Dynamique des Génomes Végétaux

FIFTH WORKSHOP ON
PLANT GENOME DYNAMICS AND EVOLUTION

14-15 Novembre 2019
INRA Bordeaux Nouvelle-Aquitaine

Amphithéâtre Colette et Josy Bové,
Centre INRA de la Grande Ferrade, Villenave d'Ornon

Département des Sciences de l'Environnement

UMR 1332 Biologie du Fruit et Pathologie

PROGRAMME

JEUDI 14 NOVEMBRE 2019

9h00-9h15 Opening meeting (Amphithéâtre Colette et Josy Bové, INRA, Site la Grande Ferrade, Villenave d'Ornon)

Session 1 : Genome dynamics / origin / adaptation	
9h15	Reconstruction of wheat origin and evolutionary trajectories from modern and ancient DNA **Jérôme Salse**, *GDEC, INRA, Université Clermont Auvergne, Clermont-Ferrand, France*
10h00	Exploring the species origin of haplotypes in the complex sugarcane genome using NGS data **Nicolas Pompidor**, *CIRAD UMR AGAP, Montpellier, France*
10h30	Genome ancestry mosaics reveal multiple and cryptic contributors to cultivated banana **Guillaume Martin**, *CIRAD, UMR AGAP, Montpellier, France*
11h-11h30	***Coffee break and poster***
11h30	Evolution of a region of agronomical interest in the context of polyploidy in strawberry. **Béatrice Denoyes**, *UMR BFP INRA Bordeaux* , France
12h00	A high-quality sequence of *Rosa chinensis* to elucidate genome structure and ornamental traits **Laurence Hibrand Saint-Oyant**, *IRHS, Agrocampus-Ouest, INRA, Université d'Angers*
12h30	Mutation in the tropical tree canopy: a research plan **Myriam Heuertz**, *UMR Biogeco, INRA Bordeaux* Aquitaine, France
13h15-14h30	**Lunch at INRA villenave d'Ornon (optional)**
14h30	Oak genome: structure function evolution **Christophe Plomion**, UMR *BIOGECO INRA Bordeaux Aquitaine, France*
15h15	Impact of evolutionary processes on the genome architecture of a perennial species **Véronique DECROOCQ**, *UMR BFP INRA Bordeaux Aquitaine, France*
15h45-16h	***Coffee break and poster***
Session 2 : Genome regulation / function / epigenetics	
16h	Evolutionary dynamics of genes duplicated by old paleopolyploidization events **Mathieu Rousseau-Gueutin**, *IGEPP, INRA, Agrocampus Ouest, Université de Rennes 1, France*
16h30	Exploring the putative role of gene duplication in species adaptation: example from the milkweeds (*Asclepias* genus) **Julien Boutte** *IGEPP, INRA, Agrocampus Ouest, Université de Rennes ; France*
17h	**dégustation vins**

20h15 RESTAURANT -Bordeaux (optional)

