Adaptation in plant pathogenic fungi

For 15 years, the team has used combinations of various approaches (laboratory experiments, field work, modeling, population genetics and genomics) for contributing to our understanding of the fungal diversity and of how organisms evolve and adapt to their environment. The question of adaptation is fundamental to understand the living world, and it also has applied importance within the context of current global changes (climate change but also movements of organisms worldwide and habitat disturbance) and increasing food demand. In addition, microbial diversity and its evolution are still poorly known, while fungi have paramount ecological and economic importance. Indeed, fungi ensure many services in ecosystems (like recycling the organic matter or establishing essential symbioses with plants), they can be the dangerous pathogens in natural populations and crops, and they were domesticated for a variety of uses, such as food fermentation and antibiotic production.

For some years, the team has developed genomic studies, to obtain new perspectives on the mechanisms of adaptation. Fungi are good eukaryotic models for these questions, given their small genomes, their accessible haploid phase, their experimental tractability and the functional data available. We could obtain a complete assembly of the genome of the fungus *Microbotryum lychnidis-dioicae*, thanks to the recent improvements of the Pacific Biosciences technology, which produces very long reads. This assembly was the first eukaryotic genome published with chromosome-length assemblies using new sequencing technologies. The analysis of the *M. lychnidis-dioicae* genome revealed features linked to its obligatory biotroph lifestyle, with in particular a repertoire of putative effectors in the form of small secreted proteins and of expansions of gene families, such as lipases which could contribute degrading cuticular waxes of the host plant.

We also have sequenced the genomes of fifty individuals to study the prevalence and genomic bases of adaptation in *M. lychnidis-dioicae*. A recent episode of adaptation leaves particular signatures: for example when a beneficial allele is selected for, the frequency of this allele in the population increases quickly, as that of the linked alleles, creating a local decrease in diversity. This is called a selective sweep. Our population genomic study revealed selective sweeps in ca. fifty regions of the genome in *M. lychnidis-dioicae* (17% of the genome was affected by recent selective sweeps). In the genomic regions of selective sweeps, the most represented functional categories were related to interactions with the host. **This represented one of the first studies of selective sweeps at the level of a completely assembled eukaryotic genome, and the results showed that an unexpected proportion of the genome was involved in recent adaptation events and identified key functions. Overall, these results are important for our understanding of the evolution of new species of emerging pathogenic fungi, and their adaptation onto new hosts.**

We also have sequenced several genomes of the fungus *M. lychnidis-dioicae* parasitizing plants in areas more or less contaminated around Chernobyl and studied the frequency of the disease, the capacity of spores to germinate and grow. We did not find any footprints of deleterious changes in the genomes or decrease in viability which would be correlated with the radiation levels in the field, likely because the red pigments of the fungus protect it from radiations. In fact, many fungi with pigments are very resistant and can grow close to the damaged reactor. On the other hand, the frequency of the disease decreased with the rate of radiation in the field, likely because the abundance of the pollinators decreased, while pollinators transport the spores of the fungus, and pollinators are sensitive to radiations.

We will also use approaches of comparative genomics to understand the mechanisms underlying adaptation and the formation of new species using as models the *Microbotryum* pathogenic fungi, causing the anther-smut disease sterilizing plants of the Caryophyllaceae family. Indeed, there exists more than 100 species in this genus causing anther-smut disease, the various species being phylogenetically close but specialized on different host plants. There was thus an adaptive radiation, with specialization events on very different and well identified ecological niches, which makes it an excellent model to study the underlying genomic mechanisms of adaptation. We already obtained genome assemblies of exceptional quality for 20 pathogenic species of ***Microbotryum* specialized on different plants. This constitutes a unique material, making it possible to study poorly known processes, like the role of the gene gains and losses, chromosomal rearrangements, and localization in particular genomic regions.** Indeed, new sequencing technologies did not allow until one or two years ago to obtain information on the genomic structure, whereas it is essential to understand whether certain genomic areas, such as regions rich in transposable elements, telomeres, or chromosomal inversions, play a significant role in the processes of adaptation, by generating variability or by protecting beneficial association of alleles from recombination. **This constitutes a new frontier of research regarding the genomics of adaptation.**

Evolution of disease virulence and multiple infections

We have studied the factors influencing the virulence of diseases, i.e. the severity of their symptoms, to try to understand how and why pathogens adapt to become lethal or on the contrary benign. By using again the *Microbotryum* fungi as biological models, we carried out one of the first theoretical studies validating some important theoretical expectations: 1) multiple infections (i.e. the presence of several genotypes of a given pathogen in the same host) increase virulence, while 2) genetic relatedness between pathogens within a given host decreases virulence by increasing kin cooperation; 3) castrating parasites are selected to increase the survival of their host, to be able to be transmitted longer.

