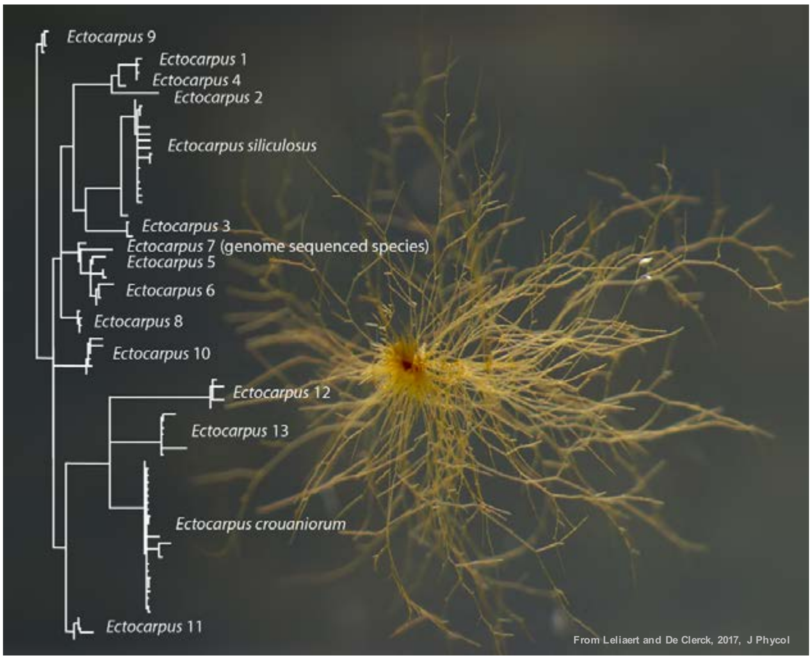
Campagne d'Evaluation 2017

Unité Mixte Internationale Franco-Chilienne

EVOLUTIONARY BIOLOGY AND ECOLOGY OF ALGAE

UMI 3614

Direction Myriam VALERO / co-direction Sylvain FAUGERON



From Leliaert and De Clerck, 2017, J Phycol

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1. Overview of the international joint research unit UMI 3614

Background and history

The creation of the UMI 3614 Laboratory of 'Evolutionary Biology and Ecology of Algae' (EBEA) is anchored in a long history of scientific collaboration between the Roscoff Marine Biological Station (SBR) and the Chilean universities Pontifical Catholic University of Chile (PUC) in Santiago and Austral University of Chile (UACh) in Valdivia. Some of the members of this research unit (namely Juan Correa, Christophe Destombe, Sylvain Faugeron and Myriam Valero) have been working together for nearly 20 years (1997 PICS-CNRS, ECOS-CONICYT projects, European INCO-DEV project).

The ties between SBR and Chile were further strengthened in 2003 with the creation of the International Associated Laboratory (LIA) between SBR and PUC. These interactions proved to be very fruitful, particularly for publishing papers and for training early career researchers. Five research-lecturers specialising in population genetics who currently hold a position in Chilean universities (S. Faugeron (PUC), L. Cardenas and M-L. Guillemin (UACh), A. Brante and F. Tellier (UCSC) are either former joint PhD students or former post-doctoral researchers from the LIA. Based on the fruitful LIA experience, UMI 3614 was created and is now completing its first five-year contract period.

UMI 3614 is spread across three sites: one located in Roscoff, France (UPMC-CNRS SBR) and two located in Chile: in Santiago (PUC) and in Valdivia (UACh).

UMI 3614 staff and financial resources

Changes in staff

Our two-pronged, theoretical and experimental approach continues to underlie our research owing to the complementary expertise of the six UMI 3614 research-lecturers and researchers, bridging the fields of modelling, experimental ecology and population genetics. The research plans laid out in the UMI 3614 project for its first contract period called for additional expertise in population genomics and quantitative genetics. The PhD students and post-doctoral researchers recruited during this period effectively have skills matching these profiles, and the goal now is to preserve this know-how within UMI 3614. Expertise in population genomics was enhanced in Chile (Valdivia) with the recruitment of Pablo Saenz at UACh as a research-lecturer who will join UMI 3614 upon renewal of the contract. A position for a research-lecturer specialising in seaweed ecology and/or evolution is currently being advertised for PUC in Santiago. Although this position is not specifically ear-marked for UMI 3614, the recruit will be strongly encouraged to join our research unit. In Roscoff, UMI 3614 has benefited from the arrival of Stéphane Loisel at SBR in September 2016; he is an assistant engineer in natural, geological and human-modified environment studies. This position is shared with two other research teams from UMR 7144 (DIVCO – Diversity and Connectivity in

Coastal Marine Landscapes and EFEB – Ecogeochemistry and Benthic Ecosystems Functioning) and meets our strong need to implement, optimise and ensure long-term *in situ* experiments. Moreover, the CNRS Institute of Ecology and Environment (INEE) provided the funds to recruit Barbara Raffenne for a part-time employment contract to handle administrative matters and develop the international cooperation actions at UMI 3614. This position has been crucial for ensuring good working conditions at UMI 3614 and for promoting the cohesiveness of the unit. This contract period has thus been very positive for our staffing needs which have been met, although the future of these positions is uncertain. We now have an operational team of personnel in France and in Chile to maintain algal cultures, carry out crosses and set up controlled experiments. However, the position of administrative manager for international cooperation needs to be stabilized for next five years. For the future, we have proposed to mutualize this position with the FR2424.

Since early 2014, the number of permanent positions has grown from 11 to 12 and should reach 14 or 15 by the beginning of the next contract period. In total, UMI 3614 staffs 30 people within its walls each year. In terms of contractual or temporary personnel, UMI 3614 has hosted 36 people during the past five years: 10 post-docs, 16 PhD students (co-supervised or joint PhD degrees), 10 contractual employees as well as Master's (first- and second-year) student research interns.

Changes in financial resources

In terms of laboratory and office space, SBR has provided an office for UMI 3614 to accommodate short-stay Chilean researchers and students. In addition, two laboratories continue to be shared with the DIVCO team at SBR in perfect harmony: one dedicated to genotyping analyses and the other to microscopy. However, there is no specific office space for French short-stay visitors at UACh in Valdivia or at PUC in Santiago. Nevertheless, French visiting scientists always have use of office space at both universities during their stays.

The total annual financial resources have varied from €540,000 to €730,000. The creation of UMR 3614 has fostered international visibility for the unit and has unquestionably contributed to our high success in obtaining research grants. A large proportion of the budget (90%) comes from research grants (from the French National Research Agency (ANR), the French 'Investment for the Future' expenditure programme (PIA) and national Chilean programmes). However, the CNRS and UPMC subsidies are nonetheless crucial for the unit's operations. These funds finance the exchanges (virtual or visits) between the different sites as well as exploratory studies. Since 2017, we have secured European or international funding for three research projects.

Scientific strategy

UMI 3614 is an international research laboratory with French CNRS researchers and UPMC research-lecturers and Chilean research-lecturers at PUC and UACh. The research conducted at UMI 3614 focuses on the basic precepts of ecology, evolution and genomics in marine algae and develops links with resource management and seaweed farming. There is much to gain from pursuing basic research on seaweeds at SBR and at our Chilean partner institutions. SBR offers unique facilities and a stimulating environment for studying algae, particularly in the fields of biology, evolution, chemical ecology, genetics and genomics. Our Chilean partners have complementary expertise in experimental field ecology of seaweeds along with extensive experience in uni-algal cultures, and international renown in the management of marine protected areas, the analysis of ecosystem services and aquaculture. Moreover, they have access to a remarkable coastal ecosystem – one of the world's most productive due to the Humboldt current, but nonetheless subject to the recurrent El Niño phenomenon – and diversified algal resources and in particular the red seaweed *Gracilaria chilensis* and the giant kelp *Macrocystis*, both of which are key engineering species of economic interest. Collaboration with Chile has been successful in terms of exchanges (researchers, post-docs, PhD and Master's students), scientific production and recruitment. This success encourages UMI 3614 to apply for a renewed contract.

The UMI's visibility as a successful example of long term collaborative research and student training was improved, in particular through the activity of Juan Correa as Dean of the Faculty of Biological Sciences in the PUC, Santiago Chile. Within the PUC, Juan Correa actively contributed to the definition of an institutional long term policy aiming at providing financial support to consolidated international programs that have demonstrated a long lasting and fruitful relationship. In fact, our UMI has been used as a model to be followed by other initiatives at the University. These efforts have been successful, and the PUC has established a competitive fund that consists in an initial stage that provides ca 13.000 euros, and following an evaluation, the possibility to apply for additional 130.000 euros. Our UMI obtained the first fund and we will apply for the second funding during this September. As the result of the existence of the UMI, J. Correa is now a designated member of the Internationalization Committee set up by the PUC. This Committee has the responsibility of develop a modern, active and supportive structure and policy to help international collaboration of our Faculty members. It is clear that the success of our UMI has, and will have, enormous impact in helping to define the International Collaboration Policy of the PUC, and, as a result, it is highly likely that the UMI will benefit from that policy.

The UMI road map that we have followed over the past five years is summarised in Figure 1. The first objective of UMI 3614 is to develop basic research on the dynamics of seaweed biodiversity. In particular, UMI 3614 pursues basic evolutionary questions such as the evolution of life-history traits and the consequences of the diversity of life cycles on adaptive processes (response to abiotic and biotic stress) to understand the ecological and evolutionary limits of adaptation. The theoretical models and empirical approaches of population genetics provide the tools for addressing these basic questions. The consequences of the ecological and evolutionary processes that lead to local adaptation logically lead to questions on the evolutionary mechanisms behind reproductive isolation and speciation. The second objective of UMI 3614 is to meet societal demands on issues regarding the preservation and the management of biodiversity in the context of resource depletion, habitat destruction and global change in general. Along these lines, we have carried out research on the significance of host-parasite interactions during toxic algal blooms and on the importance of local adaptation in seaweed cultivation and the management of algal resources. One of the features of UMI 3614 is its efforts to effectively bridge the gap between basic research and societal issues.

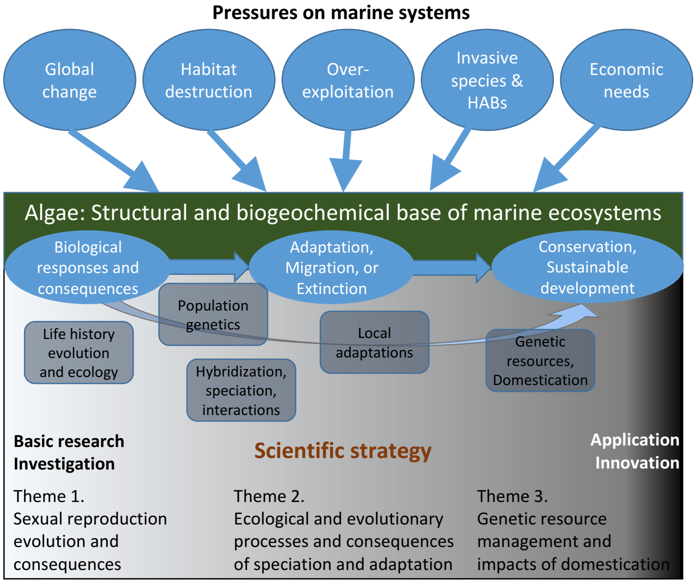


Figure 1 : Road map of the UMI 3614

The following three topics were studied during the first contract period:

(1) Evolution of sexual reproduction and its consequences (Leaders: Denis Roze & Marie-Laure Guillemin).

(2) Speciation and adaptation: ecological and evolutionary aspects (Leaders: Christophe Destombe & Sylvain Faugeron).

(3) Domestication, management of genetic resources and environmental impacts (Leaders: Sylvain Faugeron & Myriam Valero).

UMI 3614 has a unique potential for conducting research on seaweeds owing to its expertise that spans the fields of biology and evolution, genetics and genomics and experimental field ecology. This broad expertise has allowed us to address evolutionary questions using a combination of theoretical approaches (mathematical modelling) and experimental approaches in the field and in the laboratory. Our models are relatively little studied organisms (mainly micro- and macroalgae) that nonetheless have particularly interesting features, such as a wide variety of life cycles and mating systems.

Finally, the *raison d'être* of UMI 3614 is to promote the collaborative links between France and Chile mainly in basic sciences, but also in more applied fields such as aquaculture (seaweed farming) and the management of marine resources. UMI 3614 has reaped the fruit of its endeavours. It has encouraged and facilitated exchanges between the unit's three sites with 9 to 12 bilateral visits every year, striking a balance in the number of reciprocal Chile-France and France-Chile visits (see Figure exchanges). At least one researcher or research-lecturer at each of the three sites has travelled to and stayed for at least three weeks at one of the other two sites. These exchanges ensure the smooth coordination of research projects, the drafting of review articles and the initiation of new projects. UMI 3614 has also made it possible for each of the partners to seek new collaborations with European and South-American scientific communities, particularly through the organisation of summer schools or workshops. Moreover, the creation of UMI 3614 has given a high profile to French-Chilean collaboration in marine biology, resulting in an invitation to participate in two academic and scientific French-Chilean forums, one in Paris in 2015 and the other in Santiago in 2017. Moreover, UMI 3614 is currently applying for the creation of a National Reference Laboratory (NRL) for seaweed resources, under the auspices of the Chilean State Secretary for Fisheries and Aquaculture. The NRL will have a two-fold mission to coordinate research for the development of standards and regulations for the sustainable management of algal resources in Chile and to preserve (and improve if necessary) their genetic resources. UMI 3614 will thus be placed at the heart of the Chilean National Strategy on algae, which constitute a key economic sector for the country.

Biological models

The main biological models studied at UMI 3614 are marine algae. The choice of target species has been made over the course of our research, based on two main objectives: exploring the fundamental questions of evolutionary biology and addressing societal issues regarding the development of seaweed farming and the preservation of biodiversity. More recently, we have begun to develop experimental approaches for the study of evolution, as part of our 'Evolution of Sex' research topic, leading to the adoption of a new model lacking chlorophyll: rotifers.

2. Scientific production and research activities
(by topic)

Our scientific production and research activities are detailed below according to the three main research topics pursued at UMI 3614:

Topic 1: Evolution of sexual reproduction and its consequences

Theoretical approaches

The causes and consequences of the evolution of mating systems have been explored using theoretical and empirical approaches as part of four ANR projects: collaborative projects Clonix (coordinators: Sophie Arnaud-Haond, IFREMER Sète), Bicycle (coordinator: Mark Cock, CNRS-UPMC UMR 8227, Roscoff) and TRANS (coordinator: Sylvain Glémin, ISEM Montpellier) as well as the early career researcher project SexChange (coordinator: Denis Roze).