PROGRAMME

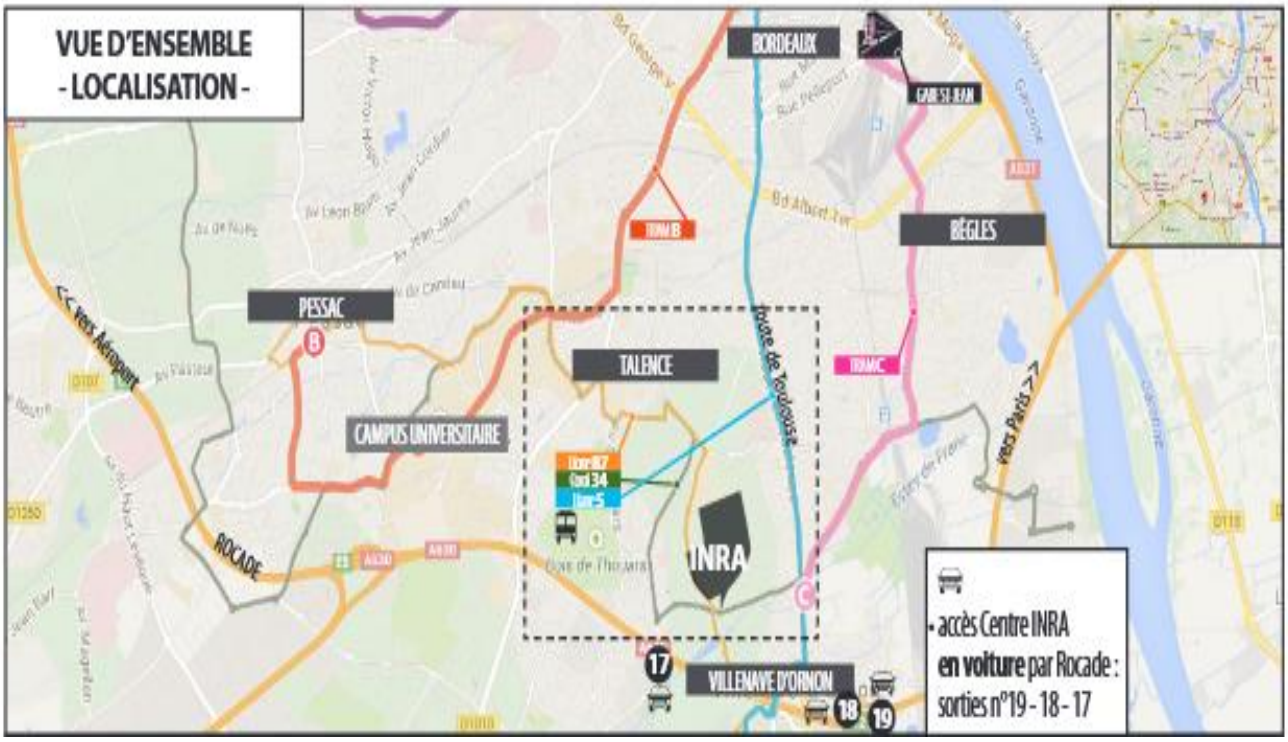
VENDREDI 15 NOVEMBRE 2019

Session 2 continued : Genome regulation / function/ epigenetics	
09h00	DNA methylation control in tomato **Philippe Gallusci**, *EGFG ISVV Université de Bordeaux, INRA Bordeaux Aquitaine, France*
09h45	From hills to mountains: modifying the recombination landscape in *Brassica* AAC allotriploids **Franz Boideau**, *IGEPP, INRA, Agrocampus Ouest, Université de Rennes 1, France*
10h15	Experimental hybrid speciation in *Brassica* **Elvis Katche**, *Plant Breeding Department, Justus Liebig University, Giessen, Germany*
10h45	*Coffee break and poster*
11h15	Transcriptome and (endo)polyploidy: food for thoughts **Christian Chevalier**, *UMR BFP INRA Bordeaux Aquitaine, France*
11h45	The symbiosis signalling pathway is conserved in plant lineages forming diverse intracellular symbiose **Jean Keller**-*LRSV, Université de Toulouse, CNRS, UPS, Castanet Tolosan, France*

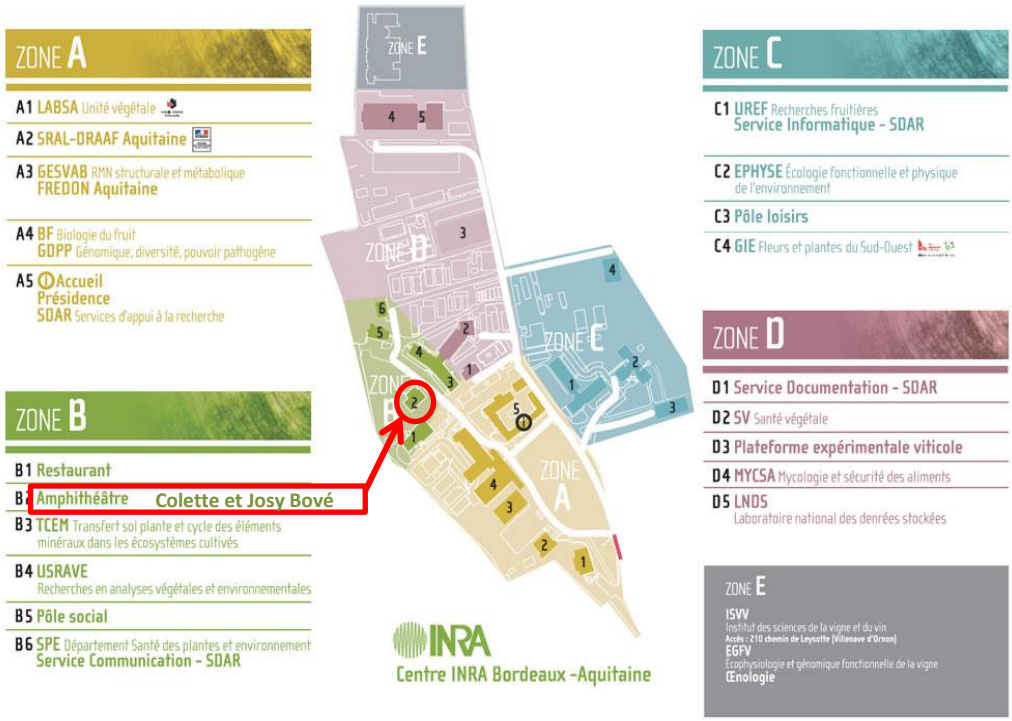
12h15 **Lunch at INRA Villenave d'Ornon (optional)**

Plan d'accès à l'INRA Bordeaux-Aquitaine site de la Grande Ferrade

71 Avenue Edouard Bourlaux, 33882 Villenave d'Ornon, Cedex France.



Plan du Centre INRA de la Grande Ferrade, Villenave d'Ornon



RESUMES/ABSTRACTS

Reconstruction of wheat origin and evolutionary trajectories from modern and ancient DNA.

