

Sorbonne Université/ China Scholarship Council program 2020

Thesis proposal

Title of the research project: **Application of cytogenetic and genomic approaches to investigate the origin and evolution of the sexes in the brown algae**

Keywords: **sex determination; sex chromosomes; brown algae; evolution; cytogenetics**

Joint supervision: ~~yes (name/surname)~~ /no

Joint PhD (cotutelle): ~~yes (name/surname)~~ /no

Thesis supervisor: Susana Coelho

Email address of the thesis supervisor: Coelho@sb-roscoff.fr

Institution: **CNRS**

Doctoral school (N°+name): ED **Complexité du Vivant**

Research laboratory: **UMR8227**

Name of the laboratory director: **Stephane Egée**

Email address of the laboratory director: egee@sb-roscoff.fr

Subject description (2 pages max):

1) Study context

Sexual reproduction is an extraordinarily widespread phenomenon that assures the production of new genetic combinations in nearly all eukaryotic lineages. Although the core mechanisms of sexual reproduction (meiosis and syngamy) are highly conserved, the control mechanisms that determine whether an individual is male or female are remarkably labile across eukaryotes. Sex can be determined by the environment or be genetically controlled. Genetic determination of sex is mediated by sex chromosomes, whose key feature is the sex-determining region (SDR) that carries the sex-determining factor(s) and does usually not recombine in the heterogametic sex. The SDR can be as small as a single locus or as large as an entire chromosome². Sex chromosomes are subject to unique evolutionary forces including sex-specific selection, asymmetrical sheltering of deleterious mutations, hemizygoty, and dosage compensation. Besides sex determination, sex chromosomes play a prominent role in several evolutionary processes such as speciation and adaptation but also are thought to be associated with the evolution of anisogamy and with the regulation of key developmental transitions during the life cycle. Traditionally, most of our knowledge on the biology

and evolution of sex chromosomes comes from a few well-studied model organisms, notably mammals, birds, *Drosophila* and the plant *Silene latifolia*. We know very little about this process in organisms outside the Opisthokonta and land plants, and the universality of the mechanisms driving the evolution of the sexes is difficult to assess without a broader view that takes into account the scope of the tree of life. Moreover, while studies so far have focused on diploid sex determination systems (the classical XY or ZW), haploid phase sex-determination systems (UV systems, such as those of mosses and some algae) have been largely ignored (Ahmed et al., 2014; Coelho et al., 2018; Umen and Coelho, 2019). The evolution of the SDR is largely impacted by the genetic mechanisms of sex determination, and although UV and XY/ZW share many common features, there are important biological differences between them that have far-reaching evolutionary and genomic implications. Contrasting the properties of XY/ZW, and UV chromosomes, together with the comparison with species that lack sex chromosomes entirely, provide exceptional opportunities to assess the relative importance of the forces driving the evolution of each system.

In this context, the brown algae represent exceptional models for investigating the origins and evolution of sex chromosomes because they exhibit a bewildering variety of reproductive systems, types of life cycle and sex chromosome system in a single group (Coelho et al., 2019; Heesch et al., 2019). Phylogenetic analysis indicates that there has been considerable switching between variants of these different features on an evolutionary timescale, offering a unique opportunity to investigate the forces driving the evolution of the sexes and sexual systems in this group. The impact of this variation on sex chromosome evolution is of particular interest, allowing current theories on sex chromosome evolution to be tested in an experimental system with the aim of resolving long standing questions on forces driving the origin and evolution of the sexes, and, more broadly, to discover new trends and patterns in the evolutionary trajectory of reproductive systems. Another advantage of the brown algae, as a group, is the availability of the model brown alga *Ectocarpus*, whose SDR has been identified and for which many genetic and genomic tools are available, most of these tools have been developed in our lab (Avia et al., 2017, 2018; Baudry et al., 2019; Cock et al., 2010a, 2010b; Coelho et al., 2012a, 2012b; Lipinska et al., 2017, 2019). Moreover, in the context of the SEXSEA ERC project (starting grant 2015-2020) and Phaeoexplorer projects (France Genomique, coll. Genoscope), both coordinated in our Team, we have now access to high quality genomes, transcriptomes and genetic and physical maps for a range of brown algal species. This set of resources allows us now to gain unprecedented insights into genome and chromosome organisation across a whole lineage, with a specific focus on evolution of sex chromosomes.