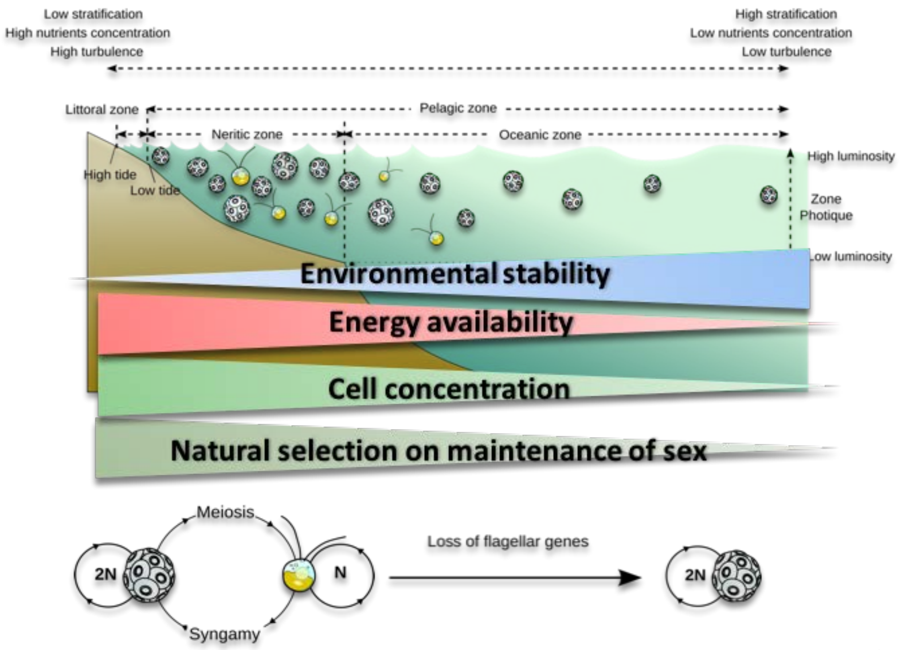
The theoretical models explored in the TRANS project have in particular helped better understand the evolution of self-fertilisation and its consequences on the effectiveness of natural selection using models that include the effects of gene interactions (Gervais et al. 2014; Roze 2015, 2016; Gervais & Roze submitted; Abu Awad & Roze 2017). Other models developed in the Bicycle project have explored the interactions between the genetic and ecological effects on the evolution of life cycles and the maintenance of two-stage life cycles, including the development of a haploid stage and a diploid stage, as observed in many seaweeds (Rescan et al 2016, Scott & Rescan 2017). Finally, models of the evolution of quantitative traits are currently being used to study the selection pressures that can favour sex in stable environments, or in those that change in time or space (SexChange project: Roze & Blanckaert 2014; Vanhoenacker & Roze in prep.). Moreover, one of the first methods able to quantify the proportion of clones in natural populations – and not just detect the presence of asexual reproduction – was developed in the Clonix project (Becheler et al. in press). The major advantage of this Bayesian approach lies in its ability to estimate the proportion of real clones for datasets with low sample sizes or numbers of loci (30 populations and 10 loci are sufficient), making it very versatile even for species for which genomic population data are not available.

Empirical approaches

For our empirical approaches, developed in the Bicycle project, temporal *in situ* monitoring of two *Ectocarpus* species showed that ecological niche differentiation between haploid and diploid individuals cannot account for the maintenance of the haploid-diploid life cycle (Couceiro et al, 2015).

In addition, as predicted by models, it was difficult to measure the frequency of asexual reproduction based on the analysis of genetic structure in the dinoflagellate *Alexandrium* *minutum* (Dia et al. 2014). The difficulty lies in the sampling effort required to obtain a good estimation of allele frequencies in its large, short-lived populations. However, it was easier to detect the presence of asexual reproduction in natural populations in two macroalgal species: in the calcareous red seaweed (maerl (rhodolith), Pardo et al. in prep) and the invasive green seaweed (*Caulerpa taxifolia*, Arnaud-Haond et al. 2017). Detection of asexual reproduction in macroalgal species is likely facilitated by predominant clonal reproduction in at least some populations, their comparatively smaller population sizes and their comparatively longer lifespans. The combined use of molecular data and controlled experiments in the red seaweed *Gracilaria chilensis* showed that drastic changes in life-history traits (clonality vs. sexual reproduction) observed in seaweed farms are related to inadvertent recurrent selection of sterile diploid individuals by seaweed farmers (Guillemin et al. 2014a).

In microalgae, the loss of sexual reproduction (and the haploid-diploid cycle), features associated with genomic erosion, was studied in the Coccolithophore *Emiliania huxleyi* (von Dassow et al. 2015). This loss was observed in offshore populations experiencing low biotic pressure and low environmental variation (Figure 2).



From von Dassow et al 2015

Figure 2: Loss of sexual reproduction in *Emiliana huxleyi.* High genome content variability in *Emiliania* relates to erosion of 1N-specific genes and loss of the ability to form flagellated cells. Analysis of 185 E. huxleyi strains isolated from world oceans suggests that loss of flagella occurred independently in lineages inhabiting oligotrophic open oceans over short evolutionary timescales. This environmentally linked physiogenomic change suggests life cycling is not advantageous in very large/diluted populations experiencing low biotic pressure and low ecological variability.

However, sexual reproduction as well as the ability to produce a haploid stage seem to have been maintained in coastal populations experiencing strong biotic pressure from viruses (that attack only the diploid stage). The *E. huxleyi* model was also used to study the physiological differences between the haploid and diploid stages (Rokitta et al., 2014, 2016). Although the categories of functional genes responding to nitrogen and phosphorus deficiencies are similar between the haploid and diploid stages, the genes involved in each stage are very different (Rokitta et al. 2014, 2016). To further study this issue, we used new transcriptomic and genomic approaches for diatoms to study the evolutionary conservation of meiotic recombination mechanisms in the eukaryotic nucleus (Patil et al., 2015).

Finally, we have been developing a new biological model (monogonont rotifers) to study the advantages of sexual reproduction using experimental evolution approaches (SexChange project).

In conclusion, as a recognised player in this field, UMI 3614 – namely Denis Roze – organised along with Tanja Schwander (University of Lausanne) a Jacques Monod conference on the evolution of sex and genetic systems in April 2013, which led to a special issue of the *Journal of Evolutionary Biology* (July 2014). Another conference on this topic will be held in Roscoff in Spring 2018.

Topic 2. Understanding speciation processes and the evolutionary and ecological limits of adaptation

Importance of cryptic species in the marine environment

As part of the national research network (GDR) Marco that assembles researchers in evolutionary genetics and ecology working on marine organisms (coordinator: S. Arnaud-Haond, IFREMER Sète), we noted a wide gap between the work of taxonomists who describe species and molecular ecologists whose gene flow analyses based on species delimitation models. This issue is particularly important in the marine environment where the inventory of biodiversity is lagging compared with that of terrestrial environments. Scientific exchanges within this network led to the publication of a review article on this subject (Pante et al., 2015). This article had a strong impact on the scientific community, being recommended by Ferdinando Boero in *F1000Prime* in January 2015. In this article, we build a conceptual framework in which species delimitation can be formalised as a falsifiable hypothesis and show how studies on gene flow and connectivity can foster integrative taxonomic work and vice versa (Figure 3).

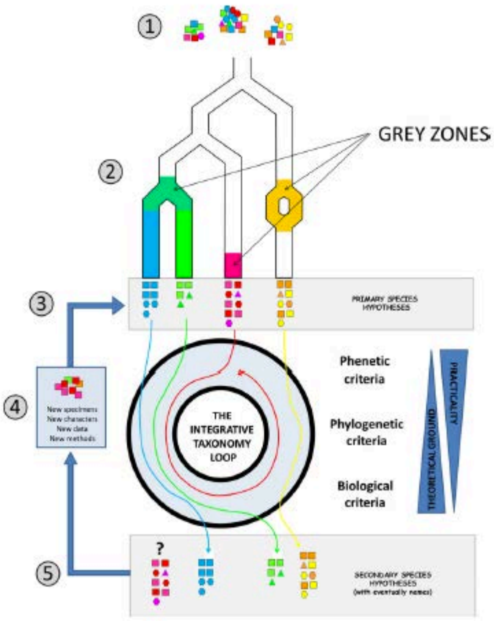


Figure 3: The integrative taxinomy loop. Integrative taxonomy consists in analyzing different characters, with different methods, and applying different criteria of species delimitation to propose species hypotheses that are as robust as possible. The different families of criteria (as discussed in Samadi & Barberousse 2015) are listed right of the loop, with the more theoretically grounded on the bottom, and the more operational (i.e. easy to test practically) on the top. Within each family of criteria different kinds of characters (i.e. morphology, ethology, ecology, biochemical, genetic, etc.) and methods (i.e. distances, maximum parsimony, maximum likelihood, population genetics inferences, crossing experiments, observations, etc…) may be applied. The different steps are as follows. 1. Population and phylogenetic sampling. 2. Sampled species may be highly differentiated (blue and green), recently diverged species that are still in a "grey zone" (see Glossary) with most characters undifferentiated (pink and red), or a single species that went through a temporal split into several temporary lineages (yellow/orange). 3. Primary Species Hypotheses (PSH) are proposed, for example using morphology or a single molecular marker. 4. PSH are engaged in the integrative taxonomy loop and are evaluated, possibly with the addition of new material, using different criteria for species delimitation. The more theoretically-grounded biological criteria can be tested directly using cross experiments or indirectly with unlinked markers, and complemented with more operational criteria. 5. When possible, taxonomic decisions are taken by turning PSH into Secondary Species Hypotheses – SSH, and are named. Some lineages (i.e. the pink/red lineage) may stay in the loop, needing more conclusive data before being turned into SSHs. Most of the literature and methods for species delimitation focus on species that are currently in the grey zone (cf. Carstens et al. 2013), even though most delimitation cases fall outside of this range.

During the first contract period for UMI 3614, we carried out work to genetically delimit cryptic species and describe their distribution for some large seaweed taxa of high economic or ecological interest (*Porphyra* and/or *Pyropia* (commonly known as nori): Ramirez et al. 2014, Guillemin et al. 2016a; *Gigartina*: Billard et al. 2015; *Mazzaella:* Guillemin et al. 2016b and *Pylaiella*: Geoffroy et al. 2015).

Hybridisation and speciation: empirical and theoretical approaches

In the collaborative PIA project IDEALG (coordinator: Philippe Potin, UMR 8227, Roscoff) involving research scientists and private businesses established mainly in Brittany, the objective of the WP2 coordinated by UMI 3614 is to gain basic knowledge on seaweed cultivation and domestication, primarily by studying the variation in life-history traits and hybridisation mechanisms to develop techniques for seaweed breeding and selection programmes. The first step in this project was to use *Ectocarpus* whose genome has been entirely sequenced as a proof of concept to address these questions.Following the demonstration of many cryptic species within this genus (Montecinos et al. 2017a), we studied hybridisation mechanisms to better understand the underlying causes of speciation in this species complex (PhD thesis, Alejandro Montecinos, 2017).

We showed that in this haploid-diploid species, the study of the genetic structure of haploid and diploid individuals offers a unique opportunity to distinguish pre- from post-zygotic barriers (Figure 4). We used a method (XPloidAssignment) that can identify hybrids in populations that harbour individuals with different levels of ploidy (Montecinos et al. 2017b). Our ‘life-cycle’ approach to the study of hybridisation barriers was featured in the News and Views section in *Molecular Ecology*.

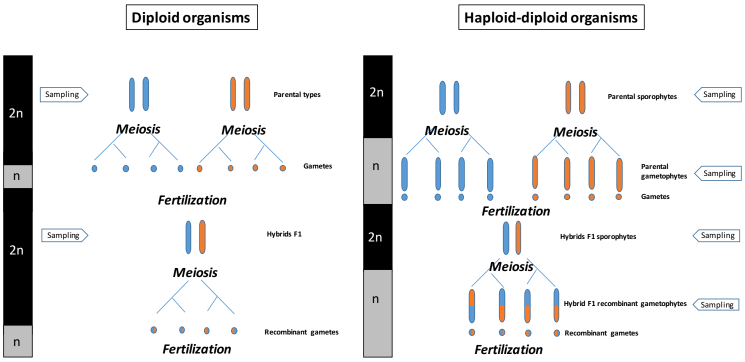


Figure 4: The haploid–diploid life cycle model to study hybridization and reproductive barriers. In diploid life cycles, mitotic cell division and somatic development occur entirely in the diploid phase. The haploid stage is reduced to a single cell (i.e. gamete) produced through meiosis in the diploid phase, and fertilization occurs immediately after release of gametes to recreate the diploid phase. Therefore, population genetics studies based on diploid life cycles are focused only on diploid individuals. On the other hand, in haploid–diploid life cycles, somatic development occurs in both haploid and diploid phases and there is an alternation between two types of independent functional individuals: haploid gametophytes produced by meiosis and diploid sporophytes resulting from fertilization. Haploid–diploid life cycles provide a unique opportunity to estimate the frequency of hybrid genotypes in diploid sporophytes and meiotic recombinant genotypes in haploid gametophytes allowing the effects of reproductive barriers that prevent fertilization to be distinguished from those that prevent meiosis.

These new data were also used to re-assess the phylogeographic and biogeographic patterns observed in European seaweeds (Neiva et al. 2016) and South Pacific seaweeds (Guillemin et al. 2016c, Figure 5) and the historical events that created them. The parapatric process of speciation appears to be recurrent along coastlines, and not vicariance, which is the most frequently suggested process of speciation for marine organisms.