Jérôme Salse
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For more than 10,000 years, the selection of plant and animal traits that are better tailored for human use has shaped the development of civilizations. During this period, bread wheat (*Triticum aestivum*) emerged as one of the world's most important crops. We use two complementary approaches to explore the origin and evolution of modern bread wheats. The synchronic approach were based on exome sequencing of a worldwide panel of almost 500 genotypes selected from across the geographical range of the wheat species complex, whereas the allochronic approach involved ancient DNA from wheat archaeobotanical remains. We investigated genetic variations at the genic, chromosomal and subgenomic levels to decipher the likely origins of modern day wheats, the consequences of range expansion and the allelic variants selected since its domestication.

References
-Tracing the ancestry of modern bread wheats Nat Genet. 51(5):905-911.
-Paleogenomics: reconstruction of plant evolutionary trajectories from modern and ancient DNA. Genome Biol. 20(1):29.

Exploring the species origin of haplotypes in the complex sugarcane genome using NGS data

Nicolas Pompidor, Olivier Garsmeur, Catherine Hervouet, Angélique D'Hont

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Modern sugarcane cultivars (*Saccharum spp*.) are high polyploids, aneuploids and interspecific hybrids (2n~12x~120). Their recent history is well documented, they are all derived from a few interspecific hybridization events performed a century ago between the formerly cultivated groups *S. officinarum* (2n=8x=80, x= 10) and the wild *S. spontaneum* (2n=5x=40 to 16x=128 and aneuploid; x=8), followed by backcrossing with *S. officinarum.* However, the genome structure and evolutionary history of these species involved in cultivars is still largely unknown.

We identified and sequenced all hom(oe)ologous haplotypes (BAC clones) from two distinct genomic regions of cultivar R570. Phylogenetic analysis showed the existence of three groups of haplotypes suggesting three founding genomes (A, B and C) in modern cultivars instead of two previously thought. We exploited available target sequence genotyping data from 307 sugarcane germplasm accessions representative of the *Saccharum* genus to explore the origin of the three groups of haplotypes. Our results suggested that two genomes (A, B) were contributed by *S. officinarum and* one (C) by *S. spontaneum*. The A and B genomes were found in *S. officinarum* and in its supposed wild ancestor *S. robustum* contradicting the previously thought of an autopolyploid origin for these species.

Genome ancestry mosaics reveal multiple and cryptic contributors to cultivated banana

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Banana cultivars are derived from hybridizations between species and subspecies of the *Musa* genus that have diverged in various tropical Southeast Asian regions and archipelagos. Among the diploid and triploid hybrids generated, those with seedless parthenocarpic fruits were selected by humans and thereafter dispersed through vegetative propagation. *Musa acuminata* subspecies contribute to most of these cultivars.

We analyzed sequence data from 24 *M. acuminata* wild accessions and *M. acuminata* based cultivars, including diploids and one triploid, to characterize the ancestral origins along their chromosomes.

We identified five ancestral groups as contributors to the selected set of cultivars. Four of the ancestral groups corresponded to known *M. acuminata* subspecies. A fifth group present only in cultivars was defined based on one of them and likely represented more than one uncharacterized genetic pool. Diverse ancestral contributions along cultivar chromosomes were found, resulting in mosaics with at least three and up to five ancestries.

This showed that cultivated banana origins involved multiple hybridization steps and were more complex than expected, with yet unidentified ancestors.

Evolution of a region of agronomical interest in the context of polyploidy in strawberry.

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The cultivated octoploid strawberry (*Fragaria x ananassa*) is the most consumed small fruit worldwide. Since its creation in the 18th century in botanical gardens in Europe by fortuitous hybridization between the two New World octoploid strawberry species *F. chiloensis* and *F. virginiana*, strawberry has been continuously improved to fit the needs of both producers and consumers. One of agronomical target for producers is the period of fruit production controlled by a single allele out of the 8 expected alleles, the FaPFRU 'allelic variant' (Perrotte, Gaston et al. 2016). To identify this 'allelic variant', we constructed a bacterial artificial chromosome (BAC) with 'Capitola' and 'CF1116' genotypes, which show contrasted period of flowering. This library covered about 1.5 fold the genome of the octoploid species. We focused our screen of the BAC library in the region of the most relevant candidate gene included in the *FaPFRU* locus, the FLOWERING LOCUS T (FT). About 15 BACs were sequenced and annotated. These BACs were analyzed in the light of the polyploidy of the cultivated strawberry and attributed to one of the four sub-genomes. Results highlight important structural rearrangement between homeo-BACs such as deletion and insertion.