Overall, these studies contributed to a better understanding of 1) how new diseases can emerge in natural ecosystems and agrosystems, 2) how pathogenic fungi adapt to new hosts or global changes, 3) what are the factors controlling disease virulence. These questions are not only academic exercises; they have important applied consequences, in agriculture and medicine, but also for the maintenance of the biodiversity.

Biological invasions of plant pathogenic fungi

The team has studied in particular biological invasions of pathogenic fungi which lead to new emergent diseases. Our work on biological invasions was at the forefront of research, showing that biological invasions can redistribute the genetic diversity from the area of origin and allow hybridizations between populations previously isolated, creating new genotypes and fostering rapid adaptation. Invasions of pathogenic fungi however can occur successfully starting from a very low number of genotypes while causing serious emerging diseases on natural ecosystems or crops. The team studied in particular of the castrating anther-smut on Caryophyllaceae, the invasions of the grapevine downy mildew (in collaboration with INRA Bordeaux, F. Delmotte), and of the oilseed rape pathogen *Leptosphaeria maculans* (in collaboration with INRA Bioger, T. Rouxel), by using genetic markers and sophisticated methods of population genetics, making it possible to test evolutionary scenarios. For the grapevine downy mildew, we showed that its introduction in Europe from the United States at the beginning of the 20th century had resulted, by a ‘leap-frog’ event, in two differentiated populations in Europe, separating Western and Eastern Europe, because of reduced commercial and human exchanges. For the castrating anther-smut fungus *Microbotryum lychnidis-dioicae*, the team showed that the invasion of the disease in the United States on the white campion at the beginning of the 20th century could be successfully achieved by the introduction of only 2 individuals from Scotland. **These studies thus overall contributed to a better understanding of how invasions by pathogens can occur, which has important applied consequences in agriculture but also for the maintenance of the balance of natural ecosystems in the face of global changes.**

Speciation in plant pathogenic fungi

We also developed studies on the general evolutionary mechanisms at the origin of new diseases on plants (in natural ecosystems or crops) caused by fungi, by using various approaches. We used mathematical modeling to show the possibility of an evolutionary mechanism unrecognized so far, allowing the rapid emergence of new pathogenic species by specialization onto new hosts: the model showed that the life cycle of fungi mating within their host largely facilitated adaptation, specialization and genetic divergence. Comparative analyses carried out by the team confirmed that fungi reproducing within their host plant diversified more easily by adaptation to new hosts, without requiring evolving other mechanisms of reproductive isolation. The monitoring across several years of populations of *Venturia inaequalis*, a pathogenic fungi responsible for apple scab, having recently circumvented a resistance introduced in cultivated apple trees, corroborated the existence and the importance of this mechanism of host shift and specialization (in collaboration with INRA Angers, C. Lemaire). This monitoring indeed showed that, as predicted by the model, mating within its host facilitated adaptation and differentiation, and allowed rapid emergence of new pathogenic lineages on new hosts.

Phylogenetic and comparative studies conducted by the team showed in addition that pathogenic fungi diversify mainly by host shifts and not by co-divergence (diversification following that of their hosts), which was long the admitted hypothesis. Another aspect of research on the emergence of new species specialized on different hosts consisted in carrying out experiments on the *Microbotryum* anther-smut pathogens, closely related but specialized on different host plants. Experimental crosses revealed cryptic species and showed that the genetic incompatibilities between species, responsible for reproductive isolation, evolved linearly with genetic distance and were mainly due to chromosomal rearrangements, and not to incompatibilities between alleles, as it is more often thought.

Domestication of cheese and dry-cured meat fungi

A project developed recently in the team focuses on the **study of the diversity and the domestication of the cheese *Penicillium* fungi, which are excellent eukaryotic models to understand the genomic processes of adaptation,** thanks to their small genomes, the strong and recent selection exerted humans for known characters (color, metabolism,…), phenotypes that can be rather easily measured in vitro and the possibility of functional validation. Humans made cheeses since the Neolithic era, and the selection was accentuated and globalized more recently. These fungi thus constitute excellent models to understand the evolutionary mechanisms generating biodiversity, or reducing it, and to understand the mechanisms of rapid adaptation to a new environment.

