowing for a larger number of viral genes to act as cheatable public goods (Sicard et al., 2019).

Cheating could also help to explain current problems in multipartite viruses, not just their origins. In many multipartite viruses, different genome segments accumulate at different rates, with some segments becoming nearly twenty times as abundant as others (Sicard et al., 2013; Hu et al., 2016; Wu et al., 2017). This presents a problem, because unequal frequencies should substantially reduce the likelihood that at least one copy of every segment is transmitted to a new host by a vector. One explanation for this is that the multipartite virus needs different gene products at different rates, and so variation in segment copy number could be an adaptation to allow for effective multipartite virus infections (Wu et al., 2017). An alternative explanation is that some segments allocate more time to replicating copies of themselves and less time to producing gene products (Chao & Elena, 2017). In this latter case, variation in segment frequency could reflect selfish selection on individual segments, potentially to the detriment of other segments (cheating), rather than selection for a set of frequencies that is optimal for the multipartite virus as a whole. There is the potential here for experimental work to clarify the relative importance of cooperation and conflict in multipartite virus evolution.

Where should cheats not evolve?

Mutual benefit but not cooperation. The term cooperation is sometimes used differently in the evolutionary and virology literatures. Cooperation is defined in the evolutionary literature as when a trait provides a benefit to another individual, and has evolved at least partially because of this benefit (i.e. the social consequences matter for the evolution of the trait) (West et al., 2007b). In contrast, in the virology literature, cooperation is sometimes used more widely, to describe cases where interactions between strains are mutually beneficial, but can be explained from purely selfish benefits (i.e. the social consequences don't matter for the evolution of the trait in question) (Vignuzzi et al., 2006; Shirogane et al., 2012; Stiefel et al., 2012; Skums et al., 2015; Xue et al., 2016a; Leeks et al., 2018). This difference matters, because we would not expect selection for cheats that exploit the 'cooperative' trait in the latter scenario, where the social consequences do not shape the trait.

To give a specific example, viral infections with two different variants can be more effective than infections with either of those variants alone. In the H3N2 strain of influenza A there are two variants, D-151 and G-151, that each encode a different version of a protein required for cell exit (neuraminidase) (Xue et al., 2016a). The version of neuraminidase encoded by one variant makes virions more effective at entering cells, but less effective at leaving them, whereas the converse is true for the other version. However, virions from cells infected by both variants have both forms of neuraminidase, so they benefit from both enhanced cell exit and enhanced cell entry. This phenomenon has widely been called cooperation, because both viruses benefit from the presence of the other. However, in this case, the benefits of a more infective virion immediately go to the offspring of both strains. Consequently, any 'cheat' variant that did not encode the mutually beneficial trait would suffer an individual fitness cost, and so we not expect such cheats to be favoured, even over the short term. More broadly, this highlights the potential pitfalls that can arise when different fields use the same term to mean different things (West et al., 2007b).

Defective but not interfering. Defective interfering genomes emerge in a wide variety of viruses due to errors in viral replication. However, error-prone viral replication does not always produce cheats. If defective mutants lack genes that are not potentially cooperative, and therefore that cannot be compensated by a functional copy in a coinfecting virus, then they are unable to spread. Defective mutants that are not interfering cannot spread may still have important effects on the outcome of the infection, such as by triggering host immune responses (Manzoni & López, 2018). However, they cannot be replicated inside cells and so they do not have the potential to evolve as cheats. Consequently, defective interfering genomes are cheats, but non-interfering defective mutants are not (Huang & Baltimore, 1970; Ghoul et al., 2013).

Should evolutionary biologists care?

Opportunities for empirical work. Viruses offer excellent opportunities for studying cheating. It is often relatively simple to link genomic changes to obligate social phenotypes, and coevolution between cooperators and cheats can occur over rapid observable timescales (DePolo et al., 1987).

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These factors facilitate a range of experimental manipulations, across many different virus systems. Furthermore, experiments can be complemented with the large amount of clinical genomic data available on viruses, which often reflects the 'natural' environment that viruses are evolving in.