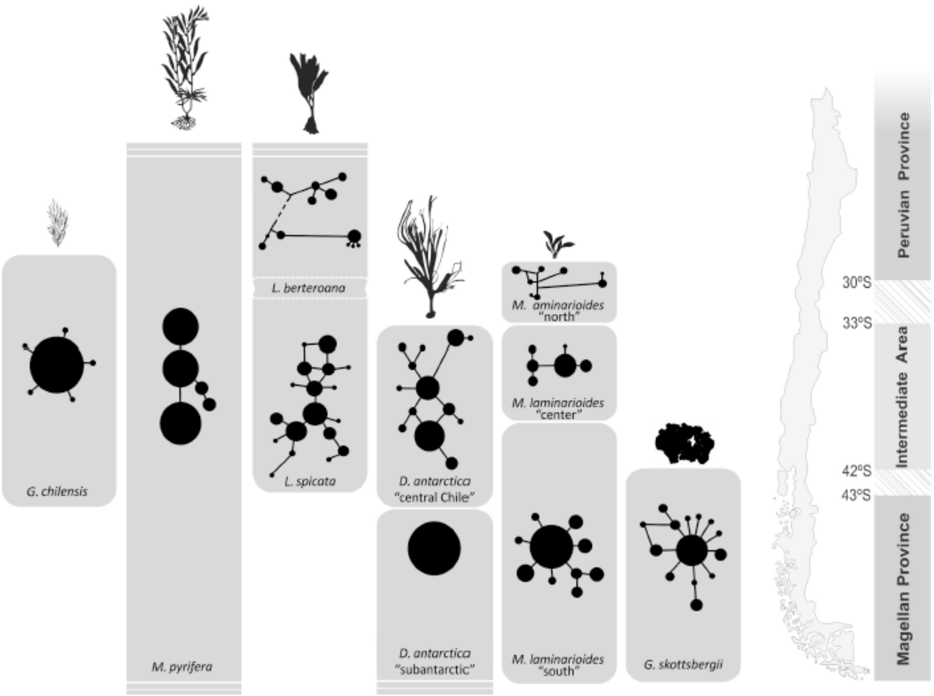


Figure 5: Phylogeographic and biogeographic patterns of chilean macroalgae. Schematized median joining haplotype networks of 10 seaweed taxa along the South East Pacific coast for which extensive phylogeographic studies have been published. Range distribution of each taxon is represented by a grey box. In all haplotype networks each circle represents a haplotype and, while the sizes of circles are proportional to haplotype frequencies in each taxon, they are not comparable across studies. Black lines represent mutational steps between haplotypes and the length of each line is proportional to the number of different base pairs between them.

Our phylogenetic approaches on microalgae (Haptophytes, Coccolithophores) showed a reticulated phylogeny for *Emiliania-Gephryocapsa*, revealing mismatches between genes from different cytoplasmic compartments. These results suggest that during its colonisation of the ocean over the past 292 thousand years, *E. huxleyi* underwent hybridisation and introgression with local species (Bendif et al., 2015; 2016). More recent (unpublished) results on the genetic diversity of *E. huxleyi* show that mitochondrial phylogenies do not match morphotype or thermal optimum patterns.

Finally, theoretical work has been carried out in the ANR HySea project (coordinator:

Frédérique Viard, UMR 7144, Roscoff) to understand to what extent current data on the genetics of speciation are compatible with classic models of adaptation, such as Fisher's geometric model (Fraïsse et al 2016).

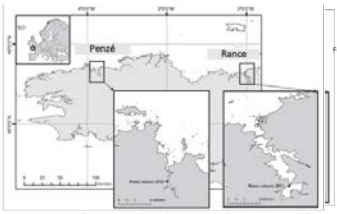
Coevolution and local adaptation

As part of the ANR Paralex project (coordinator: Laure Guillou, UMR 7144, Roscoff), we focused on the process of local adaption by studying the host-parasite interactions in the dinoflagellate *Alexandrium minutum*. Until now, parasites were considered to play a role in controlling phytoplanktonic blooms, but it was unknown whether the host and parasite could coevolve during this short lapse of time. Using a crossed inoculation design, with different time points (during a bloom) and different origins (two estuaries separated by 150 km), we showed that there is strong selection for parasites and high genetic variability for infectivity in the two tested parasite species as well as in host resistance. Our results suggest that the two parasites are well adapted to their host, reminiscent of the 'Red Queen' effect (Figure 6,

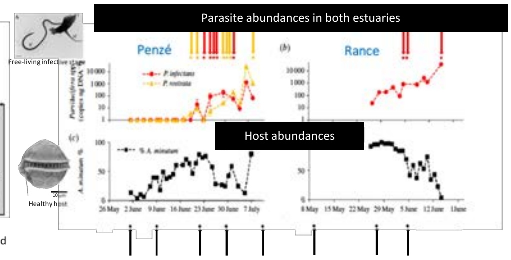
Figure 6: Evidence of parasite-mediated selection during short-lasting toxic algal blooms

From Blanquart *et al.* (2016) *Proceedings of the Royal Society B: Biological Sciences* **283**.

Harmful algal blooms (HABs) result from the rapid increase (few weeks) and accumulation of opportunistic and sometimes toxic unicellular algae. Several microeukaryotic parasitoids are known to efficiently control these blooming microalgae. Both hosts and parasites are suspected to have a huge population size associated with high reproduction rate, and both are susceptible to rapidly coevolve. However, whether coevolutionary dynamics occur in field during the rather short time of a bloom remains an open question.



Seawater samples were collected every two days to monitor the dynamics of two *A. minutum* blooms that occurred between May and June 2011 in the two estuaries separted by a distance of 170 km.

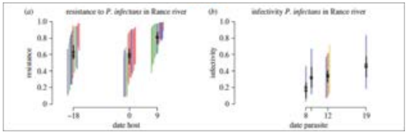


The decrease of *A. minutum* populations parallels the continued growth of *Parvilucifera* species suggesting that these parasitoids accounted for a large proportion in the demise of the bloom. Hosts (**115 strains of *A. minutum***) and parasites (**22 strains of *P. rostata*** and **15 strains of *P. infectans***) were sampled at different dates during the bloom (arrows) in order to perform 3711 cross-infections

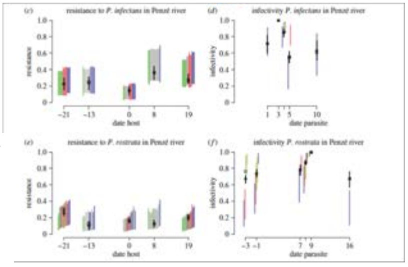
Evolution of host resistance and parasite infectivity in Rance and Penzé

For each graph, mean resistance or infectivity are given as a function of the date of collection of host or parasite strains and coloured lines represent the population varaibility for resistance and infectivity

Rance



Penzé



In Rance: (only *P infectans* is present), the host tends to increase its resistance: resistance does not change at the beginning of the bloom (-18 to 0 days) then increases when the parasite becomes prevalent (day 0 to 9)

- The host adapted to the population of parasites in about 10 days (3 generations of hosts) but no coevolution has taken place. (Perhaps because the genetic diversity of the parasite was not sufficient to allow selection to act).

In Penzé: the co-evolving dynamics are very similar between the host and its two parasites. The resistance of the host shows a temporal variation in *P infectans* and *P rostrata*. Hosts are on average more infected when confronted with their contemporary parasite rather than those of the past or the future. The two parasite species show a high variability between strains for their level of infectivity.

- The presence of high intrapopulation variability and the evidence of temporal fluctuation in infectivity and resistance suggests a co-evolutionary dynamics.

Coevolution is possible over a period of time as short as the time of a bloom (1 month). However, the processes are variable from one estuary to the other, which can have as a result a geographical variation in the dynamics of the blooms of this toxic species

Blanquart et al. 2017). These results show that coevolution is possible during a single bloom, i.e. over a very short period of time (on the order of several weeks), but varies in space (geographically).

Following work initiated during the ANR Ecokelp (coordinator: Myriam Valero) from 2007 to 2010 on the dynamics of biodiversity in kelp forests confronted with global change, the network of collaborations established locally at SBR (with UMR 7144 and UMR 8227), regionally (with IFREMER Brest, the Iroise Marine Park, and the seaweed trade union association), nationally (with the University of Lille and the National Museum of Natural History (MNHN) in Paris) and internationally (e.g. with Ester Serrao's research team at the University of Algarve, Faro, Portugal) led in particular to a review article on the status of the kelp forests in Europe (Araujo et al. 2016). UMI 3614 was also invited to participate in several projects (contract with the Iroise Marine Park, PIA IDEALG, the European BiodivERsA ERA-NET project MARFOR, the H2020 project GENIALG) to continue to study the processes that underlie the biodiversity of these forests. Our objective is to better understand the evolutionary and ecological role of local adaptation in these species but also, as described below, to propose measures that make their cultivation compatible with long-term functioning of these ecosystems.

One of the strong points of our work was to characterise the spatio-temporal variation in the biodiversity of kelp forests at the intra- and interspecific levels (PhD thesis, Marine Robuchon, 2014). Population genetic diversity of two sister species, *Laminaria digitata* and *Laminaria hyperborea*, and the specific diversity of the macroalgal understorey community were studied in Brittany. One of the important results is the demonstration that, on the scale of the Brittany coast, (1) there is a mosaic of abiotic conditions with contrasting spatio-temporal characteristics (Gallon et al. 2014); (2) this variability can explain some of the differences in the genetic diversity patterns observed in the two kelp species (Robuchon et al. 2014) as well as the associated understorey species diversity (Robuchon et al. 2017) and (3) the correlation between the spatial patterns of genetic and species diversity is generally positive, suggesting that neutral evolutionary processes predominate in these communities (Robuchon in prep). This mosaic, characterised by a pocket of cold water in north-western Brittany bordered by warmer waters in the south and the north-east, may be a refuge zone, but also may ultimately act as a barrier to dispersal for the associated understorey communities that are 'trapped' in this pocket of cold water.

In conclusion, our results show that there is a mosaic of different environments at a relatively small spatial scale (10 to 250 km), which is often ignored in niche models that predict changes in species distributions. Thus, populations are not all interchangeable; this fact influences the strategies proposed for defining protected marine areas (PMAs) or Natura 2000 zones (Couceiro et al., 2013). Finally, local mal-adaptation patterns were demonstrated in *L. digitata* in southern Brittany, at the southern limit of its distribution (Oppliger et al., 2014). Although diversity remains high in the harvested populations of this species, their location near the southern limit of its species distribution area exposes them to the threat of sea temperature rise, in addition to that of harvesting. Based on these results, we set up in conjunction with the SBR Observatory a long-term monitoring programme on the genetic variability in these two main kelp species on the Brittany coastline (10 sites were selected for regular sampling every 5 years).

Topic 3. Domestication, management of genetic resources and environmental impact

Domestication and cultivation

In contrast to many land plant species, relatively few studies have explored the domestication process in seaweeds, considered to be very recent (Duarte et al., 2007). We reviewed the literature on the subject and highlighted that the importance of the 'pre-domestication' process is often underestimated and, for some seaweed species, domestication history is older than previously thought, dating back to prehistoric times (Valero et al., 2017, Figure 7). In this review, intended for researchers working with seaweed

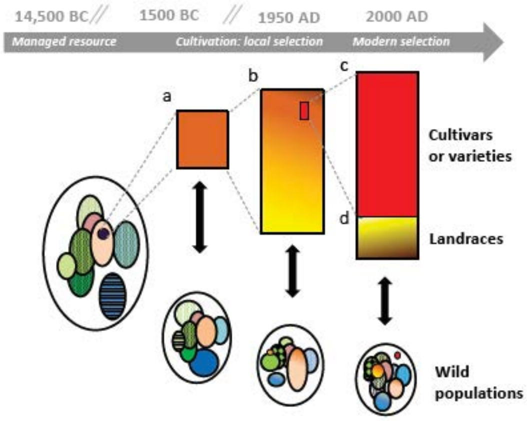


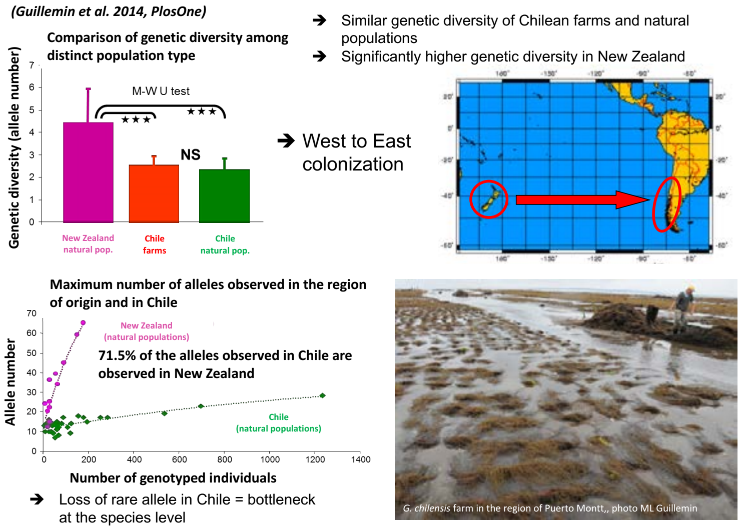
Figure 7: An overview of the domestication process in seaweeds, and the demographic, genetic and phenotypic changes involved. A schematic outline of the demographic history of a seaweed crop including a series of nested bottlenecks from wild populations (represented as ovals), and from successive cultivated strands (as rectangles). Gene flow between the seaweed crop and wild populations is indicated with black double-arrows. Both bottleneck and gene flow are integral parts of seaweed domestication history and have opposing effects on genetic diversity. Colours and motifs represent genetic diversity.
a: the first bottleneck corresponding to the cultivation of a few individuals collected from natural populations and able to grow in a new human-influenced environment; b: extension of the cultivated genotypes by farming and selection of individuals that present the best characteristics according to local conditions; c: the second bottleneck corresponding to the selection of cultivars and varieties from a few cultivated individuals; d: selection of a mixture of individuals adapted to local farming practices (landraces).
For seaweeds cultivated exclusively through vegetative propagation, i.e. repeated cutting of young branches from the same plant to form 'plantlets' (as performed for *Kappaphycus* and *Gracilaria*), the domestication process can be far more faster and completed in less than 50 years. Further to their domestication, these two seaweeds represent a limited genetic stock (Hurtado et al. 2014, Guillemin et al. 2008).).

harvesters and farmers as well as with businesses that process seaweeds, we underline the importance of using approaches that integrate ecology and population genetics on wild and cultivated stands, with characterisation of the selective pressures arising from cultivation practices. Moreover, we highlighted the issues regarding open-sea cultivation systems, in which it is impossible to control gene flow and difficult to standardise the environmental conditions compared with the terrestrial environment. This review insists on the importance of selecting local strains rather than focusing on breeding just one super-genotype for all cultivation systems and locations. The concept of local adaptation is thus the key issue and helps make seaweed professionals aware of the risks of transferring strains over large distances. It also raises the question of the propagation system for each species to ensure sustainable use of genetic resources. Finally, we also stress the importance of controlling transfers in the open sea regarding the risk of pathogen and/or herbivore proliferation.