The overarching goal of the PhD project is to use cytogenetic and genomic approaches to understand the evolution of sex chromosomes across the brown algae. Specifically, the student will focus on the spatial organisation of genomes of several brown algal species presenting contrasting sexual systems (separate sexes versus hermaphroditism).

2) Details of the proposal

The student will use a range of cytogenetic, molecular and genomic tools to perform comparative analyses across several brown algal species in order to understand the evolution and turnover of UV sex chromosomes. This PhD program will be performed in the context of a recently awarded ERC Consolidator Grant (TETHYS) to the Principal Investigator (Susana Coelho).

The project will be divided in three parts:

I. The PhD student will work on ten selected brown algal species for which we already have full genome sequences and transcriptomic data available. These species span the phylogeny of the brown algae, and represent both species with separate sexes (with UV sex chromosomes) but also species which have derived monoicy (hermaphroditism). In collaboration with members of the team, the student will use bioinformatics pipelines to detect sex-linked contigs among the male and female genomes. He will validate sex linkage using PCR approaches on several male and female

samples. He will then reconstruct the sex chromosomes of the ten species, being helped by long read sequencing (Pacbio and Nanopore) and use of genetic and physical maps (HiC). He will identify the former sex chromosomes on the hermaphrodite species.

II. In parallel, the student will adapt and develop cytogenetic approaches (fluorescence *in situ* hybridization) to the ten brown algal species in order to visualise, using microscopy, their chromosomes. He/she will map several sex-linked, pseudo-autosomal and autosomal genes using FISH approaches onto metaphase chromosomes in order to understand sex chromosome and autosomal rearrangements that have occurred during the evolution of these species. Note that protocols for cytogenetics already exist for some of the brown algal species (e.g. Liu et al., 2012), and the student will adapt these protocol to the other species.

III. Sequence data alone is unable to provide insight into how the genome is organised into chromosomes, the position and interaction of those chromosomes in the cell, and how chromosomes and their interactions with each other change over time. The student will investigate how chromosomes and their interactions with each other change during the transition from separate sexes to hermaphroditism. The student will combine chromosome conformation capture (3C), information already available in the laboratory (Baudry et al. 2019) and fluorescent *in situ* hybridization to investigate the spatial genome organisation in pairs of species where a transition from separate sexes to hermaphroditism occurred. Our group has recently collaborated the laboratory of Romain Koszul at the Pasteur Institute in Paris to develop 3C approaches for the brown algae, and we will use this tool and combine it with FISH to investigate genome 3D organisation.

Taken together, the comparative analysis using these complementary technologies that will be accomplished during the PhD thesis is expected to make great advances on our understanding of genome and sex chromosome evolution.

Planning

The following table provides a provisional planning for the PhD thesis. Note that the relative effort expended on the proposed approaches will be continuously reviewed during the project, taking into account factors such as problems encountered.

	Year 1		Year 2		Year 3	
Analysis of genomes and transcriptomes						
Pipeline bioinformatics to detect sex-linked contigs						
PCR validations						
Reconstruction of sex chromosomes						
Cytogenetic approaches						
Setting up of the FISH in several species						
Combining FISH and 3C on several species						
Paper writing						
Thesis writing						

3) References

Highlighted in blue are the references from my group

Ahmed, S., Cock, J.M., Pessia, E., Luthringer, R., Cormier, A., Robuchon, M., Sterck, L., Peters, A.F., Dittami, S.M., Corre, E., et al. (2014). A haploid system of sex determination in the brown alga *Ectocarpus* sp. *Curr. Biol.* 24, 1945–1957.

Avia, K., Coelho, S.M., Montecinos, G.J., Cormier, A., Lerck, F., Mauger, S., Faugeron, S., Valero, M., Cock, J.M., and Boudry, P. (2017). High-density genetic map and identification of QTLs for responses to temperature and salinity stresses in the model brown alga *Ectocarpus*. *Sci Rep* 7, 43241.