References

Perrotte J, Gaston A*, Potier A, Petit A, Rothan C, Denoyes B. 2016. Narrowing down the single homoeologous FaPFRU locus controlling flowering in cultivated octoploid strawberry using a selective mapping strategy. Plant Biotechnol J. 14: 2176-2189 doi: 10.1111/pbi.12574. (*, these authors contributed equally to the paper)*

A high-quality sequence of Rosa chinensis to elucidate genome structure and ornamental traits

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Rose is the world's most important ornamental plant with hedonic, economic, cultural and symbolic value. Roses, genus Rosa, are cultivated worldwide and sold as garden roses, cut flowers and potted plants. The genus Rosa contains around 200 species with high heterozygosity and various level of ploidy (2 to 10n).

Our objectives were (i) to develop a high-quality reference genome sequence for the genus Rosa by sequencing a doubled haploid, combining long and short reads, and anchoring to a high-density genetic map and (ii) to study the genome structure and evolution and (iii) to characterize the molecular and genetic basis of major ornamental traits as double flower, continuous flowering, self-incompatibility and prickle density.

We produced a doubled haploid rose line, obtained from R. chinensis 'Old Blush'. Using a combination of long and short read sequencing and genetic map anchoring, we generated a rose genome assembly anchored to seven pseudo-chromosomes (512 Mbp).

We delineated hallmark chromosomal features including the pericentromeric regions through annotation of transposable element families and positioned centromeric repeats using Fluorescent In Situ Hybridization (FISH).

The rose genome displays extensive synteny with the Fragaria vesca genome, as we delineated only two major rearrangements. By combining genetic and genomic approaches, we were able to identify potential genetic regulators of key ornamental traits as the number of flower petals. A rose APETALA2/TOE homologue is proposed to be the major regulator of petal number in rose. A misregulation of the APETALA2/TOE homologue is responsible for the increased number of petals in double flower rose.

We will present the last results in rose genomics and the new opportunities opened for genomics studies in rose with the production of this high-quality reference genome sequence.

Mutation in the tropical tree canopy: a research plan.

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We will present an overview of recently submitted grant applications on genome sequencing in tropical trees.

Genetic diversity is an essential prerequisite for the capacity of all life to adapt to an ever-changing environment, and trees are notorious for their large standing genetic variation. There are several ways by which genetic variation can be incorporated or reshuffled in a species, but the only way of generating truly novel genetic diversity is through mutation. Scientists have dedicated considerable efforts to understand the roles of phylogeography, local adaptation, speciation, and hybridisation in modulating genetic diversity of tropical trees, however, our current knowledge of mutational processes in tropical trees is very limited. Recent advances in DNA sequencing techniques now allow us to detect single point mutations with unprecedented precision, which opens up exciting possibilities in mutation research.

We propose to sequence the hologenomes of tropical rainforest trees in French Guiana, providing, for the first time, reference genomes of three Neotropical rainforest tree species. We will test intra-individual and inter-specific effects of low to extreme canopy sunlight exposure on the accumulation of novel genomic mutations and the composition of tree endophytic communities. The tree architecture (i.e., the branching pattern) will represent the null hypothesis for the pattern of accumulation of mutations and colonisation patterns of endophytes. We will test the existence of a soma vs. germline segregation in tropical trees and the transmission of novel mutations and endophytes from parent to offspring. The genomic data and knowledge to be generated in the project represent crucial information towards understanding genome evolution and its drivers in long-lived organisms and elucidating interaction with endophyte communities. The comparison of mutational processes among tropical species, combined with the potential retention of mutations in offspring should be invaluable in assessing the importance of mutation rates in the creation and maintenance of Neotropical diversity.

Impact of evolutionary processes on the genome architecture of a perennial species

Véronique Decroocq[1], Shuo Liu[1], Alexis Groppi[2], Aurelien Barre[2], Quynh Trang Bui[1], Stéphane Decroocq[1], David Tricon[1], Nathalie Rodde[3], Sandrine Arribat[3], Olivier Bouchez[4], Céline Lopez-Roques[4], Rémy-Felix Serre[4], Jean-Marc Aury[5], Corinne Cruaud[5], Erwan Denis[5], Caroline Menguy[5], Macha Nikolski[2]

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In the past few decades, scientists postulated that some of the evolutionary processes differ significantly between perennial and annual species, most probably because of different life traits, i.e. the juvenility phase, extended lifespan, overlapping generations, vegetative propagation etc… In addition, under the long-term process of selection, perennial tree plants have experienced complex historical events, such as hybridization or bottleneck, together with domestication and adaptation to new environments and climatic conditions. This is expected to have impacted the perennial population dynamics but also gene and genome evolution. However, the extent of this impact is still under question.