We addressed these issues in the PIA IDEALG, H2020 GENIALG and Chilean National Fund for Scientific and Technological Development (FONDECYT) Macrocystis projects in three species characterised by different domestication histories (the Chilean red seaweed *Gracilaria chilensis* and two brown seaweeds: *Macrocystis pyrifera* found along the Eastern Pacific coast and the European species *Saccharina latissima*). During this contract period, our work focused on sampling campaigns, preparation of materials, optimising crossing methods and protocols for measuring the response to thermal stress and the development of population genomics tools. These details are not given here; instead, the expected outcomes are described in the research project section of this report.

In *G. chilensis*, the only intensively cultivated seaweed in Chile, our work led to the partial reconstruction of the evolutionary history of this species by combining archaeological information and population genetic and phylogeographic data (Guillemin et al. 2014b). Our study showed that the genetic diversity is much lower in Chile than in New Zealand, where the species has never been harvested or cultivated (Figure 8).

Figure 8: A very low genetic diversity of the cultivated red alga *Gracilaria chilensis* in Chile



(Guillemin et al. 2014, PlosOne)

Comparison of genetic diversity among distinct population type

- ➔ Similar genetic diversity of Chilean farms and natural populations
- ➔ Significantly higher genetic diversity in New Zealand

➔ West to East colonization

Maximum number of alleles observed in the region of origin and in Chile

71.5% of the alleles observed in Chile are observed in New Zealand

➔ Loss of rare allele in Chile = bottleneck at the species level

G. chilensis farm in the region of Puerto Montt,, photo ML Guillemin

We also showed that this reduction in diversity was primarily due to trans-Pacific colonisation at the end of the last glacial period and then due to the more recent effects of overharvesting and domestication by Chilean fisher communities. In *G. chilensis* in Chile, a founder effect arising from the long-distance colonisation event and human activities have clearly contributed jointly to decrease in genetic diversity in this seaweed and, far from representing a model of sustainable seaweed farming, this species may soon enter (or already be in) an extinction vortex.

The second objective of UMI 3614 in the PIA IDEALG project is to develop techniques to implement seaweed breeding and selection programmes. First, the model species *Ectocarpus* belonging to a sister group to kelps was used as a proof of concept to build a QTL map of the tolerance to two sources of environmental stress (temperature and salinity) on a segregating family of 89 individuals and their two parents. We chose the same family as that used by Heesch et al. (2010) to build a genetic map of AFLP markers and microsatellite markers. Several thousand genetic markers have been developed using ddRADseq techniques to construct a genetic map of 3588 SNP loci (Avia et al. 2017). In parallel, by exposing the parents and the 89 offspring of this family to more or less stressful temperature and salinity conditions, we identified 39 QTLs for growth-linked traits as well as plasticity traits (Avia et al., 2017, Figure 9). Phenotyping will continue on this family to characterise other traits of interest for IDEALG partners.

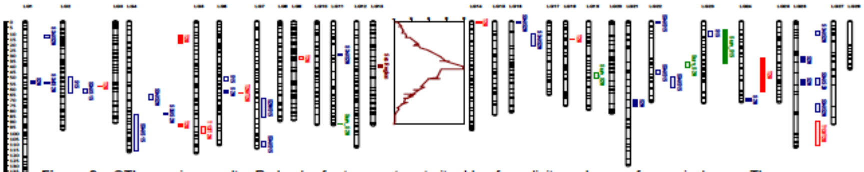


Figure 9: QTL mapping results. Red color for temperature traits, blue for salinity and green for survival ones. The curve on LG13 indicates sex QTL by Kruskal-Wallis non parametric method using MapQTL.

In addition, an F2 family was produced for *S. latissima.* This F2 family was obtained using the techniques that we have extensively developed in the laboratory: gametophyte isolation, sex determination and crosses. Sporophytes produced from these crosses were then cultivated in seawater on long-lines in plots rented from seaweed farmers. Two years were necessary to obtain this F2 family, which is currently growing in open seawater, to construct a genetic map and to screen for QTL traits of agronomic interest. A part of the new collaborative European H2020 project GENIALG, our goal is to compare the QTL analysis on haploid F2 offspring with that of F2 diploid offspring to study the genetic correlations between the haploid and diploid stages and to explore the epigenetic events related to the difference in ploidy. In Chile, similar approaches are currently underway on the brown seaweed *M. pyrifera* with a delay in obtaining the F2 generation due to the difficulty in producing fertile individuals in seawater (the first experiment led to an F2 with 19 males and 19 females from a single F2 sporophyte, but the second experiment failed to produce more sporophytes and gametophytes due to technical climate-related problems: a series of thermal and biotic stresses at sea).

Scientific outreach

UMI 3614 plays an important role in France-Chile scientific cooperation actions because it gives high visibility to the work that is done in ecology and evolution in marine biology. L'UMI was involved in the organization of the two France-Chile Forums, where we have presented our experience to other collaborative but less consolidate initiatives. It was for example actively involved in organising and moderating the round tables on 'Coastal sciences' during the France-Chile presidential forum in 2017. Chilean laboratories, at PUC and at UACh, regularly participate in scientific outreach events organised by the Chilean National Commission for Scientific and Technological Research (CONICYT), including the annual open-house event for schools, conferences for the general public in schools and at universities (e.g. participation of S. Faugeron and M-L. Guillemin as invited speakers for the 10th year anniversary of the Chilean Society for Evolution), discovery days and supervising research work for small groups of secondary school children (organised by the CONICYT Explora science outreach programme and the Chilean Antarctic Institute (INACH)) and the Antarctic Science Festival (INACH).

In parallel, SBR personnel are involved in scientific outreach for the general public in France, participating in various annual events (e.g. Researcher Night, National Science Week, the International Maritime Festival in Brest 2016) and giving conferences for the general public (at the Oceanopolis aquarium, the French Botanical Society, 'Entretiens de Port-Cros' symposium, 'Sciences à Coeur' science & society conference series at Sorbonne University). A bilingual (French-Spanish) book for young people (8-12 years) on the biodiversity of kelp forests in Brittany and in Chile will be produced by UMI 3614 (M. Valero, C. Destombe and V.L. Oppliger). This work aims to spark interest in these exceptional ecosystems to life and raise awareness in children and their parents on the protection of the coastal environment. It also endeavours to explain the scientific concepts behind ecology and evolution.

In 2014 and 2015, UMI 3614 hosted an Art & Science project instigated by a French-Chilean team of artists who envisaged a memory piece. Using natural seaweed-based pigments, they produced very well-defined images in negative on the surface of seaweeds without using any chemical substances, effectively rematerializing the souvenir of those who perished at sea during the Pinochet regime. Trials on various Breton and Chilean seaweeds were carried out and the project is currently seeking additional funds to come to fruition. An exhibit is to take place in Roscoff and in Chile.

As part of the IDEALG project and the European projects GENIALG and MARFOR, UMI interacts closely with seaweed professionals and private businesses marketing seaweed-derived products, marine park managers and NGOs involved in biodiversity protection. Through these projects, UMI 3614 contributes to innovation and the results of our research have an impact on the economy and on society. As mentioned above, the major interests of UMI 3614 lie in the processes driving biodiversity, the effect of global change on this biodiversity and in particular the consequences of seaweed cultivation on the environment. The objective is to guide the sustainable development of seaweed harvesting and cultivation for all the players in the seaweed trade. The harvesting pressure on natural ecosystems has stimulated seaweed farming projects around the world. The potential impact of these cultures on the genetic diversity of wild seaweeds and more generally on the ecosystem is an important issue.

Education and Training

UMI 3614 is very involved in education and training activities and actively participates in teaching courses for undergraduate and graduate degree programmes in France and in Chile. The research-lecturers at UMI 3614 (SF,CD, MLG, PVD) are head instructors for eight Master's degree courses (or their equivalent in Chile) that are taught in French, English or Spanish as part of the UPMC Master of Science of the Universe, Environment, Ecology (SDUEE) and two courses in Genetics and Evolution at both UACh and PUC. Researchers as well are actively involved in the organisation and teaching courses in genetics and evolution or on marine model organisms (seaweeds and invertebrates).

AT UPMC:

- Two courses for the SDUEE Master with a concentration in Ecology, Biodiversity and Evolution: (1) Molecular Ecology and Evolutionary Genetics of Marine Populations (Head instructors: C. Destombe & C. Lejeusne); (2) Evolution of Reproductive Cycles (Head instructors: C. Destombe & A. Frantz)
- Two courses for the SDUEE Master with a concentration in Oceanography and marine environments: (1) Marine Biodiversity: Identification of Marine Flora (Head instructors: N. Simon & C. Destombe); (2) Measuring Species Diversity and Distribution (Head instructors: E. Thiébaut & C. Destombe)
- As well as two courses in the Biology-Mathematics Bachelor's degree programme: (1) Population Genetics (first-year course) (Head instructor: C. Destombe); (2) Genetic Systems and Evolutionary Mechanisms (Second-year course) (Head instructors: C. Destombe & D. Roze)
- Participation in the continuing education course on Seaweed Taxonomy offered at SBR, through UPMC and MNHN (Head instructor: N. Simon)

At PUC:

- Graduate-level courses: (1) Molecular Ecology (Head instructors: S. Faugeron & E. Poulin); (2) participation in the Fundamentals of Evolution course (Head instructor: MF Perez; S. Faugeron and P. von Dassow ensure roughly one-fourth of the course volume)
- Undergraduate-level courses: (1) Genetics and Evolution (Head instructors: S. Faugeron &

P. Carvallo); (2) Introduction to Aquaculture (Head instructors: R. Sà, F. Bas & S. Faugeron; General Oceanography (Head instructor: P. von Dassow); (3) Physical and Biological Oceanography (Head instructor: P. von Dassow).

At UACh:

- Graduate-level courses: Advanced genetic (Head instructor:. M-L. Guillemin). M-L. Guillemin also participate to Population genetic and quantitative genetic (Head instructor: L. Cardenas) and Molecular Ecology (Head instructor: P. Saenz)
- Undergraduate-level courses: Genetic and evolution (Head instructor:. M-L. Guillemin), Biogeography (Head instructors: S. Paula, M-L. Guillemin in charge of the module of "speciation and historical biogeography")

UMI 3614 staff are involved in various international courses. In particular, they organised the Austral Summer School 'New challenges for research in biodiversity and ecology of micro- and macroalgae' (27 November – 6 December 2015, ECIM, Las Cruces, Chili), with the participation of 9 speakers and 19 students from 7 countries. Organisers: S. Faugeron and M. Valero.

Moreover, UMI 3614 members are involved in designing and coordinating Bachelor's and Master's courses by participating in meetings and in Master's thesis committees at various partner universities. Christophe Destombe represents the Roscoff and Banyuls-sur-Mer marine stations in the MNHN Doctoral School ED227 (Natural and Human Sciences: Evolution and Ecology). Marie-Laure Guillemin was the director of the Master's degree programme in Genetics at UACh in 2014 and 2015.

From 2012 to 2017, UMI 3614 members have supervised 23 Master's (second-year) research interns and 16 PhD students, of which 5 are still underway.

UMI 3614 members have been solicited to participate in 23 PhD thesis committees and 8 HDR thesis committees.

Finally, Denis Roze participated in writing a chapter on the evolution of sex for the graduate-level textbook *Biologie Evolutive* (de Boeck, 2nd edition published in 2016).

Scientific collaborations at the local (SBR), regional, national and international levels

At SBR, the creation of UMI 3614 has not altered its relationships with the other SBR research units and support services, namely UMR 7144, UMR 8227 and FR 2424. Our daily interactions with them involve logistic, administrative and, of course, scientific aspects. UMI 3614 is represented in the SBR Management Board and thus participates in defining the scientific strategy at SBR. Scientific collaborations with the other SBR research teams take place through common research projects (3 ANR, 1 PIA and 1 H2020 projects) and have produced 20 co-authored articles, co-supervision of 3 PhD students and 2 post-doctoral researchers. Our research draws on the expertise, infrastructures, services and facilities offered by FR 2424 (bioinformatics, genomics, biological resource centre, marine operations and facilities, etc.). The local research teams have strongly supported the functional organisation of the FR 2424 into common core service departments and facilities. It is thus essential that these departments and facilities continue to benefit all the on-site research teams. To maintain and preserve this type of collective, shared organisation of services and facilities, the SBR research units contribute 4% of their funds to the FR2424 budget.

The regional and national scientific collaborations (IFREMER Brest and Sète, MNHN, University of Montpellier II, University of Aix-Marseille) and international collaborations with Portugal (University of Algarve, Faro), Germany (Alfred-Wegener Institute), the United States (University of Arizona) and Brazil (University of Sao Paolo - USP) were fostered by the various funded projects (6 ANR and 13 Chilean national projects, including 6 coordinated by UMI 3614, 1 Inter-ministerial Fund (FUI) and 3 European projects) and led to 10 co-supervised and 3 joint PhD theses with Chile (including 2 with a laboratory belonging to the France/Chile/Brazil international research network (GDRI)).

In addition, owing to the bilateral Cofecub project (French Evaluation Committee for Academic and Scientific Cooperation with Brazil) and the creation of the France/Chile/Brazil GDRI (10 funded mini-projects and exchanges), collaborations between UMI 3614 and other Brazilian laboratories (USP) and Chilean laboratories, such as the two universities in Concepción (UDEC and UCSC), the team headed by L. Conteras at the Andrés Bello National University in Santiago, the iMar centre in Puerto Montt, the University of Magallanes in Punta Arenas as well as internal collaborations at UACh and PUC have been very fruitful in terms of scientific production and joint degree students (2 Master's degree students and 1 PhD student). These collaborations are supported by national Chilean projects and have benefited from project funds during the first UMI contract period (e.g. CONICYT projects sponsored by FONDECYT, INACH projects). Moreover, the Millennium Institute of Oceanography project joined scientists from the UDEC, the Pontifical Catholic University of Valparaiso, the University of Antofagasta and the CEAZA research institute. Lastly, large Chilean projects, such as the FONDAP funds' IDEAL research centre project and the Millennium Institute, have provided further support for national collaboration.

