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The great viral team-up

Viruses are no lone wolves. They have social lives and work together in ways we ignore at our peril, finds Graham Lawton

If your social life has suffered during the coronavirus pandemic, you may not want to know that the virus has a social life too. And it is probably better than yours right now.

It may seem odd to say that viruses fraternise when they arguably aren't even alive, but virologists are discovering just how rich this aspect of their existence is. Far from being lone operators, viruses cooperate and compete with one another; they can be altruists, freeloaders or cheats. These discoveries are rewriting the virus rule book and suggesting novel ways to tackle viral diseases, and that includes the newest one, Covid-19, caused by SARS-CoV-2.

Understanding these complex and sometimes strange interactions could be the key to getting our own lives back to normal.

The classical view of a viral infection doesn't create much opportunity for social interaction. A single virus particle, or virion, encounters a target cell and breaks and enters. Once inside, it disassembles like a cat burglar unpacking tools and then executes its potentially deadly genetic program.

This program is designed to do one thing: build an army of virus clones to move on to the next victim. To this end, the virus requisitions the cell's protein and genome production facilities, churning out millions of copies of its constituent parts. These viral genomes and proteins assemble into virus particles and, once they reach a critical mass, disgorge out of the host cell, killing it in the process. The infection cycle then begins anew.

This view isn't wrong, but is vastly simplified. Viral attacks are rarely solo missions. "The virion has been traditionally viewed as the minimal viral infectious unit," says Rafael Sanjuán at the University of Valencia in Spain. "However, single virions often fail to establish productive infections."

In truth, virions usually hunt in packs, and can infect cells en masse, alongside other species of virus. And this creates hitherto underappreciated opportunities for virus-virus interactions. The microbiologists who study such interactions in the new field of sociovirology say they can be understood using the same concepts developed to describe those between animals, plants and, more recently, bacteria.

Social evolution theory, which seeks to explain these interactions, grew out of attempts to incorporate complex animal behaviours such as cooperation and competition into evolutionary theory. One especially thorny problem was altruism, such as cooperative breeding systems, in which some individuals forgo the opportunity to reproduce in order to help rear others' offspring. Why make such a costly sacrifice? The answer turned out to be self-interest. Individuals will make sacrifices, but only for close relatives, and hence help to usher genes that are effectively their own into the next generation. As the great evolutionary biologist J. B. S. Haldane put it: "I would lay down my life for two brothers or eight cousins." This is known as kin selection.

Classical virologists did have some inkling that viruses interact with one another. They knew, for example, about superinfection exclusion: how, once a cell has been infected, other viruses are often blocked from entering it. But most weren't schooled in social evolution and were unaware that there were ready-made concepts to explain such things. And in any case, the "single infectious virion" dogma didn't require much in the way of social skills. True, the infected cell would end up full of viruses, but as they were all clones of the original invader there was little opportunity for complex relations to arise. >
colleagues of Sanjuán in Valencia made a surprise discovery about a phage called phi 6, which is among the most extensively studied viruses on Earth. They found that if bacteria were simultaneously infected with two slightly different genetic variants, the infection was initially more successful, but becomes less so over time. Exactly why wasn’t clear, but the researchers realised that the decline in fitness could be described using a mathematical approach called game theory to explain interactions, specifically a classic thought experiment known as the prisoners’ dilemma.

And contending with other viruses turned out to go way beyond shutting them out. For example, even when a host organism is infected by a single virus, it can end up harbouring a vast array of different viral genomes. Viruses have very high mutation rates; those with genetic material made from RNA have the highest error rate of any known biological entity. Newly minted genomes aren’t always proofread and mutations in their genetic code can accumulate at an alarmingly high rate. A single host organism can thus harbour thousands of different variants of the “same” virus, creating ample opportunities for competition and cooperation between them to evolve.

Another key discovery was that viruses often get together to hunt. They form a variety of “collective infectious units”, which can be as simple as an aggregation of identical viruses, or as complex as a bubble-like vesicle crammed full of many virions of two or more unrelated viruses. Viruses interact within the unit, and can also coinfect the same cell, again setting up the conditions for complex interactions. These coinfections turn out to be extremely common. “Coinfection is the default condition of humans,” says Díaz-Muñoz. “We always have several viruses in our cells.”