Avia, K., Lipinska, A.P., Mignerot, L., Montecinos, A.E., Jamy, M., Ahmed, S., Valero, M., Peters, A.F., Cock, J.M., Roze, D., et al. (2018). Genetic Diversity in the UV Sex Chromosomes of the Brown Alga *Ectocarpus*. *Genes (Basel)* 9.

Baudry, L., Marbouty, M., Marie-Nelly, H., Cormier, A., Guiguelmoni, N., Avia, K., Mie, Y.L., Godfroy, O., Sterck, L., Cock, J.M., et al. (2019). Chromosome-level quality scaffolding of brown algal genomes using InstaGRAAL, a proximity ligation-based scaffold. *BioRxiv* 2019.12.22.882084.

Cock, J.M., Sterck, L., Rouzé, P., Scornet, D., Allen, A.E., Amoutzias, G., Anthouard, V., Artiguenave, F., Aury, J.-M., Badger, J.H., et al. (2010a). The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature* 465, 617–621.

Cock, J.M., Coelho, S.M., Brownlee, C., and Taylor, A.R. (2010b). The *Ectocarpus* genome sequence: insights into brown algal biology and the evolutionary diversity of the eukaryotes. *New Phytologist* 188, 1–4.

Coelho, S.M., Scornet, D., Rousvoal, S., Peters, N.T., Darteville, L., Peters, A.F., and Cock, J.M. (2012a). *Ectocarpus*: a model organism for the brown algae. *Cold Spring Harb Protoc* 2012, 193–198.

Coelho, S.M., Scornet, D., Rousvoal, S., Peters, N., Darteville, L., Peters, A.F., and Cock, J.M. (2012b). Immunostaining of *Ectocarpus* cells. *Cold Spring Harb Protoc* 2012, 369–372.

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Coelho, S.M., Mignerot, L., and Cock, J.M. (2019). Origin and evolution of sex-determination systems in the brown algae. *New Phytologist* 10.1111/nph.15694.

Heesch, S., Serrano-Serrano, M., Luthringer, R., Peters, A.F., Destombe, C., Cock, J.M., Valero, M., Roze, D., Salamin, N., and Coelho, Susana M (2019). Evolution of life cycles and reproductive traits: insights from the brown algae. *BioRxiv*.

Lipinska, A.P., Toda, N.R.T., Heesch, S., Peters, A.F., Cock, J.M., and Coelho, S.M. (2017). Multiple gene movements into and out of haploid sex chromosomes. *Genome Biology* 18, 104.

Lipinska, A.P., Serrano-Serrano, M.L., Cormier, A., Peters, A.F., Kogame, K., Cock, J.M., and Coelho, S.M. (2019). Rapid turnover of life-cycle-related genes in the brown algae. *Genome Biol* 20, 35.

Liu, Y., Bi, Y., Gu, J., Li, L., and Zhou, Z. (2012). Localization of a female-specific marker on the chromosomes of the brown seaweed *Saccharina japonica* using fluorescence in situ hybridization. *PLoS One* 7, e48784.

Umen, J., and Coelho, S. (2019). Algal Sex Determination and the Evolution of Anisogamy. *Annu. Rev. Microbiol.*

4) Profile of the Applicant (skills/diploma...)

The applicant should have a diploma degree in biology, with excellent grades, an interest in sex chromosome evolution, and have a background experience in molecular biology and cytogenetics approaches (FISH, chromosome painting, etc). The student should also have notions of bioinformatics. An interest and experience

with manipulating brown algae would be also important. The student must be able to communicate in English as he/she are expected to participate in meetings and conferences and participate actively in writing the papers. The working language in our group is English. The student will be working in close collaboration with the rest of the group, bioinformaticiens, postdocs, and technicians and therefore must be able to work in a team.

Contacts:

Thesis supervisor

Susana Coelho

Email address of the thesis supervisor: **Coelho@sb-roscoff.fr**