Scientific renown and academic attractiveness:

The renown and attractiveness of UMI 3614 can be gauged by the participation of its researchers and research-lecturers in four editorial boards (D. Roze: Associate Editor for *Evolution*, C. Destombe: member of the editorial board of the *European Journal of Phycology*, section Molecular Biology, S. Faugeron and M.-L. Guillemin: members of the editorial board of *Cryptogamie Algologie,* M.-L. Guillemin: Associate Editor for *Scientific Reports*) and several scientific expert assessment bodies (active involvement in research and teaching assessment and administration, C. Destombe is member of section 67 of the CNU, S. Faugeron and M.-L. Guillemin are members of the committees that allocate CONICYT Master's and Doctoral scholarships) and as heads of thematic networks (M.-L. Guillemin: Chilean leader of the France/Chile/Brazil GDRI).

The reputation of UMI 3614 can also be measured by the 11 post-doctoral researchers and many visiting scientists hosted, its invitations to speak at national and international scientific congresses or seminars (6) and the organisation of 5 scientific congresses and 3 symposia, its participation in national or international schools and its scientific production of 79 articles in international, peer-reviewed journals, of which 71 have been published since the creation of UMI 3614 in 2014, giving an average of 4.5 articles per year per full-time equivalent researcher (average IF: 3.5).

Scientific report

Highlights

- Optimisation of a new biological systems for an experimental evolution approach;

- Development of genotyping tools for high-throughput sequencing and several thousand SNP loci in several macroalgal species (*G. chilensis, S. latissima* and *Ectocarpus*); bioinformatics analyses of population genomics are underway and will be completed during the next contract period.

- Genetic mapping and development of QTL markers for resistance to abiotic stress in the model species *Ectocarpus*;

- Development and consolidation of micro- and macroalgal culture collections;

- Theoretical work combining multi-locus analysis results and simulations for the development of original models on the evolution of sex

- Regional, national (in France and in Chile) and international reputation for expertise on genetic diversity of kelp forests, and public policy support showing the genetic and ecological factors to take into account in the development of rational seaweed cultivation practices;

- Importance of cryptic species in the marine environment and the conceptual framework in which species delimitation can be formalised;

- Review of the domestication of brown and red seaweeds in light of the recent development of seaweed farming;

- Population genetic diversity of microalgae and demonstration of the possibility of host-parasite coevolution during a single bloom;
- The success of our UMI has enormous impact in helping to define the International Collaboration Policy of the PUC University as well as of the Station Biologique de Roscoff.

3. Organisation of UMI 3614 and its activities

UMI 3614 is a research unit spread across three different geographic locations and has a staff of around 30. The relatively small size of UMI 3614 did not require setting up any formal councils. Decisions are made collegially during the monthly unit meetings. UMI 3614 unit meetings are done by videoconferencing (French RENATER network services). All UMI 3614 members are invited to participate in the first part of the meeting that is dedicated to current news and a scientific presentation of 20 to 30 minutes. The second part of the meeting, reserved for permanent staff, is centred on discussions of scientific strategy, administrative and financial issues and preparation for the various SBR council, board and committee meetings. The organisation of unit meetings (including diffusion of the agenda, writing up and distributing the minutes) is handled by the UMI 3614 administrative manager (Barbara Raffenne). She is also in charge of regularly sending out information by email. Moreover, meetings of the representatives of the three sites (Marie-Laure Guillemin, Sylvain Faugeron and Myriam Valero) are organised regularly, roughly about once a week by videoconference.

The CNRS-UPMC subsidies for the unit go mainly to ensuring exchanges and trips between France and Chile. A large part is also dedicated to SBR overhead operations (for example, contribution to the FR 2424 budget). The rest of the subsidy is dedicated to funding exploratory research projects (not yet funded externally) upon request of UMI 3614 researchers.

The web site of UMI 3614 is regularly updated by the administrative manager and plays an important role in the dissemination, visibility and cohesiveness of UMI 3614.

Yearly trips of the representatives of the three site also ensure the cohesiveness of UMI 3614 (Figure 10).

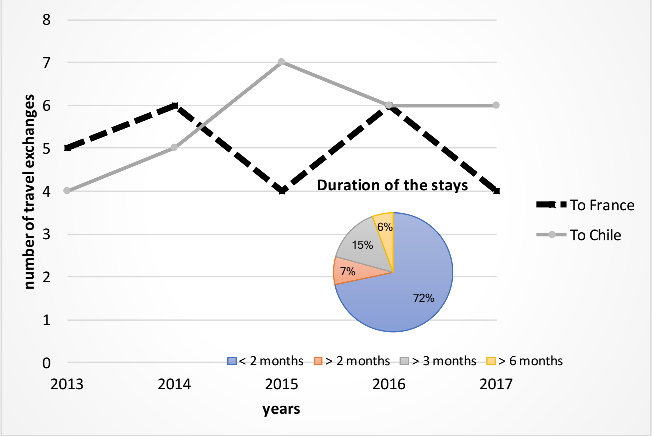


Figure 10: UMI Travel exchanges 20113-2017 (total 53)

Materials available at UMI 3614

Equipment needs (culture chambers, microscopy equipment and computing capacity) have been met by the CNRS at SBR. In Chile, the facilities for micro- and macroalgal cultures have been enhanced, with PUC investing in the organisation of its live strain collection, thus facilitating its integration with the Roscoff Culture Collection (RCC). Moreover, at PUC as well as UACh, genotyping and sequencing facilities have greatly improved upon securing Chilean funds (e.g. FONDEQUIP) for the purchase of capillary and Illumina sequencers. In addition, FONDEQUIP funding made it possible to acquire an advanced flow cytometer/cell sorter coupled with 488 nm and 640 nm lasers, with an additional 405 nm laser funded by well a Moore Microbiology Initiative ‘Experimental Model Systems’ grant. This acquisition has vastly improved strain isolation and phytoplankton analysis at UMI 3614. In addition to isolating phytoplankton, this apparatus can also be used to analyse macroalgal spores and isolate gametophytes.

Health and Safety, quality assurance

At SBR

Health and Safety (H&S) actions at UMI 3614 are part of the larger H&S network at SBR as defined by the SBR director. The local H&S committee is headed by Dominique Marie of UMR 7144, seconded by 6 H&S Liaisons from all the research units, each being specialised in one domain: Ludovic Delage (chemical substances), Nathalie Desban and Daniel Poher (infrastructures), Laurence Dartevelle (radiological protection), Yann Fontana (marine operations), Stéphane Mauger (molecular biology and H&S Liaison for UMI 3614).

Risk assessment and analysis at UMI 3614 is carried out by the H&S Liaison, who writes up and regularly updates the professional risk assessment reports, which are then submitted to the UMI 3614 director for validation. Corrective actions have been implemented by the H&S Liaison (inventory of chemical substances, systematic labelling of hazardous chemical products, installation of drip trays in storage cupboards for liquid chemical products, etc.).

There are no accidents to report for UMI 3614 during the 2012-2017 period.

Laboratory waste is collected by TERIS, a firm specialising in the elimination of chemical waste, and by SITA-OUEST, a firm specialising in the elimination of biological waste.

An H&S folder containing key safety information sheets specific to SBR is provided for all new SBR arrivals. The training programme for new personnel has been revamped and is now carried out via the on-line application NEO, an educational platform that provides training in safety for new personnel in CNRS research units. Each teaching module lasts around 20 minutes and ends with a quiz to test knowledge acquired. At the completion of the training session, a certificate, signed by the director of the unit, is given to the new recruit via the

H&S Liaison.

Along with this training programme, the FEVAR application is also used by the H&S Liaison to produce a radioactivity and chemical exposure assessment sheet for each staff member. Each year, refresher first-aid courses are organised, as part of continuing professional development training, as well as fire drills.

Since the arrival in 2015 of Frédéric Claude, the new Regional Prevention and Safety Officer (IRPES) at the CNRS Regional Office (DR17), meetings take place regularly at SBR between the IRPES and all the SBR H&S Liaisons (at least once every two months). Thus, many H&S issues have been addressed, such as:

- Visits to the units' laboratories before submission of risk assessment reports;
- Drafting and displaying specific information sheets in laboratories;
- Implementation of procedures for personnel working alone or after SBR opening hours;
- Drafting a new information sheet for new personnel.

In the coming contract period, two important events will take place:

1. Replacement of some the windows at SBR whose seals contain asbestos. Given that more than 50% of the UMI offices and laboratories will be affected by these works, in collaboration with the IRPES, the SBR technical departments and all the H&S Liaisons participate in the meetings to prepare for these works planned for late 2018.
2. Two UMR 7144 teams (ABICE and DIVCO) are to merge; the possibility of sharing some of the laboratory and office space with this new team is being reviewed. Some of this space has been shared between UMI 3614 and DIVCO since 2008. If this shared space is expanded, the hazardous chemical substances of UMI 3614 will be stored in UMR 7144 safety cabinets, located in the joint space shared by the ABICE+DIVCO and UMI 3614 teams.

At PUC:

In 2013, PUC set up a Biosafety and Bioethics commission to evaluate all research projects designed by its research-lecturers (including UMI 3614 staff) and if necessary guide the corrective actions and improvements required before the project begins. The project must be validated by this commission before signing any research grant agreement. This represents a major improvement in monitoring research activities with the creation and application of standards and protocols that aim to ensure the safety of all personnel as well as compliance with bioethics principles.

In parallel, during the current contract period, measures were taken to improve safety (in a country in which standards are not always precise or rigorous):

- Standardisation of signage regarding emergency evacuation procedures, with the designation of a safety contact person on each floor of the building to assist in emergency evacuation, particularly in the event of a strong earthquake. Jessica Beltran is the designated contact person for the 4th floor (which houses the algal laboratory and the main UMI 3614 offices); Gioconda Peralta is in charge of the 5th floor (which includes the genotyping laboratory and the sequencing facility which UMI 3614 staff manage). Both women have received special training in these procedures.
- Fire extinguishers are now installed in all laboratories and all permanent staff are trained in their use.
- A disposal management process has been set up for laboratory biological waste, which is incinerated twice a week.
- A disposal management process has been set up for laboratory chemical waste, which is stored according to standard protocols and disposed of once every 2 months by a specialised firm.In the UMI 3614 laboratories, improvements have been made to the organisation and management of space and personnel, with in particular:Storage of toxic and/or inflammable reagents in separate and clearly labelled cabinets;
- Writing up of a information manual, given to each new user of the algae laboratory that stipulates the main work and safety standards.

At UACh:

As in the case of the PUC, all national projects including a researcher of the UACH need to be revised by two internal commissions before being sent to funding agencies: the bioethic commission and the health and safety committee.

- All general, chemical and biological residues have to be managed in the UACh following a protocol established since 2003 and actualised each year (See "Manual de Procedimiento de Manejo de Residuos de la UACh"). Within the laboratory of Molecular Ecology (i.e. M-L Guillemin) Nelida Kohler is in charge of the organisation of the lab security and residues removal. Alcohol and flammable reagents are keep apart as are other toxic reagents, both in labelled and special containment facilities as demanded by the UACH health and safety committee.
- Each semester, a small course is organized to give information about health and safety procedures within the laboratory of Molecular Ecology. This course is obligatory for undergrads, postgrads and research assistants that are beginning their work in the lab.
- At least twice a year the UACH organize a real life test of the employees and students response when following emergency evacuation procedure, which, in our case, include both fire and earthquake.

Gender equality

Regarding permanent staff, there is a strong (but non significant) male bias. This imbalance remains the same regardless of the level of the position. In Chile, there are no gender equality rules for recruiting students or permanent staff. Positive discrimination tends to operate in the attribution of research grants, particularly in the Millennium Science Initiative programme. Moreover, women benefit from an inalienable right to a six-month maternity leave. In the case of female students and post-doctoral researchers with scholarships or a fellowship, their scholarship or fellowship is automatically extended for 6 months.

4. SWOT analysis

Strengths

- Multi-disciplinary partnership characterised by the original and historical position of national and international leaders in the fields of theoretical modelling of the evolution of mating systems, molecular ecology and population genetics in seaweeds, experimental ecology and reproductive ecology in the marine environment, environmental genomics and biodiversity of seaweeds;
- A long history of scientific collaboration between the Chilean and French research teams, with a special focus on studies of reproduction cycles. This collaboration has greatly benefited from complementary experimental and genetic approaches and theoretical models;
- International standing, with strong collaborations (outside of France and Chile): Canada, the Netherlands, Portugal, Brazil, New Zealand and the United States, and participation in national and international networks (France/Chile/Brazil GDRI on seaweed genetics; network of marine stations: EMBRC, Assemble+ and Euromarine) that integrate additional skills and expertise and foster the implementation of ambitious projects;
- Research projects centred on the genetics and evolution of seaweeds that meets societal demands for public policy support on domestication, resource management and aquaculture. The proactive research conducted at UMI 3614 is a real asset regarding the current emergence of many seaweed farm projects in Brittany, France and in Chile. These projects are conducted constructively with other SBR research units.
- Significant involvement in education and training programmes (undergraduate and graduate degree programmes) in France, Chile and Brazil, including supervision of Master's and PhD students with three universities that have already signed joint-degree agreements;
- Ability to organise international research training for Chilean Master's students (joint degrees, intensive collaborative courses, short- and medium-term internships in international laboratories); Master's students in Chile, in contrast to PhD students, have little access to national funds for this type of training;
- High success rate in obtaining research grants in Chile, France and in Europe, with clear participation of members from both countries in most of these projects;
- New expertise in the research fields that UMI 3614 set out to develop in 2014: (1) experimental evolution; (2) development of NGS tools and population genomics analyses; (3) genetic mapping and QTL markers;

Weaknesses

- Vulnerability of new expertise within UMI 3614, to be countered by sharing know-how across SBR research units;
- Weak harmonisation between the European Bachelor/Master/Doctorate system and the Chilean higher education system;
- Videoconference equipment in need of renewal and specific facilities; videoconferencing is a veritable lifeline between the three sites of UMI 3614.