At Bordeaux, our model plant is the perennial species, Prunus armeniaca L. (apricot). Apricot is a temperate stone fruit tree, it belongs to the family Rosaceae, to the genus Prunus and is one of the five species of the section Armeniaca. The species P. armeniaca refers to both the crop species (also called 'common apricot') and its wild progenitor which still grows in the forests of Tian Shan and Pamir Mountains in Central Asia (Liu et al, Molecular Ecology, 2019). Taking advantage of the small size of the apricot genome (< 220 Mbp for 2n=16), we assembled de novo a high-quality reference sequence of P. armeniaca but also of P. sibirica and P. mandshurica wild related species, endemic in China. Through several local and national fundings1, we resequenced by ILLUMINA technologie the complete botanical Armeniaca germplasm collection held at INRA Bordeaux (~400 Individuals) as well as part of the cultivated repository (CRB UGAFL Avignon) (~200 individuals).

From this extensive resequencing data, we first focused on how selection has influenced the genomic architecture in apricot. We inferred the demographic history of P. armeniaca and its wild related species, using SMC++. To test for common or distinct signatures of selection, we took advantage of the parallel history of domestication in the European and Chinese apricots and compared with their wild, Central Asian progenitor. Through the use of seven different statistical tests, we detected evidence for artificial selection at a genome-wide scale, both for European and Chinese apricots, with a significant number of homologous genomic signatures of domestication, thus indicating convergent yet independent selection of a common set of genes during two geographically and culturally distinct domestication processes. We also identified signatures of selection which could be associated with local adaptation in either wild or cultivated apricots. We are currently performing comparative genomic analysis of the Armeniaca genomes assembled to date as well as of domesticated and wild apricot genomes and thus question the impact of domestication and of inter- and intra-specific (wild-to-crop) gene flow into diversification and adaptation of this long-lived perennial species.

[1] We thank the University of Bordeaux (ATT G2P "SWAGMAN" and AAP interdisciplinaire "ABXING") and , the ANR CHEX "ABRIWG" and France Génomique "SWAG" project

[1] *We thank the University of Bordeaux (ATT G2P "SWAGMAN" and AAP interdisciplinaire "ABXING") and , the ANR CHEX "ABRIWG" and France Génomique "SWAG" project*

Evolutionary dynamics of genes duplicated by old paleopolyploidization events

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Recurrent cycles of polyploidization (Whole Genome Duplication) and diploidization have shaped the genomes of all extant flowering plants and are at the origin of an astonishing plant biodiversity. While WGD instantly duplicates all genes, a diploidization will consequently occur and will notably engender the progressive loss of duplicated copies via fractionation and chromosome number reduction. These combined processes create countless gene combinations that may contribute to species diversification. With the increasing number of sequenced genomes, it is now possible to decipher the long-term evolutionary dynamics of duplicated copies. To date, a good system to explore duplicated gene evolution belongs to the Brassica genus as species from this genus underwent a whole genome triplication event about 22 million years ago and the genomes of several diploid and polyploid species are now available. Taking advantage of these genomes, we notably compared in B. rapa (turnip) and B. oleracea (cabbage), the number of copies retained in each species and how these old duplicated copies (also referred as paleologs) are functionally regulated after a novel episode of polyploidy. To validate that paleologs may retain the same function and act synergistically, we performed CRISPR-Cas9 experiments on a duplicated gene and started to explore the impact of the loss of one or several copies on a specific biological function.

Exploring the putative role of gene duplication in species adaptation: example from the milkweeds (*Asclepias* genus).

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Asclepias (*Asclepias* L., Apocynaceae Juss., 2n=22) is an ecological and evolutionary plant model system for the study of reproductive biology, plant defenses, plant-animal interactions, or plant adaptation. Recently, the first genome and transcriptome of *Asclepias syriaca* were assembled, facilitating the exploration of the genetic mechanisms that are involved in the adaptation of some North-American *Asclepias* to arid habitats. In this genus, two non sister clades developed different strategies to adapt to such extreme environment. Indeed, the Sonoran Desert clade (SDC) species have narrow and ephemeral leaves whereas the Mexican species harbor large leaves and an increased adaxial trichome density to retain water. To identify the putative genomic mechanisms behind these independent adaptations, we analyzed shotgun data from 29 American *Asclepias* species (including 14 SDC, 8 Mexican, and 7 other species growing in temperate environments). Using these data, we explored Copy Number Variation (CNV) of 65,356 *Asclepias* transcripts as duplicated genes are known to play a major role in plant adaptation. Taking advantage of the recently elucidated evolutionary history of this genus, it was possible to infer 694 gene duplication events. Gene Ontology enrichments of these latter revealed an increased number of genes related to plant organ and shoot system development in the SDC clade, whereas species from the Mexican clade were enriched for genes involved in the vacuole, an organelle notably storing water and regulating the osmotic pressure. To validate our method to identify CNV between *Asclepias*, we performed fluorescence *in situ* hybridization (FISH) on a CW-type Zinc finger protein gene, whose number of copies deeply varies within SDC *Asclepias* species. Additionally, taking advantage of RNA-Seq data obtained from bulks of organs in a few individuals, we were able to infer the functionality vs. pseudogeneization of each copy of this gene.

