

Viruses and Biodiversity

Before my career switch to work on the Nectandra Project, my professional training was first as a molecular biologist, followed by a postdoctoral stint in medical virology. In every phase of my training, my understanding of the role of viruses was strictly that of pathogens. Within the last ten years, new data and astounding information led me to think of viruses in a brand new light, one that every conservationist (myself included) should be aware of, but probably isn't, due to the newness of the information. So, even if the topic is slightly off track from our usual newsletter subjects, now is a pertinent time to talk about viruses and where they fit in the big picture.

In the past year humans had to take a jolting, compulsory crash course on the novel virus (SARS-CoV-2) that causes the disease known as Covid-19. Millions have suffered its effects, with no end yet in sight. We are struggling to learn the evolving lessons, but the course is far from over. The final exam is still ahead of us. By now, everyone on this planet has acquired some knowledge about viruses.

Our current obsession with Covid-19 has shadowed our view of viruses in general. Their importance centers on how they affect our health and welfare. Historically since the turn of the 20th century when viruses were first discovered, they were studied mainly as pathogens of humans, of animals, and agricultural crops. Even as recently as 2005, viral biodiversity was poorly known. We didn't know where most of them were, their numbers or their roles in our planetary ecosystems.

What are Viruses?

Structurally, viruses are basically capsules containing viral genomes and hardly anything else. They have none of the machinery to survive or reproduce on their own and must parasitize other organisms. Some biologists do not even consider them as living organisms, but something in between live and non-living.

To survive and reproduce, they must first come in contact with susceptible prey, attach and inject their genetic material into the host, then commandeer the host cellular

machinery to replicate, and finally, be released from the killed host for another cycle of infection. The average replication cycle takes from 20 minutes to two days depending on the virus. Not all viruses kill and lyse their host cells. One category of viruses is more sinister. Upon entry, the viral genome is integrated into host genetic material. The virus, in effect, becomes a "mole" hidden in the host genome to escape detection while waiting for optimal conditions, indefinitely if necessary. From time to time, these latent viruses can reactivate and cause havoc from within. Examples of these include the herpesviruses (causing cold sores, infectious mononucleosis) and the retroviruses (HIV) in mammals.

Viruses are difficult to study because they are small, very small. For example, the SARS-CoV-2 virus is 3000 times smaller than a grain of salt. So almost all viruses are virtually invisible. There are three main ways to find them:

- Grow them in test tubes seeded with susceptible cells. Viruses, unfortunately, are host specific and cannot grow in cells when there is a mismatch — a good thing biologically, but a huge research challenge when working with a virus of unknown origin, such as the situation with Covid-19 at the beginning of the pandemic. This exacting biological method is therefore of limited value as a tool to study viruses at large.
- See them highly magnified (~100,000 x) by electron microscopy. For this, virus samples are first concentrated down to a tiny volume, then inspected by microscopy using an electron beam, which acts analogous to a light beam in standard microscopy. In transmission electron microscopy, the samples are cut into ultra-thin sections so that the beam can pass through for viewing and contrast (Fig 1).

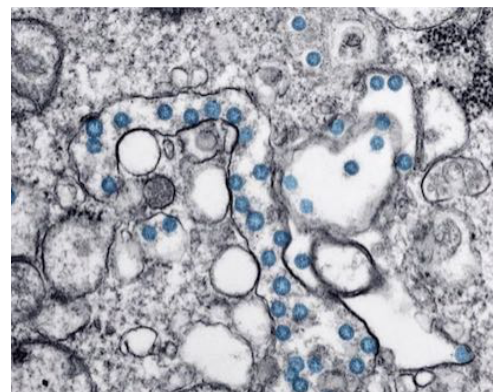


Fig.1 Transmission electron microscopic image of an isolate from the first US case of COVID-19. The spherical viral particles are colored blue. Credit: Centers for Disease Control.

In scanning electron microscopy, secondary electrons reflected off the sample surfaces are detected. The

samples can be thicker but only external contours are visualized (Fig. 2).



Fig. 2. Scanning electron microscopic image of SARS-Cov-2 (colored in magenta) emerging from the surface of cells cultured in the lab. The virus was isolated from a patient in the US. Credit: NIAID-RML.

Electron microscopic inspection, however, is relatively insensitive and requires a lot of concentrated viral particles. Imagine the difficulty of detecting 3-4 viral particles in 100 liters of water (the average concentration of human pathogens along the world's popular beaches) for electron microscopy.