We have analyzed the genomes of *Penicillium camemberti*, used for the production of Camembert and Brie cheeses, and *P. roqueforti*, used for the manufacturing of blue-veined cheeses, and compared them with other *Penicillium* genomes absent from the cheese environment. We showed that there had been rapid adaptation to the cheese medium, the populations used for maturing cheeses growing better in cheese media, rich in salt, lipids, proteins, and better excluded other micro-organisms. We also showed that the rapid adaptation to cheese has occurred bymultiple horizontal gene transfers between cheese species, although very distantly related. We provided evidence in particular for very recent horizontal transfers of genes involved in the metabolism of lactose and in the competition against other micro-organisms in cheese. These horizontally transferred regions were indeed identical over hundreds of kbs between the species present in the cheese-making environment, very distant genetically, and absent from all other *Penicillium* species and from public databases. We studied in addition genetic diversity within *P. roqueforti*, and we identified several differentiated genetic groups, two of which being inoculated in cheese, one being made up of strains collected in habitats other than cheese, such as wood or silage, and the last consisting of food contaminants. **This work improved our understanding of the genomic processes allowing rapid adaptation to new environments, in particular by stressing the importance of gene duplications and horizontal transfers, even in eukaryotes.**

We currently develop studies of comparative genomics and population genomics to study parallel adaptation of *Penicillium* species used for the fermentation of sausages (*P. salami* and *P. nalgiovense*). As for the cheese *Penicillium* fungi (see past achievements), they represent cases of parallel, recent and rapid adaptation events, in two phylogenetically distant lineages, on the same new human-made medium, rich in salt, lipids and proteins. We are carrying out experiments to test the existence of differentiated traits between the populations from sausage and those from other environments, which would indicate that an adaptation actually occurred. Using comparative genomics, we have detected very recent and specific horizontal gene transfers between the two *Penicillium* fungi used for the fermentation of sausages, and we are studying the functions of the genes they contain. We are also analyzing ca. ten genomes of each *Penicillium* species from cheese and sausage to study genetic diversity, to detect traces of selection, and to identify genomic regions involved in adaptation. We also develop approaches of genetic transformation to test the role of gene candidates identified in *Penicillium* fungi from cheese and sausage in the adaptation to the new human-made media.

We will also continue to develop our studies on the fungi used for cheese maturation to understand the genomic processes involved in the parallel adaptation of phylogenetically distant species to the same ecological niche. We will in particular sequence the genomes of other species used for cheese maturation, phylogenetically distant to each other and to previously studied species. Thanks to the study of parallel adaptation events (of at least five independent lineages), our project will make it possible to test the existence of convergent adaptation events, to test if they occurred through shared genomic mechanisms and/or if the same traits/genes/genomic regions were targeted.

Domestication of apple trees and gene flow with wild apple trees

Another project developed in the team relates to the study of the domestication of the apple tree. Since the discovery in Central Asia of a crab-apple tree (*Malus sieversii*) carrying relatively large apples and closely related to the cultivated apple tree (*M. domestica*), it was admitted that this Asian wild species had given rise to our cultivated apple tree. We showed, using hypervariable genetic markers (microsatellites), that the domestication history of the apple tree was in fact more complex: we revealed that the varieties of *M. domestica* carried in their genomes footprints of recent gene flow from other wild species present along the silk roads, through which the apple tree was brought back to Europe by Romans during their commercial exchanges with Asia. In particular, we detected frequent and recent traces of genetic introgressions (i.e., imports of genomic fragments from a species into another by hybridizations followed by back-crosses) in the genome of the cultivated apple tree coming from the European crab-apple tree, *M. sylvestris*, disseminated in our European forests. The European crab-apple tree was thus a secondary contributor, but an important one, to the genome of the current cultivated apple tree, to the extent that the varieties of apples currently cultivated in Europe are genetically closer to the European crab-apple tree than to its initial Asian ancestor. Reciprocally, the cultivated apple tree also introgressed the European crab-apple tree to the extent that this threatens the integrity and the genetic resources of the European crab-apple tree. We could show that the introgressions were more frequent in areas more affected by human activities. In addition, we showed that private firms sell seeds of wild apples which are in fact mainly hybrids, or even domesticated apple trees, under ambiguous names. The seeds distributed by the ONF correspond to wild genotypes, but with a very restricted genetic basis, so that a very low genetic diversity is replanted. **Our work has fostered new laws for the conservation of the European crab-apple and the set-up of orchards aiming at producing crab-apple seeds for replanting pure genotypes in forests.**

By using the same genetic markers, we studied the genetic structure of the European crab-apple tree throughout its geographical distribution in Europe. We showed that this species displays three genetic groups, a group occurring in western Europe, another group more in the east in the Carpates and the last group in the Balkans. This kind of genetic structure is typical of glacial refugia in the south of Europe during the last glaciation: the north of Europe having been covered with an icecap, the plants could persist only in the south in the Iberian, Balkan and of Carpates peninsulas, between which there was no more gene flow, fostering genetic differentiation. During the postglacial warming, the populations recolonized the north of Europe, keeping their genetic structure. Such knowledge of the genetic structure of the crab-apple tree is essential for developing a conservation program for this species endangered by human activities, to preserve its genetic diversity and its potential local adaptation. **Our work thus brought a new perspective on the evolutionary history of the apple tree domestication, which has a great economic and cultural importance. We revealed in addition that the European crab-apple tree was threatened by gene flow from the cultivated apple tree and we unraveled its genetic structure, which reflects responses to the past climate changes, thus contributing to predict and maybe mitigate the consequences of the current climate changes.**