Keywords: Milkweeds, Apocynaceae, Copy Number Variation (CNV), CW-domain, Gene Evolution, Sonoran Desert Clade.

DNA methylation control in tomato

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Epigenetics encompasses the complement of genetic information carried in chromatin beyond the DNA sequence. For example, DNA methylation which occurs on the 5th carbon of cytosine (5mC) is involved in the control of gene expression and transposon mobility. Studies in Arabidopsis, tomato and other plants, have demonstrated the relevance of epigenetic mechanisms in the control of development, impact on agronomic traits and genome stability. Effects of DNA methylation can last over several generation as demonstrated using Arabidopsis epigenetically modified recombinant inbred lines (epiRILs) generated by crossing a wild type with a mutant line, in the same genetic background, impaired in DNA methylation maintenance.

We have generated various transgenic cherry tomato RNAi lines that are impaired in the control of DNA methylation that present either reduced SlMET1 or demethylase (DML) gene expression. SlMET1 RNAi plants display several phenotypes including limited root development, smaller fruit, modified flowers, and premature death together with reduced level and altered patterns of DNA methylation as compared to WT and increased transposon mobility. In contrast SlDML RNAi plants present defect in the fruit ripening process associated with altered methylation and gene expression profiles. Additionally some SlDML RNAi present modified plant and fruit shape.

By crossing WT and the RNAi SlMET1 plants of the same genetic background we have now created a unique population of EpiRILs consisting of 115 lines that were propagated by single seed descent over 8 generations. Observable epigenetic and phenotypic differences are evident within the population. Methylome analysis of F5 plants indicates a methylation profile in part corresponding to the WT or SlMET1 RNAi lines. This epiRIL population should now provide a valuable epigenetic community resource to evaluate the contribution of epigenetic variations to traits of agronomic interest including chemo/phenotypes and other fruit quality traits.

From hills to mountains: modifying the recombination landscape in *Brassica* AAC allotriploids

Franz Boideau[1], Gwenn Trotoux[1], Loeiz Maillet[1], Virginie Huteau[1], Maryse Taburel[1], Frédérique Eber[1], Marie Gilet[1], Anael Brunet[1], Jérôme Morice[1], Julien Boutte[1], Cyril Falentin[1], Olivier Coriton[1], Mathieu Rousseau-Gueutin[1] and Anne-Marie Chèvre[1]

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Meiotic recombination via crossovers (COs) is a major mechanism generating new genetic diversity at each generation by reshuffling alleles inherited from the parents. However, COs are highly regulated both in frequency and in distribution, with rarely more than three COs per chromosome that are almost exclusively present in subtelomeric regions. In few polyploid species, such as in *Brassica* or *Gossypium*, it has been shown that polyploidy can increase the homologous recombination rate by almost two folds compared to their diploid progenitors. However, it remains to be deciphered the impact of the polyploidy level on the distribution of COs along the chromosomes. To that purpose, we produced two *Brassica* F1 hybrids having two different levels of ploidy but the same A genotype: one allotetraploid (AACC) and one allotriploid (AAC). These hybrids result from crosses between oilseed rape (*Brassica napus*, AACC, 2n=4x=38) and a synthetic oilseed rape line or one of its diploid progenitors (*B. rapa*, AA, 2n=20) used as males, respectively. As these two hybrids of different ploidy levels share the same A sub-genome, it is possible to compare without any bias their frequency and distribution of COs between A chromosomes. These comparisons were performed using genetic maps established from thousands of SNP markers that we physically anchored on *Brassica* reference genomes. Our preliminary results indicate that despite the AAC *Brassica* allotriploid hybrid presents a lower ploidy level compared to the allotetraploid hybrid, it shows a higher recombination rate and more interestingly a modified CO distribution, even in the vicinity of centromeres that are normally deprived of COs. We are currently investigating the origin of these modified recombination rules in allotriploids and if they can persist for several generations or revert back to normal. Altogether, this study will provide new insights on meiotic recombination regulations in an interspecific and inter-ploid hybrid and new ways to more efficiently enhance the narrow genetic diversity of a major polyploid crop.

