Ten things we learned in 2010-2019 (aside from everything else)

Sophien Kamoun

The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich NR4 7UH, UK

He who has studied himself is his own master. –Sri Lankan proverb.

By the time this gets posted, you'll probably be sick and tired of all those retrospective articles looking back at the 2010-2019 decade. I feel your pain. But hey, we're still early in the new decade and I have a good reason for writing this. This last decade has been such an exhilarating period of exploration and discovery for me, my team and my collaborators that I just can't resist the urge to write this post. The decade took us through unexpected research paths that I would have never imagined ten years ago. As I'm drafting these words during my holidays break in Sri Lanka—in between tasting the local milk rice curries and soaking the soft Indian ocean December sunshine—I'm reflecting on the local proverb above and I'm using it as my lame excuse to offer you yet another list of decadal achievements.

Please note that this is my personal highly biased perspective on ten things we have learned in 2010-2019. This list is by no means meant to be comprehensive review of advances in our research field but rather a reflection of my own personal take on the scientific topics we investigate.

2010. Two-speed genomes, everywhere? What started as a loose metaphor inspired from economics went sort of viral at some point in this decade, sometimes to comical effects (one speed genome anyone?). To those who struggle with metaphors, the idea is that there is an uneven distribution in the rates of gene evolution across the genome, not that there are *precisely* two-rates of gene evolution. The term actually dates back to 2009 press releases associated with the Haas et al. paper on the genome of *Phytophthora infestans*. We noted at the time that it was a catchy term that does illustrate the point we were making, and I really liked the French translation into the poetic "génome à deux vitesses". By 2010, we were confident enough that we formally used the term in the Raffaele et al. paper on genome evolution after host jumps. What was even more exciting about the "two-speed genome" concept is that it turned out to apply not just to *Phytophthora* genomes but also to many other plant pathogens. Ten years later, we start the new decade with a paper on the two-speed genome architecture of the blast fungus *Magnaporthe oryzae*.

2011. WY fold—commonalities amid diversity. Our collaboration with Mark Banfield started yielding its fruits with the 2011 Boutemy et al. paper. This and related papers by the Staskawicz and Shirasu Labs in the US and Japan, respectively, marked the discovery of the WY-fold of oomycete effectors. This has now expanded into the LWY-fold and an effector of the tobacco blue mold pathogen *Peronospora tabacina* has 18 WY units. The finding that pathogen effectors share structural features despite limited primary sequence similarity has also extended to other filamentous pathogens, for example the MAX-fold of *M. oryzae* effectors. This is very useful because it improves prediction of effector genes from pathogen genomes and sets the stage for effectoromics.

2011. The haustorial interface—where it all happens? I'm a big fan of this Bozkurt et al. paper because it was very challenging for me to get outside my comfort zone into the murky world of plant cell biology (where many people seem reluctant to quantify their observations…). Kudos to Tolga Bozkurt and Sebastian Schornack for leading the way and taking me through this journey. As often, the effectors gave us the first clue and the discovery that some *P. infestans* effectors accumulate at the haustorial interface (perihaustorial) turned out to be a starting point for many cool projects. Thanks in part to a nudge from an anonymous reviewer who was dissing the novelty of studying effectors that suppress PAMP-triggered

immunity (the "it has all been done with *Pseudomonas syringae*" type of reviewer), we decided to focus on perihaustorial effectors. This resulted, in many important findings, notably the discovery of the ATG8-binding effector PexRD54 and that the host autophagy machinery is diverted to the haustorial interface during infection by *P. infestans*. This also led us to study plant ATG8 proteins and how they have specialized throughout evolution.

2013. Genome editing made easy. Ten years ago, geneticists were dreaming about gene editing. What if there was a tool that would allow facile gene editing. TALENs popped up first in 2009 but, in our hands, applying them turned out to be anything but simple. Vlad Nekrasov noted that the AvrBs3 backbone of standard TALEN constructs wouldn't generate transgenic tomatoes because they elicit Bs4-mediated cell death. That frustration was one motivation in early 2013 to ditch the TALEN work and focus on the newly reported CRISP/Cas9 system. That was a wise decision and Vlad got CRISPR/Cas9 to work in what seemed like weeks. The rest is history with Vlad's CRISPR/Cas9 plasmids have been distributed >500 times via Addgene. Vlad, in collaboration with Detlef Weigel's lab, went on to engineer the transgene-free powdery mildew resistant mutant Tomelo in less than a year. This work ended up being highlighted by the BBC as one of "four good things that happened in 2016".

2013. Field pathogenomics—just sequence it! It was the ash dieback outbreak that gave us our first opportunity to combine sequencing of field collected tissue with open science and crowdsourcing to mount a rapid response to plant health emergencies. Back then it did feel like plant pathology was lagging behind in immediately applying genome sequencing to emerging plant pathogens. Diane Saunders, Kentaro Yoshida and Dan MacLean managed to put OpenAshDieback together and release a draft of the pathogen's genome just weeks after the outbreak was detected in Norfolk. Diane then applied the approach to yellow rusts and we later used field pathogenomics to identify the origin of the pathogen that caused the 2016 wheat blast outbreak in Bangladesh. That project kicked off a very inspiring collaboration with Tofazzal Islam and Nick Talbot and further strengthened my dedication to advocate for open science. It also changed the research direction of my lab, especially after the BLASTOFF project was funded by the ERC.