- Molecular methods since the 1990s bypass the above limitations. They directly test for viral genomes in the samples, therefore circumventing the difficulties of growing the viruses or of visualization by electron microscopy. Unlike the previous methods that focus on one specific virus, it is possible to search for many at a time, including unsuspected viruses. These techniques completely revolutionized the way we look for viruses, microbes, or for that matter, any life form. They spawned a brand new scientific discipline known as metagenomics — the study of genetic material recovered from the environment.

Where are the viruses?

Current information is still vastly incomplete, but the picture is rapidly coming into focus.

The general emerging picture is that 90% of the world's viruses are associated with oceanic plankton (*i.e.*, collections of microscopic drifting organisms at the ocean surface), and land microbes in animals, plants, and soil.

In 2004, armed with newly developed molecular tools, Craig Venter and his team ambitiously sampled, in a Herculean effort, marine microbes at a subtropical study site off Bermuda in the Sargasso Sea. From just 1500 liters of ocean, they recovered 1.2 million new genes, representing at least 1800 species of microbes, including hundreds of new species of bacteria and viruses. By 2010, similar surveys showed around 5000 novel viral types per 200 liters of seawater. Since then, the explosion of discovery of new viruses from similar environmental studies has been mind-boggling.

Viruses are ubiquitous everywhere on the planet. They vastly outnumber their preys. For example, in a study of just one bat species (*Pteropus giganteus*)— the Indian flying fox, known to harbor many human pathogens — at least 58 different viruses, including a dozen novel ones, were found. Multiplied by the number of known species of fauna and flora, the estimated global viral load is astounding. Just in the ocean alone, there are a staggering 1 nonillion (with 31 zeros!) viruses.

Another example is the human virome, *i.e.*, the total collection of viruses in the human body, roughly calculated to be about 0.5 quadrillion (with 15 zeros). The more we look, the more we find. These numbers will likely turn out to be gross underestimates.

Viruses in the Ocean

Metagenomics began with the sea because the ocean covers three-fourths of the planet and the sampling of water is relatively straightforward. We now know that the most abundant oceanic organism is a large group of yet unclassified bacteria currently designated as



Fig. 3 Photosynthesizing plankton bloom off Argentina. Image: MODIS Land Rapid Response Team at NASA GSFC.

SAR11 (for Sargasso clone 11), which constitutes 40% of the oceans' plankton. SAR11 bacteria, in turn, are With the ensuing viral-bacterial tug of war, the lysed bacterial cells release an enormous quantity of fresh nutrients for the next round of microbial growth. In essence, viruses act as a biological pump for oceanic carbon cycling.

It is not difficult to guess that the warming of ocean water with climate change will greatly affect the health of our planet.

Viruses in Soil

There is a lot less information on viruses in soil communities, as soil virology is more challenging due to the heterogeneity of soil types, ranging from crop lands, to deserts, wetlands, hot springs and permafrost. Only a handful of studies have been done to date. It is impossible, for example, to sample an equivalent of 1200 liters of soil, as was done with the Sargasso Sea survey.

Soil viruses are associated with the root microbiome in the interface between plant and soil. Unlike the ocean, where bacteria dominate, local soil varies in richness of microbes which include the archaea (bacteria-like organisms incapable of living with oxygen), bacteria and fungi, which in turn influence the diversity of the local viral communities.

Per gram of soil, viral content ranges from less than one particle in deserts to billions in wetlands. In the more extreme environments, viruses are the only microbial predators, attesting to their adaptability to harsh conditions.

Viruses in Fauna

Most animal viruses are associated with gut microbes. In humans, there may be as many as 1000 intestinal viral types, many of which have not been characterized. Metagenomics show that the gut microbiome to be stable numbers on the planet should make us take notice of their effect on life of the planet and their impact on the global carbon cycle.

Viruses kill prey selectively. Their short life span means a high turnover among themselves as well as that of their infected prey. They are key drivers of biodiversity and geochemical cycles in the ocean, on land and everything in between.

Viruses are genome traders and gene movers. They exchange genomes among themselves and with their hosts. Replicating viruses can occasionally carry bits of host genes during assembly, only to leave genes behind in

infected by a group of viruses known as bacteriophages. Viral predation follows the plankton blooms (Fig. 3) brought on by warm temperature and surges of nutrients over time and idiosyncratic (specific to each individual). Each person's microbiome is analogous to fingerprints.

Recent metagenomic analyses of human blood showed that there are a number of unknown viruses in healthy human blood. Whether any of these novel viruses are

pathogenic remains to be studied. The presence of novel viruses in human blood, of course, is of great concern because of the human-human transmission risk associated with blood donation, medical procedures and insect bites.

Viruses in Plants

In 2006, there were about 2000 recognized plant viruses. By 2010, in a single viral genomic survey of the 7000 plant species in Guanacaste, Costa Rica, several thousand new plant viruses were discovered. In fact, known plant viruses were rare among several other metagenomics surveys, an indication that there are still a vast number of plant viruses to be discovered.