Another axis of research which I develop with my team relates to the studies of introgressions between the cultivated apple tree and the European crab-apple tree, at the whole genome level, and on the adaptive or deleterious role of these introgressions. Our recent work changed the view on the domestication of the apple tree and revealed a threat to the European crab-apple tree because of these introgressions (see “past achievements”). However, we had previously used only 35 genetic markers, in non-coding regions. We are now analyzing the sequences of the complete genomes apple trees. A first goal is to evaluate the importance of reciprocal gene flow between the domesticated apple tree *M. domestica* and the European crab-apple tree *M. sylvestris*, at the whole genomic level, both quantitatively and qualitatively. A second goal is to assess whether the European crab-apple tree contributed to traits selected by humans during domestication, such as disease resistance, climate or soil adaptation, or robustness. Reciprocally, we wish to determine if the introgressions of the European crab-apple tree by the domesticated apple tree threatens its persistence, or if in contrast they fostered adaptation, or if they are neutral. More generally, we wish to contribute to understand the evolutionary genomic processes of adaptation that occurred during domestication. **These questions are to the forefront of evolutionary biology and have applied consequences, in terms of conservation of endangered species and varietal improvement.**

In addition, we are collaborating with an INRA team (C. Lemaire, Angers) to study the consequences of the domestication of the apple tree on the fungus responsible for apple scab, *Venturia inaequalis*, by sequencing multiple genomes of various areas in Europe and Asia and various apple species. This is also a good model to understand the rapid adaptation to a new environment: the fungus responsible for the scab tree differentiated while adapting to the domestication of its host, and there exists several differentiated populations hybridizing in the zones of secondary contact between the various hosts. The fungal population on the cultivated apple tree appears more aggressive and more generalist than the populations on crab-apple trees. **Again, this is thus a good model to understand rapid adaptation to an anthropized environment and the generation of the biodiversity.**

Evolution of sex chromosomes and mating-type chromosomes

Another part of research in progress in the team relates to the study of the adaptive causes underlying the striking evolution of sex chromosomes, by again using fungi as useful eukaryotic models. The sex chromosomes in plants and animals often show recombination suppression (for example between X and Y in humans), which leads to differentiation and degeneration; for example, the Y chromosome is very small in humans, with very few genes and many repeated sequences. The current dominant theory to explain the suppression of recombination between the sex chromosomes in plants and animals, which often occurred in several successive steps (called evolutionary strata), is the existence of traits which are beneficial in males but deleterious in females, or conversely. There would then exist a selection to successively link the genes responsible for these traits to the sex-determinism genes. However, very little evidence of this process could be documented despite years of research. The fungi provide, here again, good models to test these hypotheses, because some have mating-type chromosomes with recombination suppression, whereas the mating types are not related to male and female functions. In fungi, the genes determining mating types control sexual compatibility, two haploid genotypes being compatible if, and only if, they have different alleles that mating-type genes, but without other different traits between the mating types.

The *Microbotryum* fungi were the first ones in which mating types were identified, at the beginning of the 20th century, and the first on which dimorphic mating-type chromosomes with suppressed recombination were described. For a long time, these chromosomes could not be assembled given their high quantity of repeated sequences. Thanks to the complete assembly of the *M. lychnidis-dioicae* genome that we have just obtained, we could show that the mating-type chromosomes are non-recombining across nearly 90% their length. Moreover, they present an exceptional degree of rearrangements and degeneration. Hundreds of genes were lost in one or the other mating type, and many deleterious changes were identified, in terms of replacement of amino-acids, sub-optimal gene expression or accumulation of transposable elements. Even more remarkably, we detected evolutionary strata on the mating-type chromosomes of *M. lychnidis-dioicae*, whereas there exist no male or female function in these fungi, all gametes displaying the same size. The mating-type chromosomes of *M. lychnidis-dioicae* thus present remarkable convergence with the sex chromosomes of plants and animals, despite lack of association with male and female functions. These results suggest that the dominant evolutionary theory explaining the evolution of sex chromosomes by differential optimal adaptation of males and females should be reconsidered. We are testing alternative hypotheses, such as a selection to shelter in a permanent heterozygous state the deleterious mutations which would accumulate in edges of the non-recombining regions. In addition, the unique material of extremely well assembled genomes of several *Microbotryum* species enabled us to show that the suppression of recombination has evolved many times independently and recently, by different chromosomal rearrangements, and with independent evolutionary strata. This is thus a case of remarkable evolutionary convergence, with likely a strong adaptive advantage, which we are trying to identify.