Experimental hybrid speciation in *Brassica*

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Interspecific hybridisation is an important path to generating evolutionary novelty, and the genus Brassica is a major agricultural genus with a history of interspecific hybridisation. The allotetraploids B. juncea, Brassica carinata, and Brassica napus were formed by pairwise hybridisation between diploid progenitors Brassica rapa, Brassica oleracea and Brassica nigra. Although crossing between these allotetraploid species is possible and has been carried out in several studies either to study chromosome behavior or to transfer useful traits between species, attempts to generate novel, stable and fertile synthetic hybrids through this method have not been reported. We generated interspecific hybrids (AABC = F1 = 37), by crossing B. juncea (2n = AABB= 36) × B. napus (2n =AACC=38), (CCAB = F1 = 36) by crossing B. napus (2n = AACC = 38) × B. carinata (2n = BBCC = 34) and (BBAC = F1 = 35) by crossing B. juncea (2n = AABB = 36) × B. carinata (2n = BBCC = 34) and self-pollinated these hybrids for generations by selecting for fertility. CCAB and AABC hybrids became infertile in the early generations (S1 and S2 respectively) while BBAC increased in fertility across generations (up to S6). In the absence of homologous pairing partners, the A and C genomes paired, restructured and stabilized to form viable and fertile offspring. This pathway can be useful for generating evolutionary novelty which can be transferred to other Brassica species and also to produce new useful crop types.

Transcriptome and (endo)polyploidy: food for thoughts

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As part of normal development most eukaryotic organisms ranging from insects to mammals and plants display variations in nuclear ploidy levels resulting from somatic endopolyploidy. Endoreduplication is the major source of endopolyploidy in higher plants. Endoreduplication is a remarkable characteristic of the fleshy pericarp tissue of developing tomato fruits, where it establishes a highly integrated cellular system that acts as a morphogenetic factor supporting cell growth. However, the functional significance of endoreduplication is not fully understood. Although endoreduplication is thought to increase metabolic activity due to a global increase in transcription, the issue of gene-specific ploidy-regulated transcription remains opened. To investigate the influence of endoreduplication on transcription in tomato fruit, we tested the feasibility of a RNA-Seq approach using total nuclear RNA extracted from purified populations of flow cytometry-sorted nuclei based on their DNA content. Our data indicated that cell-based approaches to study RNA-Seq profiles need to take into account the putative global shift in expression between samples for correct analysis and interpretation of the data. From ploidy-specific expression profiles we deduced that the activity of cells inside the pericarp is related not only to the ploidy level but also to their tissue location.

The symbiosis signalling pathway is conserved in plant lineages forming diverse intracellular symbioses

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Plants have been the foundation of most terrestrial ecosystems since they colonized land 450 million years ago[1] and this colonisation event was facilitated by the symbiotic association with arbuscular mycorrhizal (AM) fungi[2,3]. Following that founding event, plant diversification has led to the emergence of a tremendous diversity of mutualistic symbioses with microorganisms, ranging from extracellular associations to the most intimate intracellular associations, where fungal or bacterial symbionts are hosted inside plant cells. Our understanding of the molecular mechanisms underpinning these associations is currently limited to the association with AM fungi and with nitrogen-fixing rhizobial bacteria[4.] We created a database dedicated to the study of symbiosis evolution containing 248 transcriptomes and 115 genomes (http://polebio.lrsv.ups-tlse.fr/symdb/index.php), including the de novo sequenced genome of the symbiotic liverwort Marchantia paleacea. Using this powerful resource, we are able to demonstrate that the symbiosis signalling pathway specifically co-evolves with intracellular endosymbioses, including the ericoid mycorrhizae in angiosperms with ascomycetes and basidiomycetes and the ericoid-like mycorrhizal associations of bryophytes. In contrast, species including the bryophyte Blasia pusilla, that form extracellular symbioses, but lack intracellular interactions have lost symbiosis signalling. Using trans-complementation, we demonstrate that this signalling pathway has maintained its biochemical functionality across land plants with intracellular symbioses. This work unifies intracellular symbioses, revealing a conservation in their evolution across 450 million years of plant diversification.

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POSTERS/ABSTRACTS

Towards a stable and diverse *Brassica* hexaploid crop

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Interspecific hybridization and polyploidization processes are known to confer advantages such as hybrid vigor and increased environmental tolerances. Although cultivated diploid and allotetraploid *Brassica* species which contain different combinations of the A, B and C genomes exist, there is no naturally occurring allohexaploid that contains all three genomes (AABBCC). Despite this, there are traits in each of the *Brassica* species that if combined together can potentially produce a new species with many advantageous features. However, there are currently three major challenges in production of *Brassica* allohexaploids as a viable crop type: generation of sufficient genetic variability, proof of agricultural potential, and genome stability. In this study we are focusing on improving genome stability and on increasing the genetic diversity of our material. To do this, we have combined several *Brassica* allohexaploids from different origins: *B. napus* × *B. nigra* (naponigra = $A^nA^nB^iB^iC^nC^n$), *B. carinata* × *B. rapa* (carirapa = $A^rA^rB^cB^cC^cC^c$), *B. juncea* × *B. oleracea* (junleracea = $A^jA^jB^jB^jC^oC^o$), and (*B. napus* × *B. carinata*) × *B. juncea* (NCJ = $A^{n/j}A^{n/j}B^{j/c}B^{j/c}C^{n/c}C^{n/c}$). The cross-compatibility between these species combinations and genotypes varies, with many of the potential hybrid seeds ending up as aborted embryos. One of the most fertile genotypes was an NCJ hexaploid which also showed highly regular meiosis, making it a great candidate for a genomically stable genotype. Overall, the naponigra hexaploid types were the least fertile and also produced very few seeds in combination with other genotypes. From all crossing combinations, we produced 8246 new hybrid seeds, from which we selected a subset from the best combinations based on the number of seeds obtained and the genotypic diversity. In future, we aim to compare the new hybrids to the inbred parental lines for chromosome pairing behavior during meiosis and seed setting. At the same time, we are in the process of analyzing chromosome complements and chromosome segregation in the progeny of putatively stable, advanced-generation allohexaploid lines using high-throughput SNP genotyping via the Illumina Infinium *Brassica* 90K array. The identification of genotype- or species-specific factors related to fertility and genome stability in *Brassica* allohexaploids will be useful for producing a novel, stable crop species.