There is no information what roles these new viruses play in their host plants or environment, creating a huge gap in our understanding of plant viral diversity and ecology. Better information on viral presence in plants is becoming critical to conservation, food security, pest management, and international regulations concerning the transportation and exchanges of plants and their pests among trading countries.

What IS the Big Picture?

Invisible to us, the planet is bathed in a mantle of viruses. They are everywhere. Their diversity vastly exceeds those of other life forms. They dominate every biological ecosystem studied, natural or man-made. Their sheer

the next new host. In effect, viruses are gene importers/exporters that can circumvent species barriers. For example, influenza can infect more than one animal host (e.g. birds and humans), generating new recombinant viruses. This capacity to move genes contributes to diseases (and disease resistance, at least in plants), and ultimately, to evolutionary changes.

Viruses are ecosystems invaders. Some viruses are able to move in hosts across large distances and from different ecosystems (e.g., viruses from infected plants passing through human guts and then expelled with the seeds). In effect, the viruses can use their host as transport from one ecosystem to another.

Our knowledge of viral ecology is still in its infancy, but one thing is already certain. Viral diversity is immense. Viral functions, both harmful and beneficial, are only beginning to be appreciated and mined. In the meantime, we can certainly admire one example of viral contribution to the aesthetic of plants (Fig 4).



Fig 4. Two coexisting wild violets from the Ozarks Mountains in Arkansas, uninfected (left) and infected (right) by *Cucumovirus* via aphids, with no apparent foliage damage. (<http://dx.doi.org/10.1094/PDIS-11-11-0928-FE>)

— Evelyne T. Lennette —

News Highlights

June-July — Year 2020 was a providential year for plantlet production, as we put extra work with social social distancing in the outdoors. The league (Liga CUENCA) of 12 water management associations that have obtained ecoleons from Nectandra Institute (NI) started a nursery as part of the “eco-interest payment”, with the objectives of supplying and sharing native trees for reforestation of the members’ properties.



In addition to the 650 trees grown from seeds of 20 native species this year, voluntary wild seedlings along roads (which were at risk of being cut as part of the road maintenance) were bagged into growth bags to add to the nursery inventory. About 1000 seedlings of 25 native species were produced this way. They were distributed to 4 ecoleon properties this year.

A few of the properties were difficult to reforest due to a combination of steep slopes and fierce wind. After several failed attempts with direct transplantation, banana plants as nurse plants turned out to be the solution, as they were able to adapt to challenging terrains and conditions. They, in turn, protected newly transplanted native seedlings from sun and wind. Their fruits also attracted act both as seeds importers and seed dispersers. Nectandra.org

Sept — Nectandra Institute (NI) held its first virtual New Culture Water Month. The annual multi-communities event included educational and popular ludic activities. It was celebrated on the different social networks, as well as virtual meeting forums and round tables hosted by NI.

During the festivities, participants were asked “What motivated you to work and contribute to your water management associations?” “Commaraderie”, “pride”, “good experience” “responsibility”, “commitment”, “solidarity” were some of the expressed feelings. Video of event: <https://www.youtube.com/watch?v=kSg5JncGzZc>

Nov – The paper titled, *A taxonomic and molecular survey of the pteridophytes of the Nectandra Cloud Forest Reserve, Costa Rica*, authored by Joel H Nitta, Atsijjo Ebihara, Alan Smith, was published in PlosOne (<https://doi.org/10.1371/journal.pone.0241231>). It summarizes Nitta’s years of collecting and molecular studies on the exceptionally diverse fern species (176 total) at the Nectandra reserve. (See 2020-1 Newsletter)

Dec — NI helped with the planning and the meeting logistics of the VI National Water Community Management Associations Conference. This event was planned for months and took place in the first week of December. Panelists from various parts of the country participated, as well as international participants from Brazil, Ecuador and Colombia.

— Liga CUENCA's nursery has a new ally. One of three large hotels in the Balsa Watershed will purchase native trees produced by the Ligua nursery to reforest the lodge’s extensive grounds used by the lodge’s guests. The first delivery of trees to the lodge was in December.

Aug to Dec — Three neighboring federations — FEDAPRO, UNAGUAS, and the Liga CUENCA — consisting of about 40 water management associations, joined effort to propose modifications to three Congressional bills that will affect the management of the water associations. The federations met three times with the legislators, made their reports and negotiated changes in the bill that will be considered by the Legislative Assembly.

For additional information, please visit:
 Website: nectandra.org
 Blogsite: serendips.net