Sujet de thèse Unité Evo-Eco-Paléo – campagne 2018

Université : Université de Lille

Ecole doctorale: ED104 Sciences de la matière, du rayonnement et de l'environnement (SMRE)

Filière doctorale : Géosciences Écologie Paléontologie Océanographie

Titre de la thèse : Evolution of the dominance/recessivity hierarchy among self-incompatibility alleles in Arabidopsis.

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Laboratoire(s) de Rattachement : Unité Évolution, Écologie et Paléontologie, UMR CNRS 8198

Programme(s) de Rattachement : ERC NOVEL

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SUJET DE THÈSE

Dominance is a basic property of the genotype-to-phenotype map and the evolution of dominance has been one of the most hotly debated topics in genetics over the XXth century (reviewed in Billiard and Castric 2011). Biefly, the cornerstone of the debate was the possibility for natural selection to provide substantial advantage for genetic elements controlling dominance/recessivity interactions between alleles at other genes. Plants of the Brassicaceae family show a very interesting dominance/recessivity phenomenon at the self-incompatibility system, whereby S-alleles are arranged along a fully transitive, mostly linear dominance hierarchy (called "sporophytic" self-incompatibility). We previously showed that this dominance is largely explained by a set of small non-coding RNAs produced by dominant S-alleles that have the capacity to target and transcriptionally silence the more recessive S-alleles, hence providing the first documented empirical evidence for « dominance modifiers ». We now want to learn more about how these unusual genetic elements achieve their function and how they evolved in conjonction with the diversification history of S-alleles in the Brassicaceae. The aim of this PhD project is to understand how the dominance hierarchy among self-incompatibility alleles became established in the course of evolution. To achieve this goal, we will focus on two clades of closely related S-alleles that became diversified and acquired the regulatory elements controlling dominance relatively recently. We will fully characterize their dominance relationship and the regulatory network by which they are controlled. Focusing on this finer phylogenetic scale will enable us to look for signatures of the molecular events that have led to their recent formation and propose a scenario for how they arose.

Step 1. **Sampling S-alleles**. We will obtain a comprehensive representation of S-allele sequence diversity of closely related alleles by screening a large collection of individuals from natural populations available in the lab to identify individual plants carrying S-alleles from clade II and III, which are the two clades with the most closely related S-alleles (i.e. with the most recent history of allelic diversification). We will do this using a recently developed low-coverage Illumina sequencing protocol.

Step 2 : Determining the dominance relationships. Plants with S-alleles of interest will be crossed in the greenhouse to generate all possible heterozygote combinations. Controlled pollination assays will then be performed to phenotypically determine the matrix of pairwise dominance relationships and allele-specific qPCR assays will be used to compare the transcript levels of each allele of the pollen-expressed gene in each genotype.

Step 3 : Identifying the regulatory network controlling dominance. We will first use long-read sequencing technologies to sequence and assemble the full S-locus region of each S-allele of interest. In the event where the assembly fails, we will resort to BAC library construction, screening

and sequencing, as we previously used (Goubet et al. 2012). The genes encoding the cognate receptor and ligand proteins will be annotated. We will then massively sequence sRNAs from flower buds to identify the sRNA precursors expressed by each S-allele, and we will predict the targets of these sRNAs on the other S-alleles using alignment-based methods.

Step 4 : Tracking the mode of emergence of the regulatory interactions. We will compare the sequence of the sRNA precursors with that of their target site and look for evidence of recent inverted duplications in order to determine how often this mechanism of acquisition has been used. Alternatively, we will test for sequence similarity with transposable elements in these genomes. We will determine whether new dominance interactions are more frequently determined by the accumulation of SNPs in existing sRNA precursors and their target sites (as proposed by Yasuda et al. 2016) or by the de novo emergence of new sRNA precursors (Durand et al. 2014).

Step 5: Functional validation. Using a set of *A. thaliana* lines in which we have transferred the elements of the self-incompatibility system and their regulatory elements, we will test general features of the silencing network, such as the importance of functional redundancy, whereby a given dominant S-allele is predicted to transcriptionally silence a given recessive S-allele by more than one regulatory interaction.

Billiard S, Castric V. 2011 Evidence for Fisher's dominance theory: how many "special cases"? Trends in Genetics 27, 441-445.

Durand E, Méheust R, Soucaze M, Goubet PM, Gallina S, Poux C, Fobis-Loisy I, Guillon E, Gaude T, Sarazin A, Figeac M, Prat E, Marande W, Bergès H, Vekemans X, Billiard S, Castric V. 2014. Dominance hierarchy arising from the evolution of a complex small RNA regulatory network. Science. 346(6214):1200-5.

Goubet P, Bergès H, Bellec A, Prat E, Helmstetter N, Mangenot S, Gallina S, Holl AC, Fobis-Loisy I, Vekemans X, Castric V. 2012 Contrasted patterns of molecular evolution in dominant and recessive self-incompatibility haplotypes in Arabidopsis. PLoS Genetics 8, e1002495.

Yasuda S, Wada Y, Kakizaki T, Tarutani Y, Miura-Uno E, Murase K, Fujii S, Hioki T, Shimoda T, Takada Y, Shiba H, Takasaki-Yasuda T, Suzuki G, Watanabe M, Takayama S. 2016. A complex dominance hierarchy is controlled by polymorphism of small RNAs and their targets. Nat Plants. 3:16206.

Skills required

- Evolutionary biology
- Genetics
- Genomics