Plate-Forme de Cytogénétique Moléculaire Végétale

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La cytogénétique moléculaire dont l'outil principal est l'Hybridation In Situ en Fluorescence (FISH) sur préparation chromosomique a révolutionné l'approche de la cytogénétique traditionnelle. Le pouvoir de résolution permet de répondre à des projets d'étude des génomes par une analyse fine de la structure des chromosomes en méiose et mitose. A travers les différents outils FISH proposés sur la PF, l'intérêt de cette technologie sera illustré à travers d'exemples développés sur la plate-forme portant sur la dynamique des éléments transposables visant à la compréhension de l'histoire évolutive de différents systèmes végétaux ou encore la compréhension de la structure des génomes polyploïdes….

Stable, fertile lines of *Brassica napus* synthetics

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Rapeseed (Brassica napus, AACC) is a young allotetraploid species formed by the hybridization of Brassica rapa (AA) and Brassica oleracea (CC). However, resynthesized B. napus lines are often highly unstable and infertile, unlike natural B. napus, which is both fertile and stable. Meiotic stability in natural B. napus may have arisen through allele inheritance from the progenitor species or via one or more de novo mutations post-polyploidisation. We tested these hypotheses by characterizing a diverse set of B. napus lines resynthesized from the cross B. rapa × B. oleracea for chromosome rearrangements, allele inheritance, fertility, and meiotic behaviour. SNP genotyping was performed using the Illumina Infinium Brassica 60K array, and allele copy number used to infer translocation events between the A and C genomes. Approximately 52% of lines (91/174) with SNP genotyping information were homozygous as expected; most of the older resynthesized lines were putatively contaminated by outcrossing over many generations of maintenance in the field. Self-pollinated seed-set (average 611, range 0 – 3876 per plant) and genome stability (number of copy number variants) were significantly affected by the interaction between the parental B. rapa and B. oleracea genotypes. On average, 11 copy number variants were observed per plant (range 1 – 34), with variants including loss of one copy (reduced copy) or both copies (deletion) as well as gain of one or more copies (higher copy) of a chromosomal region. Some lines showed clear evidence of unbalanced translocations between the A- and C-genomes, where loss of one homoeologous region was not balanced by presence of an additional copy of the other homoeologous region. Analysis is ongoing, but our results show that some resynthesized lines are more stable and fertile than others, and support the hypothesis that allelic variants inherited from genotypes of the parent species affect genome stability in synthetic rapeseed.

Fast sequence-based microsatellite genotyping development workflow for any non-model species

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Application of high-throughput sequencing technologies to microsatellite genotyping (SSRseq) has been shown to remove many of the limitations of electrophoresis-based methods and to refine inference of population genetic diversity and structure. However, early proof of concept and species specific development studies resulted in dispersed information making it cumbersome for prospective users to identify a clear path to SSRseq approach set up in species of new interest. To overcome these difficulties, we present here a streamlined SSRseq development workflow that includes microsatellite development, multiplexed marker amplification and sequencing, and automated bioinformatics data analysis.

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Assessment of pollen size according to ploidy level in Brassica model

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It is well established that unreduced gametes play a major role to change plant ploidy level for the production of hybrids and of polyploid species (Mason et al. 2011). In spite of a very low frequency in diploid species with 0.1 to 2.0% on average (Kreiner *et al.,* 2017), their frequency depends on genotype and can be changed by environmental factors. A rapid and reliable method of detection is needed in order to assess more precise their frequency in different genetic structures. We developed a method based on imaging treatments of pollen size after Aceto-Carmine staining. Pollen area and perimeter were measured in two diploid species, *Brassica rapa* (AA, 2n=20) and *B. oleracea* (CC, 2n=18) and in their corresponding autotetraploids on very large samples. We will present this method and will discuss the perspectives that it may open.

Keywords: autoploidy, unreduced gametes

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