Threats

- Geographic distance between France and Chile, which is an obstacle to communication between Chilean and French UMI 3614 staff. The videoconference equipment must be improved to ensure good-quality research unit meetings.
- Rigid administrative policies in France and in Chile that make it difficult to host non-national interns, transport or ship biological materials and transfer funds. UMI 3614 is often powerless when confronted with Kafkaesque-like situations. The administrative manager position has been the key to progress in this area. However, this position is still only temporary and its continuity is essential. There is also a basic need for legal counsel from the various affiliated institutions.
- Earthquake risks. Chile is one of the most earthquake-prone countries in the world, with a high associated tsunami risk (2 tsunamis and 7 earthquakes greater than magnitude 7 on the Richter scale since 2010). However, there are usually few victims, owing in particular to efficient infrastructures and the earthquake awareness of the population that allows for effective and appropriate action in the event of an earthquake.
- Multiplication of biological models has proven to be inevitable and justified by the choice of organisms the best adapted to the questions raised.

Opportunities

- Outstanding scientific environments: SBR provides research vessels, diving facilities and sampling equipment, as well as core technological facilities for biological and ecological research with a staff of highly qualified technicians affording access to data analysis in histology, microscopy, imaging, flow cytometry, cell cultures, sequencing, transcriptomics, proteomics and bioinformatics. The PUC marine station, ECIM, has considerably improved its research infrastructure and enhanced its international renown as a coastal laboratory in the South-East Pacific Ocean. Both marine stations provide ideal access to rich coastal ecosystems, experimental research and algal cultures, and are instrumental for the organisation of workshops and courses. The doctoral programme in Ecology and Evolution at UACh with which M.-L. Guillemin is associated has received a grant from MECESUP to set up a high-throughput sequencing facility, bioinformatics and genotyping facility, which will facilitate the acquisition and processing and analysis of population genomics or transcriptomic data.
- Increasing Chilean financial support: A positive aspect on the Chilean side is the increasing financial support for research and innovation in the marine domain in particular in aquaculture and marine resources, which were defined as the main priorities for the country. The Chilean members of UMI 3614 are actively involved in most of the excellence initiatives set up in this field, including the Millennium Nucleo

Centre for Marine Conservation, the Millennium Institute of Oceanography and the FONDAP IDEAL research centre focused on the resilience of high latitude marine systems, the Biotechnology and Bioengineering Centre (CeBiB) and the IFAN consortium for the development of biological applications derived from seaweeds. This type of opportunity is important for UMI 3614 because it offers the possibility to capitalise on the complementarity between the basic research implemented in the UMI 3614 scientific project and applied research offered through these initiatives. Similar types of opportunities are offered in the PIA IDEALG, translating into long-term grants for basic and applied research on seaweed biology.

- Bilateral France-Chile cooperation: French science shares a long history with Chile, in many fields (agronomy, mathematics, etc.). This bilateral cooperation is now pursuing its consolidation, in particular through the creation of a sponsored two-party France-Chile collaboration in which UMI 3614 actively participates (see for example the 'Coastal sciences' roundtable moderated by UMI 3614 members during the Chile-France presidential forum in 2017).
- French scientific influence: As mentioned above, several former students with a joint degree now have permanent positions in Chilean universities. Moreover, 5 of the 7 to 8 principal population geneticists in Chile are French – we can now say that there is a French School of population genetics. This is clearly an indication that the French-Chilean collaborations have left an indelible signature on the Chilean scientific landscape. The renewal of the contract for UMI 3614 is thus a guiding force in France-Chile scientific collaboration.

5. Scientific project for the next 5 years

One of the main objectives for the coming years is to strengthen the position of the UMI within the Franco-Chilean community using the existing networks at the regional, national and international levels. For example, the UMI should be involved in the organization of the next International Phycological Congress 2021 in Puerto Varas (Chile). This objective requires to increase the number of researchers in evolutionary biology in the UMI in order to perpetuate this Franco-Chilean collaboration. In this context, two new colleagues from the two Chilean universities are joining the UMI for the next quinquennial.

The research axes of the UMI are clearly defined for the next 5 years. The collegial functioning of the UMI leaves a fairly important place to risk-taking and to the emergence of innovative subjects and/or at the disciplinary interfaces. Moreover, the flexibility of the organization of the UMI should allow to rapidly react to the evolution of its environment and its scientific field.

As our research activities are partially developed on chilean algae species, from algae sampling to the establishment of a culture collection and identification of strains of interest , we wish to be compliant with international treaties and regional commissions. Whereas Chile did not sign the Nagoya Protocol, Chile adopted its first National Biodiversity Strategy in 2003 which proposed the following strategic orientations: (i) ensure ecosystem conservation and recovery to significantly slow the loss of biological diversity before 2010; (ii) ensure the preservation of species and genetic heritage; (iii) promote sustainable production practices; (iv) strengthen interinstitutional and intersectoral coordination for the integrated management of biodiversity; (v) establish formal and informal mechanisms required for the optimal management of biodiversity; (vi) strengthen environmental education, public awareness and access to information on biodiversity; (vii) strengthen and coordinate research; and (viii) consolidate funding mechanisms. In order to fulfil our commitments regarding the access and benefits sharing arising from the utilization of marine resources in Chile, we will contact the Access and Benefits Sharing contact point to explain our research on chilean species of algae and get the related authorizations that might occur for our research[1].

During these last four years, we have successfully fulfilled the new approaches we wanted to develop within the three research axes of the newly created UMI with (1) the development of experimental evolution to test prediction of theoretical models and (2) the development of thousands of SNPs in model or non model species to carry out genome wide investigation and QTL mapping. For the next five years, the aim is to strengthen and consolidate these three research axes as detailed below.

Topic 1: Evolution of sexual reproduction and its consequences

(Denis Roze & Marie Laure Guillemin)

The study of the evolution of reproductive systems and their consequences will be pursued using a combination of theoretical and empirical approaches. In particular, we plan to use more realistic models of genetic architecture in order to investigate the effect of environmental variability on the evolution of different aspects of genetic systems. The development of experimental systems for testing such theories in the lab will also be continued. Finally, we plan to explore the consequences of the reproductive mode of organisms (in particular, clonal vs. sexual reproduction) on the response of micro and macroalgal populations to environmental change (e.g., stress episodes or fluctuating environmental conditions), and initiate new projects on sex determination systems in red algae.

The theoretical part of the project will rely on analytical and simulation methods currently developed during the SexChange ANR project. These methods are based on models representing an arbitrary number of quantitative, polygenic traits under selection (such as Fisher's geometric model of adaptation). Using such quantitative trait models presents two advantages: (1) it allows one to introduce possible compensatory effects between different mutations, or more generally, distributions of epistatic effects (on fitness) that seem compatible with the data available (Martin et al 2007). Furthermore, (2) it allows one to derive predictions in terms of phenotypic variables, which are often more easily accessible to

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[1] The contact point is Sr. Vicente Paeile Marambi, División de Recursos Naturales, Residuos y Evaluación de Riesgo Ministerio del Medio Ambiente San Martin 73 Santiago

experimental measure than the underlying genetic variables (allele frequencies at selected loci, linkage disequilibria). During previous and ongoing projects, we have used this type of model to explore the effect of the reproductive mode of organisms (in particular, their rate of clonal reproduction or self-fertilization) on the genetic variance for quantitative traits at mutation-selection equilibrium (Roze & Blanckaert 2014, Abu Awad & Roze 2017), conditioning their evolutionary response to a change in environment. We have also extended these models to add an arbitrary number of loci affecting the rate of sex of organisms, and quantified the strength of selection for sex in terms of genetic variances and selection gradients on quantitative traits (Vanhoenacker et al, in prep.). We plan to use this approach to study the selective forces acting on different aspects of genetic systems (mating systems, recombination or mutation rates, ploidy levels), exploring in particular the effects of the topography of the adaptive landscape (e.g., shape of the fitness peak) and the effects of environmental variations of the fitness function across time or space.

Other theoretical projects on the evolution of sex will be initiated during the GENASEX ANR project (coord. Christoph Haag, CEFE Montpellier, starting mid 2018). These will concern the effect of the precise mode of reproduction of asexual organisms (in particular, clonal reproduction vs. automictic parthenogenesis) on the conditions allowing the spread of asexual lineages, and on their long-term evolution. Contrarily to clonal reproduction that maintains heterozygosity, automictic parthenogenesis increases the homozygosity of offspring and may generate gradients of heterozygosity along chromosomes. This is expected to affect the mutation load of asexual lineages, and different aspects of genetic systems such as the evolution of recombination, gene expression or the fate of gene duplications.

The question of the evolutionary advantage of sexual reproduction will also be tackled using experimental evolution approaches. For this, we have been developing a new planktonic

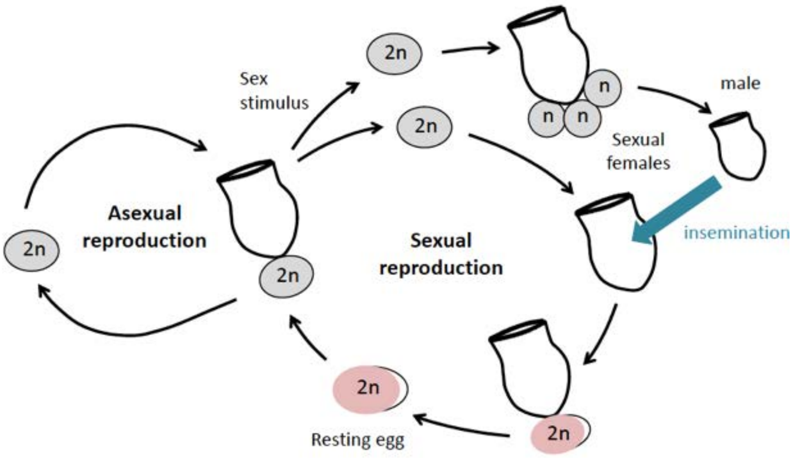


Figure 11: Monogonont rotifer life cycle. Asexual females produce diploid eggs (in grey) which develop into other asexual females, or into sexual females in response to the sex-inducing stimulus. Sexual females produce haploid eggs by meiosis, which develop into males if not fertilized. Fertilization yields dormant resting eggs.

system in the lab during the SexChange ANR project (the monogonont rotifer *Brachionus plicatilis*). The life cycle of monogonont rotifers is similar to the life cycle of aphids or *Daphnia*, involving both sexual and asexual reproduction (see Figure 11): individuals reproduce asexually at low density, and become sexual in response to a chemical stimulus indicating high density.

Interestingly, the propensity for sex (response to the sex stimulus) is genetically variable within natural populations (Carmona et al 2009), and may evolve in the lab during adaptation experiments (Becks & Agrawal 2010, 2012, Luijckx et al 2017). Preliminary experiments have been conducted in Roscoff using different strains from natural populations from Brittany and Spain (the latter being provided by M.J. Carmona, University of Valencia). In particular, we have set up a chemostat system allowing us to maintain experimental populations during a large number of generations, and observed the evolution of investment into sex within these populations (towards lower rates of response to the sex stimulus under constant environmental conditions). We plan to explore the evolution of investment into sex under different types of environmental conditions, and study the effects of sex on the fitness of offspring under these different conditions (e.g. gradual or abrupt environmental change, cyclical environment or random fluctuations).

Another axis of research concerns the evolutionary response of sexual and asexual populations of micro and macroalgae. An expectation from both theoretical and experimental evolution work with standard model organisms is that the loss of sex can result in diminished physiological performance and diminished flexibility in responses. In the cosmopolitan phytoplankton *Emiliania huxleyi*, the possible loss of sex is observed in open ocean populations which face much lower average nutrient availabilities. We plan to confirm using technologies such as RADseq that the loss of key haploid genes is indeed associated to the loss of sexual recombination, and we will test how loss of sex affects physiological performance in response to environmental stress (e.g. nutrient limitation) and fluctuating conditions.

Along a similar direction, and building on the results of the CLONIX project and the publication of our bayesian method ClonEstiMate (Becheler et al., in press), part of the postdoc of R. Becheler will be dedicated to test for differential responses of asexual and sexual populations of *Gracilaria chilensis* to the catastrophic impact of the earthquake of February 2010 and following tsunami in Chile. Indeed, the rate of clonality and the mode of vegetative reproduction of a population are two major determinants of its genetic diversity. High rates of clonality could buffer the effects of drift, but could also slow down the recovery and resilience of populations after strong bottlenecks. Indeed, genetic variation could be seen as “protected” within large clones (with many replicates of the same genotype), and large clonal lineages should be more prone to survive catastrophe, helping in maintaining diversity immediately after the impact. Temporal sampling of *G. chilensis* before and after

2010 and genotyping of microsatellite loci have already been performed during the first UMI contract. We also plan to study the effect of this catastrophic event on the genetic structure of other fully sexual marine algal species in the context of a collaborative project between ML Guillemin (UACH) and A Brante (UCSC).

Finally, within the framework of the GDRI DEBMA, we plan to develop a new consortium including researchers from France (in particular in collaboration with the team of Mark Cock and Susana Coelho, UMR 8227 Roscoff), Chile, Brazil and USA to study the evolution of sex and genetic sex-determining regions or sex chromosomes in red algae within the next five years. The genetic basis of sex determination has never been studied in depth in red algae and, for this phylum, we propose to use Gracilariales species as new biological models. In many Gracilariales the sex ratio is close to 1:1 in natural populations and in segregating progenies, suggesting a genetic control of sex determination. As in other haploid-diploid organisms, sex is expressed during the haploid phase of the life cycle in red algae, and the existence of a U/V sex-determination system has thus been proposed. In such haploid systems, neither of the sex determining regions or chromosomes (called U and V when sex is expressed during the haploid phase) does recombine, contrarily to XY (or ZW) systems where the X (or W) chromosome still recombines. This leads to different theoretical predictions concerning the evolutionary trajectory of these genomic regions, compared to diploid systems (in particular, both the male and female regions may degenerate due to the loss of recombination, but degeneration may also be limited by haploid gene expression). We plan to obtain draft genome or transcriptome of related haploid male and female (“brother” and “sister” products of the meiosis of the same diploid individual) unialgal tissues cultivated from spores in our laboratories.