A lot of the early work on sociovirology was done with a class of viruses called bacteriophages, or phages for short. These look a bit like minuscule planetary landing craft that alight on the surface of bacteria and inject their genetic material into them. The doomed bacterium then becomes a phage production plant.

Phages were once seen as the quintessential solo assassins. But in 1999, colleagues of Sanjuán in Valencia made a surprise discovery about a phage called phi 6, which is among the most extensively studied viruses on Earth. They found that if bacteria were simultaneously infected with two slightly different genetic variants, the infection was initially more successful, but becomes less so over time. Exactly why wasn’t clear, but the researchers realised that the decline in fitness could be described using a mathematical approach called game theory to explain interactions, specifically a classic thought experiment known as the prisoners’ dilemma.

In this scenario, two partners in crime are
told that if they alone confess and snitch on the other, they will get a shortish sentence and their partner a long one. If they both confess and snitch, they will both get intermediate sentences. If they both stay shtum, they will both get an even shorter sentence. The best collective outcome is to say nothing, but neither can risk it in case their partner confesses. So they both confess and produce a less-than-optimal outcome. The phages appeared to be playing a version of this game. They initially kept quiet, but then one discovered the benefits of snitching and the other followed suit. In other words, they were interacting (anti)socially.

In another key discovery, phages infecting a bacterial colony were discovered to be sending molecular messages out of the cells they were in. When there were more viruses, the levels of signalling molecules increased and this communicated the overall level of infection, and hence whether it was time to burst out or lay low for a while. This is the phage equivalent of quorum sensing, a common cooperation strategy in bacteria.

Focus soon moved from phages to the viruses that infect mammals, including humans. One of the earliest discoveries that these viruses also cooperate came in 2005. Marco Vignuzzi, then at the University of California, San Francisco, engineered poliovirus – an RNA virus with a very high mutation rate – to replicate its genome with greater precision. He found that this “improved” virus was actually much worse at infecting cells. Exactly what was going on wasn’t clear, but Vignuzzi proposed that poliovirus mutants somehow worked together to boost collective success.

Of course, “working together” doesn’t imply intentionality, says Asher Leeks, a sociovirologist at the University of Oxford. It just so happens that swarms of mutants are better at passing on their genes, so this has been selected for by evolution.

Since then, cooperation has been documented in many other viruses, including measles, flu and hepatitis B. In 2016, a team at the University of Washington in Seattle found that an H3N2 influenza virus was more successful when two genetic variants coinfected the same cell. One variant is highly efficient at entering cells, the other is efficient at exiting. Neither is very successful on its own, but when they work together they are dynamite.

Further work has also suggested other ways viruses cooperate. Mutants might produce slightly different versions of a viral protein, some of which are slightly more successful under certain circumstances. It is unlikely that a single virus will acquire all of these beneficial mutations, but no matter. Viral proteins and genomes mingle inside the cell and become “public goods”, another key concept from social evolution theory. By dipping into this pooled resource, a perfectly adapted virus will probably assemble and lead the charge out of the host cell.

Viruses have even been observed performing the ultimate social interaction: altruism. The hepatitis C virus, for example, maintains an army of mutants, some of which strongly attract the attention of the immune system and allow others to fly under the radar. These decoy mutants aren’t successful individually, but they evolved to take one for the team.

Last year, Sanjuán’s team discovered another form of viral altruism. It showed that an RNA virus called VSV, or vesicular stomatitis virus, can suppress the immune system of its host – usually a horse, cow or pig, but occasionally a human – by producing a molecule that inhibits antiviral interferons, substances released by cells that alert neighbours to viral attack.

Engaging in this sort of chemical warfare is an expensive investment for the virus and it has a detrimental effect on its reproductive fitness early on in an infection, but it paves the way for future success by keeping nearby cells susceptible.

**Farewell freeloaders**

The team then introduced a mutant VSV that doesn’t suppress interferon, and can therefore freeloade on viruses that do. As expected, this mutant took advantage and outcompeted the non-mutant, but its cheating eventually caught up with it and it was nailed by the interferon system.

Over the longer term, viruses that made the initial sacrifice are much more successful than the freeloaders.

If at this point you are thinking, “OMG, they’re ganging up on us”, here is some comforting news. Social interactions can also be a real drag for viruses.