2013. Going back to the past to better prepare for the future. It's not every day that you get lampooned by the Colbert Report. Stephen Colbert was correct, it wasn't the 1b haplotype of *P. infestans* that triggered the Irish famine disaster, it was HERB-1. Our collaboration with Hernan Burbano, Detlef Weigel and several others on sequencing *P. infestans* genomes from 19th century herbarium samples, received incredible media coverage. With Hernan having recently started a new position at UCL, you can expect more pathogen aDNA projects in the future. Stay tuned.

2014. Effector adaptation after jumping hosts. There are literally dozens and dozens of examples of rapid evolutionary adaptations in plant-pathogen interactions in which the precise mutation is known. It's no big deal to find a new one these days. But almost all of these are AVR effectors that overcome host resistance. What Suomeng Dong and others documented is an effector that has adapted to a new target after switching hosts. Suomeng showed that the protease inhibitor effector EPIC1 has undergone biochemical specialization on the protease of its new host. This paper builds up on work dating back to the 2000-2009 decade by PhD students Miaoying Tian who discovered the protease inhibitor effectors of *Phytophthora* and Jing Song who further studied the EPICs. It was also the point when we decided to center the lab around the theme of evolutionary molecular-plant microbe interactions or #EvoMPMI as it's known on Twitter.

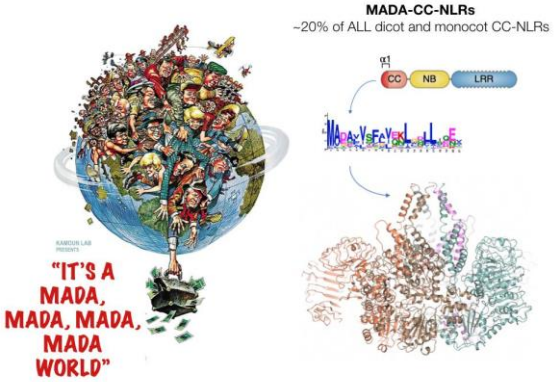
2015. The beauty of a protein complex structure. Stella Cesari and her colleagues deserve much credit for articulating the NLR integrated decoy concept, although some of us prefer to use the more agnostic term integrated domain (NLR-ID). I'm thrilled to have been the matchmaker who helped link up the amazing work of Ryohei Terauchi on rice blast effectors

and R genes with the structural biology magic of Mark Banfield. This resulted in bringing an unprecedented level of detail to Harold Flor's gene-for-gene model with Abbas Maqbool solving the structure of *M. oryzae* AVR-PikD in complex with the integrated HMA domain of the rice immune receptor Pik-D. Mark and his team went on to publish a series of trail blazing follow-up papers on how to exploit this knowledge to engineer new disease resistance specificities (De la Concepcion et al. 2018, 2019; Varden et al. 2018).

2017. Do NLRs work in pairs—it's more complicated! In what was initially a follow-up study to the AVRblb2 project of Bozkurt et al., Chih-hang Wu, Ahmed Abd-El-Haliem and Jack Vossen "accidental" discovery that NRC4 is necessary for Rpi-blb2 ended up having some very unexpected ramifications. Chih-hang's PhD took quite a turn when he followed up on a suggestion by Khaoula Belhaj to silence multiple NRC paralogs and uveil a complicated NLR network. He went on to his most insightful discovery that the NRC network is phylogenetically structured and has expanded over 100 million years ago (Mya) from an NLR pair to a network that makes up to half of the NLRs of asterid plants. All this cool stuff ended up taking over my research program by storm, with Team NRC making up half of my lab. It also led to the fascinating research question of how NLRs have evolved from singletons to pairs to networks. Meanwhile, Chih-hang is starting his new lab at Academia Sinica in January 2020.

2019. The coming of age of the plant resistosome. Courtesy of Jijie Chai, Jian-Min Zhou and their collaborators, 2019 brought us a full-length NLR structure some 25 years after their discovery in the early 1990s. But these landmark papers by Wang et al. (2019a, 2019b) had much more than that. They showed that they could activate the ZAR1 resistosome *in vitro* by flooding it with ATP. This results in the "death switch", a conformational change that generates a funnel-shaped structure that is proposed to insert into the plasma membrane and cause cell death. Beyond this extraordinary breakthrough, we had good reasons to celebrate—as we did in this video. The ZAR1 death switch model immediately explained some two-year old results that Hiroaki Adachi and Adeline Harant had produced with our own NRC4. This led Aki to discover the functionally conserved N-terminal MADA motif of NLR proteins that defines the N-terminus of NRC4, ZAR1 and at least one fifth of CC-type NLRs. We predict that a ZAR1 type conformational "death switch" is a common activation mechanism for CC-NLRs. What a way to end the decade. IT'S A MADA, MADA, MADA, MADA WORLD!

Conclusion. Over the last decade, the research topics in my lab have drifted from a focus on *Phytophthora* genome and effector biology to new interests such as *M. oryzae* and NLR biology. I heard that several colleagues find this puzzling. Some of the drift can be explained by a tendency to follow Peter Medawar's maxim of "science is the art of the soluble". Another reason is an obsession with Keplerian thinking—unexpected findings are opportunities to explore new research avenues and shouldn't be dismissed because they don't fit the current theory. Also, some of the projects moved on to greener pastures when postdocs took on independent positions at other institutions and it didn't make any sense for me to continue working on those topics. This said, there is probably more to this willingness to jettison projects and switch to new ones. I should think deeper about this. After all, "he who has studied himself…"



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