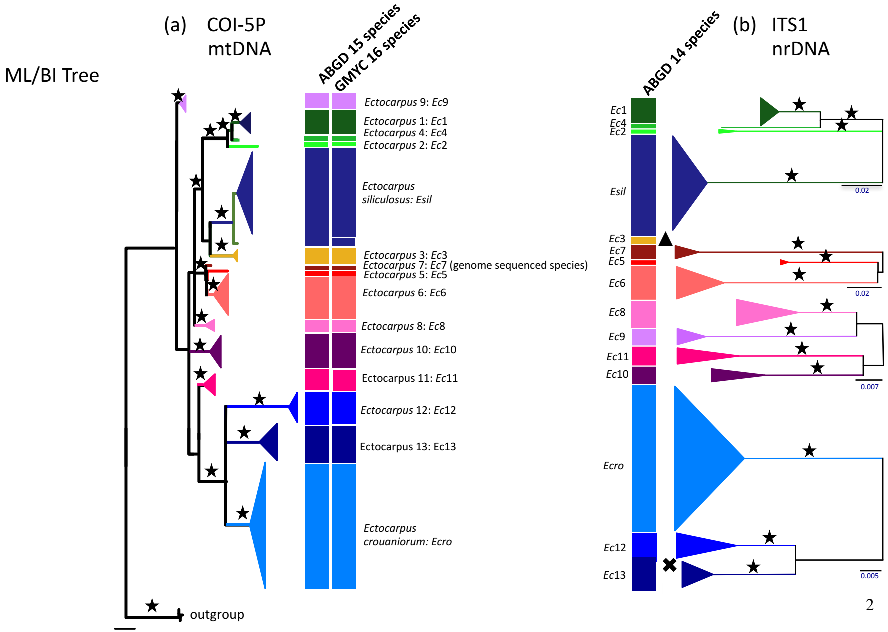
Topic 2. Understanding speciation processes and the evolutionary and ecological limits of adaptation

(Christophe Destombe, Peter von Dassow & Pablo Saenz)

Adaptation and Speciation

Speciation is one of the major focus of evolutionary biology research. Our project is in continuity with our previous research theme and aims to answer the following major questions about the mechanisms of speciation, the genetic basis of speciation and the relationship between speciation and diversity.These questions will be addressed on different models of brown and red algae (Laminariales, Ectocarpales, *Mazzaella*) as well as on phytoplankton species (*Emiliania, Alexandrium*).

The PhD thesis of Alejandro Montecinos have leaded to the publication of two main works on species diversity and distribution (Montecinos et al. 2017a) and hybridisation and barrier to gene flow (Montecinos et al. 2017b) in the cryptic species complex *Ectocarpus siliculosi* (Figure 12). However, phylogeny and species delimitation in the *Ectocarpus* group was based



Montecinos et al. (2016)

Figure 12: Proposed species in the *Ectocarpus siliculosi* group using COI-5P (a) and their consolidation using ITS1 (b). The asterisks ✶ on branches indicates statistical support values >75 and posterior probabilities >0.80. The triangle ▲ in Figure 12b indicates the sequences of *Ectocarpus* 3 that were unalignable with any other of the four alignment subgroups, and the cross ✖ indicates the two consolidated species that were merged using ABGD. The outgroup for the COI-5P analyses were three sequences of *Kuckuckia spinosa*.

on only two orthologous genes (the COI mitochondrial and the ITS nuclear) while hybridisation between the two more-common species of *Ectocarpus* (*E. siliculosus* and *E. crouaniorum*) have been done using a only a few number of molecular markers including two species specific markers and nine microsatellites. Thousands of SNP markers have been obtained via high-throughput sequencing (i.e. ddRAD) for individuals studied with more classical markers in Montecinos et al. 2017a and b within the framework of the collaborating project IDEALG. We now plan to apply populations genomic approach to re-evaluate our first conclusions on speciation and hybridisation on *Ectocarpus* and study patterns of gene flow within species across space for *E. siliculosus* and *E. crouaniorum*. First, we plan to use specimens from nine cryptic species for which SNP data are now available to obtain a better resolution of the phylogenetic relationships within the *Ectocarpus siliculosi* group. The existence of a complete genome in *Ectocarpus* (cryptic species number 7 in Montecinos et al. 2017a) will also allow us to compare different phylogenetic reconstruction methods using SNPs obtained via de novo assembly and SNPs generated using the available reference genome. Second, the study of Montecinos et al. 2017a revealed a high diversity of taxa with various levels of divergence in *Ectocarpus* opening new opportunities to study hybridization in different evolutionary contexts. Indeed, the existence of porous reproductive barriers within this group suggests that speciation is still ongoing and our previous works suggests a probable relationship between genetic distance and cross compatibility in this genus. We plan to study possible cytoplasmic introgression and estimate the strength of barriers to gene flow in distinct species groups: 1) in groups of closely related species as *Ectocarpus* 6 and *Ectocarpus* 7 distributed in parapatry in Chile on both side of a biogeographic barrier or *Ectocarpus* 12, *Ectocarpus* 13 and *E.* crouaniorum present in sympatry in various areas. Incongruence between nuclear and mitochondrial markers has been observed between the latter; 2) species pair belonging to the two highly divergent clades *E.sil* and *E.cro* and for which a strong reproductive barrier acting at the F1 hybrid generation was detected (i.e. *E. siliculosus* and *E. crouaniorum*; Montecinos et al. 2017b). We will use the RADseq data recently obtained and genome scan for genomic signatures of selection to search for genes associated with speciation processes. Furthermore, since several empirical studies and theoretical models of speciation have argued that loci involved in reproductive isolation will preferentially accumulate in regions with little recombination such as the regions near the centromeres or located in the sex chromosome (Butlin 2005), we will explore patterns of divergence in non-recombining regions (i.e. female (U) and the male (V) sex chromosomes) in comparison to the recombining autosomal regions. If true, the hypothesis of accumulation of loci associated to speciation in the sex-determining region, implies that we will obtain deeper separation between species using the sex markers than for the rest of the nuclear loci studied. At last, the effect of biogeographic boundaries and distance on genetic structure will be analysed for the two truly cosmopolitan species *E. siliculosus* and *E. crouaniorum.* Complex historical scenario of recent introduction and population expansion have been proposed for these species and will be tested using RADseq data sets and Approximate Bayesian Computation (ABC) methods. An ANR project will be submitted in Autumn 2017 to explore these questions

Strength of reproductive barriers and existence of parapatric margins and hybrid zones will be further studied not only in the brown alga *Ectocarpus* (see above) but also in the species complex of the common intertidal red alga *Mazzaella laminarioides*. In this morphological species, our previous phylogeographic study (Montecinos et al. 2012) has detected the existence of three highly divergent genetic lineages distributed in strict parapatry (named “North”, “Center” and “South”) encountered over more than 3,500km of the Chilean coast. The development of new microsatellite markers (Guillemin et al. 2016b), but tested on only six populations (two of each cytoplasmic lineage), have revealed some nuclear-cytoplasmic incongruence that could be explained by incomplete lineage sorting of the nuclear genes or asymmetric introgressive hybridization between the lineages. We will use a new unpublished data set of 24 populations genotyped using microsatellites and sequenced for the mitochondrial gene to test for mechanisms sustaining barriers to gene flow in *Mazzaella.* At first sight, distribution of genetic groups for the distinct markers allow to hypothesize that the integrity of the “Center” and “South” species could be maintained by geographic barriers to dispersion, while possible niche divergence or selection against hybrids may maintain the existence of a tension zone located at 32°S and separating the “North” and “Center” species.

Understanding genetic divergence in the highly dispersive and seemingly homogeneous pelagic environment for organisms living as free drifters in the water column remains a challenge. We have addressed this issue on different phytoplanktonic models (*Emiliania huxleyi, Pseudo-nitzschia* sp. and *Alexandrium minutum in* collaboration with Mickael Le Gac IREMER).

Phylogenetic and population genetic work is ongoing on *Emiliania huxleyi* to determine whether there are associations between phenotypic, phylogeographic, and population genetic patterns of differentiation in relation to geographic and oceanographic boundaries (Master II student co-supervised by von Dassow and Valero in 2017). Investigators von Dassow (as PI) and Faugeron (as co-PI) recently submitted a FONDECYT proposal 1181614 "Speciation and adaptation in marine diatoms and coccolithophores" to expand this work to look at how oceanographic processes determine patterns of genetic differentiation both at intra-specific and inter-specific levels. A simple and falsifiable hypothesis is that "Genetic differentiation and phytoplankton speciation are both principally regulated by oceanographic processes". We propose to test this comparing genetic differentiation of *E. huxleyi*, a ubiquitous species, to phylogeographic differentiation patterns at genus level in the cosmopolitan diatom genus *Pseudo-nitzschia*. These two groups pose interesting biological contrasts, in terms of life cycle, properties, evolution, and ecology. *Pseudo-nitzschia* is also an interesting genus, because most species in these genus produce the neurotoxin Domoic Acid, the cause of amnesiac shellfish poisoning, yet it has been very little studied in the whole Eastern South Pacific.

To understand how hybridization may work in speciation in phytoplankton, we are collaborating with other investigators to use a phylogenomic approach to understand how the incongruences between phylogenies, geographic/oceanographic distributions, and population genetics may reflect a genomic history of hybridization and introgression between the cosmopolitan *E. huxleyi* and closely related species with more restricted distributions. *Emiliania* seems to show little barriers to hybridization with closely related species. In contrast, barriers to hybridization in *Pseudo-nitzschia* are reported to be very strong. In the FONDECYT proposal 1181614, we aim to confirm whether this is true.

Finally, a major ongoing push has been to look at the limits of local adaptation in oceanic phytoplankton. We will continue whether (and at what geographic scales) *E. huxleyi* and its close relatives with more restricted ranges exhibit local adaptation to abiotic variables (e.g., ocean acidification, temperature, trace metals). In addition, we hypothesize that the propensity for hybridization impacts the capacity for local adaptation, and that organisms prone to develop barriers to interbreeding may exhibit a higher propensity for local adaptation and specialization in contrast to generalist physiologies of organisms prone to hybridization. IMO and FONDECYT proposal 1181614 (if awarded) will support this work.

In the toxic dinoflagellate *Alexandrium minutum,* using a reverse ecology approach based on the mRNA sequencing and morphology analysis we identified two diverging groups (corresponding to two pseudo-cryptic species), co-occurring in nature (Le Gac et al. 2016). The most likely scenario of divergence involved ancient divergence in complete isolation followed by a secondary contact resulting in gene flow, heterogeneous across the genome, between the diverging groups. The SNPs subjected to restricted gene flow also display an over-representation of fixed nonsynonymous polymorphism.

Our result highlights the importance of the functional aspect of the divergence and identifies selection as a potential major evolutionary force driving this event. At the molecular level, the functions associated with the divergence are especially related to toxin production and calcium/potassium fluxes with the potential implications in terms of ecological divergence and build-up of reproductive isolation that remain to be tested.

In the framework of the PhD thesis of Gabriel Metegnier (ARED Region Bretagne co-supervised by Mickael Le Gac and Christophe Destombe), we will propose to study sexual reproduction (gametes fusion and encystment) in *A. minutum* in vitro and more specifically the gene expression profiles involved in sexual reproduction. The expression patterns will then be sought in the metatranscriptomic datasets generated as part of the follow-up of the efflorescence of *A. minutum* to better identify these key moments in the life cycle of this toxic dinoflagellate *in situ*. More generally, these include determining which genes are differentially expressed during efflorescences, and whether differential expression profiles are repeated from one year to the next in order to identify which functions are performed by the cells of *A. minutum* within their natural environment, in particular functions associated with abiotic or biotic factors (competition, predation, parasitism ...), or with the life cycle (mitoses, sexual reproduction, encystment ...). In order to draw a robust link between patterns of differential expression *in situ* and functions performed by organisms, it is necessary to obtain *a priori* information on the biological functions of genes within organisms. This information will be obtained either by assigning the biological functions by bioinformatic analysis and statistics or by experimentation in the lab. Crossing experimentations within and between speudo-cryptic species will be performed (project IFREMER Alexrepro; Mikael Le Gac)

Thermal variability and evolution of local adaptation in the context of global change

A major determinant of the geographic distribution of a species is expected to be its physiological response to changing abiotic variables over its range. The range of a species often corresponds to the geographic extent of temperature regimes the organism can physiologically tolerate. Many species have very distinct life history stages that may exhibit different responses to environmental factors.

In the framework of the two recently financed projects (The EU Biodiversa A3-2015-165 project MARFOR and the FONDECYT project 1160930 MACROCYSTIS and the FCT Portuguese project GENEKELP), we will emphasize the critical role of haploid microscopic stage (gametophyte) and diploid macroscopic stage (sporophytes) of the life cycle to study local adaptation and to explain the difference of edge distribution of kelp species.

Kelps often display large distribution ranges, therefore facing important environmental gradients such as those expected by their latitudinal extension (i.e. temperature, light, seasonality). On top of large scale environmental variability, we have shown that local conditions may also deeply influence their demography and genetic diversity (Pereira et al. 2015, Pereira et al. 2017; Oppliger et al. 2014). Because of their keystone influence in coastal communities, it is highly relevant to understand, predict and eventually manage the consequences of current temperature rise at a global scale on their populations. In our current research we have demonstrated that the global patterns of intraspecific genetic diversity of European and Chilean marine forest species were shaped by the role of past climate changes (see for review in Chile Guillemin et al. 2016 and in Europe Neiva et al. 2016). In addition because kelps have limited dispersal, kelp forests are composed of distinct genetic clusters of populations with population occurring along persistent low latitude ranges retaining higher genetic diversity (e.g. Assis et al. 2014, 2015, Robuchon et al. 2014). The transition from the Last Glacial Maximum to the present-day conditions is most often accompanied by massive shifts in species distribution ranges, and by local or global population density changes. During spatial expansion, gene surfing effects contribute to the reduction of diversity in the newly colonized region, a process that leads to the presence of one or a few haplotypes over large geographic areas (Neiva et al. 2010). Alternatively, initial founders may rapidly colonize a site through local recruitment. The consequence is a density-dependent restriction to subsequent immigration by a local saturation (or monopolization) of the substratum (Waters et al 2013). After initial colonization, a density blocking effect could limit the arrival of new haplotypes, allowing for the persistence of founder effect signatures long after spatial expansion is completed (De Meester et al. 2002, Waters et al. 2013). The numerical advantage of the first colonists may be reinforced by adaptation to local conditions. Indeed, even slight fitness increases of founder genotypes may strongly contribute to the restriction of subsequent immigration, leading to an “isolation by adaptation” pattern (Orsini et al. 2013). Under this Monopolization hypothesis (De Meester et al. 2002), local adaptation is largely based on standing genetic variation present in the first few colonizers rather than being predominantly fuelled by gene flow, as expected in demographically stable populations. And therefore, both processes of spatial colonization and of adaptation to newly colonized environments may be strongly interacting.