The creation of public goods opens the door to cheating or freeload. As we have seen, this can be overridden by altruism, but that is a rare exception. More often than not, viruses fall victim to the classic “tragedy of the commons”, where everyone hoovers up public goods as fast as possible until they are all gone and everybody loses.

In a cell that is coinfected with two unrelated viruses, for example, both types may compete for a genome-replicating enzyme produced by one of them. Selection pressure will then favour freeloader genomes that can utilise the enzyme, but don’t make any themselves, at which point there isn’t enough to go around and the infection can grind to a halt.

Freeloader genomes are a major problem for viruses, especially RNA ones. “Cheating is common in the viral world,” says Leeks.

RNA viruses are such sloppy copiers of their genomes that they churn out all sorts of useless junk – half-finished ones, ones with...
crucial bits missing or mere fragments. These are known as defective interfering genomes (DIGs), and for good reason. They consume public goods such as enzymes without contributing any themselves, and even though they are assembled into virions and are ejected from the host cell in search of pastures new, they can’t establish an infection on their own.

Being significantly shorter than complete genomes, defective interfering genomes are copied at much higher rates – up to 80 times faster, says Leeks. That means they hog the replication enzymes and end up vastly outnumbering complete viruses.

At this point the virus is itself being parasitised, to the extent that – in tissue culture at least – an infection can spontaneously fizzle out. This is such a problem for viruses in general that virologists are attempting to exploit it as an anti-virus strategy. “If we can engineer or isolate defective interfering genomes, maybe they would be an effective therapy,” says Díaz-Muñoz.

So, what of SARS-CoV-2? For now, its social life remains largely unexplored as virologists focus on more pressing questions. From what we know about coronaviruses, it seems likely that important social interactions are occurring, with possible consequences for the future of the pandemic. “I have no doubt that there will be sociovirological aspects,” says Díaz-Muñoz.

For starters, SARS-CoV-2 is an RNA virus, which raises the spectre of a hyperfast mutation rate. But fortunately, it is a very unusual RNA virus, which proofreads new copies of its genome, so doesn’t generate swarms of mutants. “The genome is exceptionally stable for an RNA virus,” says Díaz-Muñoz.

Nonetheless, it still generates oodles of defective genomes. That is because the enzyme it uses to replicate its genome is highly promiscuous and frequently jumps from one part of the RNA template to another, so produces multiple incomplete fragments. It isn’t yet known whether these are interfering genomes that cheat and hog public goods, but if they do, it offers a new target for drug development via artificial DIGs. The group in Valencia recently began research into this possibility.

There are also signs that SARS-CoV-2 forms collective units, and that it takes more than a single virus to sicken a human. The current estimate of the number of viruses required – called the infectious dose – is quite imprecise, ranging from tens to tens of thousands. A clearer understanding of this could help us to stay out of harm’s way. “The infectious dose is one of the most critical pieces of data that we don’t have that could inform public health,” says Díaz-Muñoz.

There is also good evidence for coinfection. “We know that humans can have SARS-CoV-2 and other viruses – basically all your suspects for the common cold,” says Díaz-Muñoz. That includes rhinoviruses, influenza viruses, parainfluenza viruses, respiratory syncytial virus and four other coronaviruses in general circulation.

The effect of SARS-CoV-2 coinfection in the human body isn’t yet known, says Díaz-Muñoz. It may be why different people react so differently to the virus, from being asymptomatic to dying from it and a range of outcomes in between. But coinfection does open the door to more danger. Because the enzyme that makes copies of the viral genome has a tendency to jump about mid-task, it can stitch together genomes from two different coronaviruses that coinfect the same cell. This is probably what gave rise to SARS-CoV-2 in the first place, says Díaz-Muñoz: a mash-up of two different bat coronaviruses. And it could happen again, in a person coinfected with SARS-CoV-2 and common cold coronaviruses.

It sounds scary; yet another new coronavirus to contend with. But in fact a hybrid might be less virulent than SARS-CoV-2 and could ultimately outcompete it. “There’s a tendency to think that any new mutation leads to catastrophe,” says Díaz-Muñoz. “But ‘we’re all going to die’ is from the movies. It may actually confer a more mild progression of the disease, and that may be evolutionarily advantageous to SARS-CoV-2. It could spread more easily and go on to infect huge proportions of the human population.”

That would be a huge improvement to the virus’s social life – and ours.