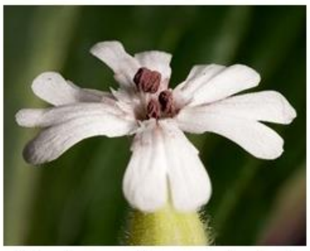
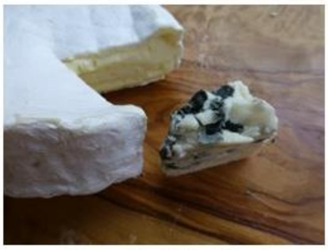
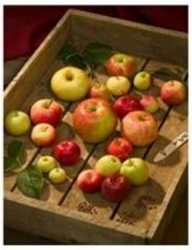
Past achievements



Microbotryum fungus producing its dark spores in anthers of the plant *Silene latifolia*



Cheese *Penicillium* fungi



Cultivated and wild apples

Apple scab caused by the fungus *Venturia inaequalis*

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.

Within this framework, 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.**

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.

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.

In addition, we 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.

A project developed more 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.**

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.**