We can also investigate the mechanisms of viral coevolution by comparing long-sighted cheats that have coevolved with cooperators over long periods of time (such as satellites) and short-sighted cheats that repeatedly and independently evolve de novo (such as defective interfering genomes). It would be possible to track both genetic and phenotypic changes in response to social adaptation in viruses, revealing both the drivers and consequences of (co)evolutionary dynamics.

New problems for social evolution. Types of social adaptation may occur in viruses that do not occur in other living things, presenting new kinds of traits for social evolution theory to explain. One example is that viral genomes could evolve to make it harder for cheats to arise through mutation (dos Santos et al., 2018). This may be the case in poliovirus, in which defective genomes that lack sections of the replicase gene are unable to be incorporated into virions, and so cannot become interfering (Novak & Kirkegaard, 1994). More broadly, after infecting a host cell, many viruses encode genes that prevent similar viruses infecting the same cell (superinfection exclusion), which could be a mechanism to avoid interacting with cheats (Doceul et al., 2010; Folimonova, 2012; Julve et al., 2013; Webster et al., 2013; Chung et al., 2014). On the other hand, it has been suggested that some viruses have evolved to produce defective and/or defective interfering genomes at higher rates. This could allow a virus to reduce the overall viral load without compromising its own relative growth rate, ultimately allowing for more transmission opportunities (Li et al., 2011; Vasilijevic et al., 2017). This latter case would challenge our notion of the evolutionary role of cheating, by suggesting that allowing mutations to cheating could be adaptive in some cases.

Should virologists care?

Cooperative traits are fundamental to the epidemiology and success of viruses. Essential viral processes such as constructing viral capsids, replicating the viral genome, and suppressing host immune defences are all potentially cooperative. At the same time, core features of viral biology, such as replication mode, collective transmission, and genome structure, can all influence the extent to which viruses interact with and are exploited by cheats. Consequently, the field of social evolution provides both conceptual and practical tools to help us understand the fundamental design and epidemiological success of viruses.

The prevalence of cheating in viruses suggests that selection on individual viral sequences can readily drive adaptation, even when this comes at a cost to other viral variants and is damaging to the infection as a whole. This challenges the idea that viruses should be defined at the group or 'quasispecies' level, since the potential for conflict is likely to prevent adaptations that are solely for the benefit of the group of viruses (Gardner & Grafen, 2009; Andino & Domingo, 2015). Social evolution theory is well placed to understand the diversity of viral infections by focusing on the interplay of cooperation and conflict between individual viral sequences.

In addition, social traits can be exploited as a mechanism to combat viruses. For example, therapeutic interfering particles (TIPs) are synthetic viruses designed to exploit wild-type virus cooperation, and to suppress viral infections by acting as a cheat (Metzger et al., 2011; Dimmock & Easton, 2014). Social evolution has a large body of work examining when cheats can spread, which could be harnessed for predicting when therapeutic interfering particles will be most effective (Brown et al., 2009; Ghoul et al., 2013). Furthermore, studying the dynamics of natural viral cheats may reveal which viruses are most susceptible to being suppressed by therapeutic cheats, how wild-type viruses may evolve resistance, and how therapeutic cheats might co-evolve in response.

Conclusion

To conclude, there are a wide range of viral cheats that exploit various forms of cooperation. Many of these cheats have the potential to play important roles in viral population dynamics, virulence, and evolution. However, much of the empirical work on cheating has been done in the laboratory, either in tissue culture or model hosts. It is not clear what role cheats play in the epidemiological dynamics of natural viruses, including those that infect humans, crops, and livestock. Can we explain the evolution of cheating in viruses with the same conceptual framework that explains cheating elsewhere in nature? Can we harness an evolutionary understanding of cheating for therapeutic purposes?

ACKNOWLEDGEMENTS

The authors would like to thank Rafael Sanjuán, Helen Leggett, and John Bruce for useful comments and discussion. A.L. was supported by funding from the Clarendon Fund, St. John's College, Oxford, and the BBSRC (grant number BB/M011224/1). This preprint makes use of the Henriques Lab preprint template.

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