For the next quinquennial of the UMI Project, we now aim to go a step forward and test the hypothesis that genetically disconnected marine forests from distinct habitats correspond to ecologically and adaptively divergent populations. We also want to investigate the importance of adaptation during spatial expansion by comparing neutral phylogeographic structure with patterns produced by markers under the influence of selection. To do so, we will develop a large-scale comparative study using several kelp species (*Laminaria digitata*, *Saccharina latissima* and *Macrocystis pyrifera*) from Europe and Chile-Peru and similar experimental designs. We aim to combine (1) ecophysiological experiments on both the gametophytes and the sporophytes in common garden experiments with (2) genome wide investigation to estimate genomic signature of local adaptation and with (3) measures of reproductive isolation using controlled crosses experiments, including measures of inbreeding and outbreeding depression.

During range shifts, populations experience drastic population size changes, eventually leading to strong genetic drift and inbreeding. Little is known about the level and consequences of inbreeding in kelps, in particular when considering the process of local adaptation. On the one hand, strong inbreeding depression can be expected, reducing average fitness and therefore hampering the success of local adaptation. On the other hand, recurrent inbreeding over generations may favour the purge of deleterious alleles and ultimately promote local adaptation. The post-doc project of Ronan Becheler and the PhD thesis of Maribel Solas are currently quantifying inbreeding and exploring its role in the process of local adaptation in Macrocystis. Due to the peculiar architecture of *Macrocystis* sporophytes (i.e. the reproductive blades are produced at the base of the individuals, leading to the release of spores very close to the substratum), spore dispersal is thought to be highly restricted, leading to the establishment of highly inbred gametophyte population near parental sporophytes. In contrast, in *L. digitata* or *S. latissima*, meiospores are produced on the blade, the distal portion of the plant, and are therefore released 1–3 m above the substratum at high tide, which may facilitate their transport in the water column. When released, spores are probably dispersed away from their parent sporophyte and related sibs. The aim of the Post-doct project of Julien Dinault and PhD project of Louise Fouqueau (that will begin in Autumn 2017) is also to quantify the mating system of these european kelps to compare with the Chilean species.

The achievements of functional and population genomics of kelps to better characterize local adaptation processes will tightly link with the goals of artificial selection of breeding programs (described below, in theme 3). In this context, the MARFOR project is organizing several workshops with stakeholders from the industry to conservation and management to provide them with solid data-driven scientific basis for habitat conservation and sustainability management, and for the blue growth economic activities that depend on marine forest species. A large-scale citizen science initiative will contribute to assess the status of marine forests, while promoting European wide awareness about marine forests. Finally, a long-term goal will be to integrate quantitative genetics approaches, in particular our capacity to detect QTLs of temperature and other environmental stressors, with population genetics of neutral and selected markers in central and marginal populations to better understand the action of natural selection (see theme 3).

Seaweed-herbivores interactions as source for adaptive (co-)evolution

Macroalgae provide habitat and food for many invertebrate species. Some of these species feed on macroalgae and can have detrimental effects on the macroalgae (a significant problem in Chile). While some of these interactions have been documented, little is known about their degree of specificity. From a population dynamics perspective, highly specific interactions should lead to congruent population structure of both macroalgae and associated invertebrates. Most macroalgae exhibit strong spatial genetic structure and are being particularly well studied by members of the UMI. By comparing patterns of population structure and demographic histories between macroalgae and their associated invertebrates, it will be possible to obtain an indirect evidence of the strength of these associations. Initial results of demographic inferences on the complex *Lessonia spicata-Scurria scurra* (PhD thesis of Andres Meynard) suggest concordant dynamics associated to post-glacial environmental changes even in unglaciated areas. We hypothesized that invertebrates quite strictly associated to their algal host (as the specialist herbivore *Scurria scurra* and its hosts *Lessonia spicata* and *L. berteroana*, or certain herbivorous amphipods that graze on macroalgae such as M*acrocystis pyrifera*) could coevolve and even co-speciate along the coast of Chile. We will further expand our research by including marine invertebrates and macroalgae associations. Pablo Saenz (Universidad Austral de Chile) who is currently working on population genomics of marine animals in Chile will join the UMI and seek funding to develop this new aspect.

In the phytoplankton, our initial results (submitted to Biogeosciences) found *E. huxleyi* inhabiting the naturally high pCO2/acidified waters of the Chilean upwellings do exhibit an extreme over-calcified phenotype, which is relatively stable in culture. As this phenotype does not appear to be an adaptation to natural acidification, despite the strong correlation, we suspect that coccolith phenotype in *E. huxleyi* in fact represents biological adaptations, perhaps to grazing by planktonic animals or ciliates. A current PhD project (candidature approved 3 July) plans to test this by implementing grazing experiments. In the longer term, we propose to test more generally for adaptations that impact grazing in *E. huxleyi*, closely related species, and in *Pseudo-nitzschia* in the context of FONDECYT proposal 1181164.

Topic 3. Public policy support for societal issues involving the conservation of biodiversity from anthropic pressures: domestication and cultivation, management of genetic resources and global change

Sylvain Faugeron, Marie Laure Guillemin & Myriam Valero

The evolutionary biology of domestication

The science of seaweed domestication also requires the development of breeding programs adapted to the haploid-diploid life cycle and the diversity of mating systems. Within the framework of the PIA IDEALG and Chilean National Fund for Scientific and Technological

Development (FONDECYT) Macrocystis projects, our current efforts are focused in three species characterised by different domestication histories (the Chilean red seaweed *Gracilaria chilensis* and two brown seaweeds: *Macrocystis pyrifera* found along the Eastern Pacific coast and the European species *Saccharina latissima*) (Figure 13). The objective is to understand the process of domestication by comparing natural populations with cultivated populations using next-generation population genomics tools and traditional genetic tools.

Macrocystis pyrifera
Native in Easter Pacific

Saccharina latissima
Native in Europe

Gracilaria chilensis
Recently in Chile

Figure 13: The three species of economic interest that are studied in the UMI

In the species *Gracilaria chilensis*, intensively cultivated in Chile since at least 35 years, we have shown that cultivation through involuntary selection could have favoured fast growing, almost sterile, heterozygous diploids, deeply modifying the life cycle and mating system of populations growing in sandy bays (Guillemin et al., 2008). We have proposed that the shift from sexual populations (i.e. recruiting from spores) to asexual population of thalli propagating vegetatively in soft substrate could be at the basis of the success of colonization from New Zealand and subsequent range expansion in South America (Guillemin et al., 2014). In Chile, the two kinds of populations coexist (i.e. clonal farm in muddy habitat and sexual populations in natural rocky shores). Within the framework of IDEALG, our current aim is to gain a better understanding of the population structure of *G. chilensis* along the Chilean coast, and to study signatures of domestication by carrying out a genome-wide analyses contrasting farms with natural populations. A total of 240 individuals was sampled (113 haploid and 127 diploid individuals) from 13 sites: seven farms and six natural populations.

In the native European *S. latissima* species, we have developed 32 microsatellite markers that revealed substantial genetic differentiation among localities distributed along the European coast from Southern Brittany (France) to Spitzbergen (Norway) (pairwise FST values ranging from 0.077 to 0.562, Guzinsky et al, 2016). The particularly high genetic differentiation between the French and Scandinavian *S. latissima* populations is in agreement with the reported ecotypic differentiation, which may reflect an important resource for genetic improvement. These first results on the pattern of genetic diversity suggest that care should be taken to avoid the transfer of strains between different geographic regions. Our current aim is to gain an estimate of the importance of genetic resources in wild populations prior to the cultivation projects that are currently developed along the European coast, by analyzing the population structure of *S. latissima* around the coast of Brittany, and along the European coast. The effect of local adaptation to different environments is also tested by contrasting populations at different tidal levels and by comparing natural populations with populations found in ports (artificial floating pontoons) that should resemble the environment of cultivated samples on the floating ropes. A total of 500 individuals were selected from 25 populations with five pairs of different tidal habitats; two trios with different tidal levels and five pairs of marina/wild populations.

In *M pyrifera*, strong genetic discontinuities have also been found associated to environmental discontinuities along the coast of Chile, and the pattern of genetic structure is highly correlated to the spatial pattern of morphological differences among individuals (based on 12 morphological traits). These results are being complemented by ecophysiological experiments to test for local adaptation among these genetic and morphological clusters.

For these 3 species, population genomics approaches are being developed to investigate the effects of selections and demographic bottlenecks associated to management and domestication of the resources. DdRAD libraries were constructed for 500 individuals in *S. latissima* and 240 individuals in *G. chilensis.* Our results show that it is an appropriate technique to be used for obtaining high numbers of genetic markers for a population genomics study. For Macrocystis, approximately 380 individuals sampled from Peru to Magellan strait are being prepared for application of the same protocols.

In addition, a new FONDECYT project involving 5 members of the UMI has begun in July 2017 and will complement IDEALG project to complete a population genomics' study of *G. chilensis*. We have proposed to use a combination of RADseq and sequence-capture genotyping via custom oligonucleotide baits on samples coming from both side of Pacific ocean and representing both individuals from sexual and asexual populations to investigate in depth the effects of (1) successive transoceanic colonization events and expansion at the scale of the Pacific, (2) recurrent bottlenecks linked to introduction, exploitation and domestication in Chile and (3) adaptation to hard and soft bottom habitats, and to habitats altered by human activities. In collaboration with S. Stoeckel (INRA Rennes, France, CLONIX project) we will develop new models and statistical tools adapted to species with complex life cycles (e.g. haploid-diploid organisms and asexual/sexual organisms) to investigate the genomic signatures of bottlenecks and selection. Our previous studies suggest that massive replication of clonal individuals produce directional selection leading to the fixation of alleles conferring advantage to this new habitat. We were not able to test this hypothesis since only a few polymorphic microsatellite loci were available for *G. chilensis.* Genomic data sets will enable identifying the local genomic regions that distinguish crops from surrounding natural populations. They will further provide the first insights about the genetic basis of domestication of a clonal organism. We will also search for trace off a hard-selective sweep in *G. chilensis* genome. Indeed, the rapid domestication process could have drove mutated alleles conferring domestication-related traits to fixation in all farms. Ultimately, we plan to use these new genomic data to better plan the future of domestication of the species (Valero et al. 2017) and wishfully start a marker-assisted breeding program for this algal crop. On a more ecological point of view, field data sets obtained over more than two years will be analysed with the non-linear regression methods developed in Vieira et al. (2016) to study the dynamic of five natural populations. Trade-offs between survival or growth and reproduction, already reported in the lab (Guillemin et al. 2013) will be tested in the field.

The UMI is involved in the FUI (Fonds Unique Interministeriel – Régions) "OCEACTIF" project, carried out by AGRIMER: a company specializing in seaweed extracts. The aim of this project is to address new challenges associated with the domestication of marine algae species, the metabolic orientation towards the biosynthesis of molecules, the extraction of metabolites by bio-mimetic green solvents, the formulation and realization of innovative and efficient biological tests (Figure 14). The first challenge is to produce seaweed of constant quality and to no longer suffer from the high variability of the raw material due to poor species identification and seasonal variations. One answer to this difficulty is the thank culture of selected seaweed strains specific to the targeted dermo-cosmetic application. This project requires know-how concerning the molecular identification of marine algae species based on the use of multilocus barcoding method associated with population genetics approaches. The target cultivated species will be selected on the one hand for their ability to reproduce vegetatively and on the other hand for their concentration of molecule of interest. The OCEACTIF project will serve also to strengthen the expertises of the UMI teams about algal biodiversity and their response to environmental stresses (climatic changes, anthropization of the environment, biotic and abiotic interactions). More specifically, the development of new sequencing methods (NGS) developed in the context of OCEACTIF will allow a better understanding of seaweed speciation and adaptation.

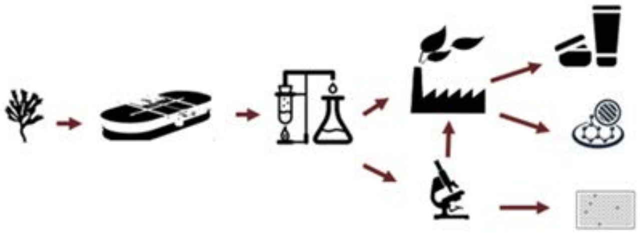


Figure 14: Seaweed extract manufacturing process diagram of OCEACTIF Project.

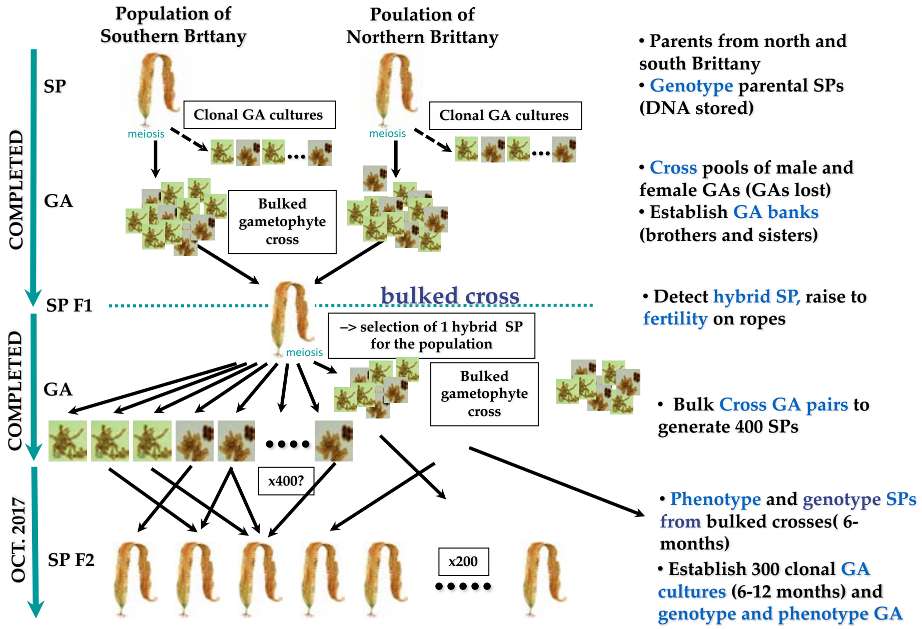
Algal breeding and genetic resources

Since January 2017, Our laboratory is also involved in the 4 year project GENIALG (Horizon 2020 Blue Growth project). GENIALG is the first industry-driven project bringing together pioneering companies in large-scale integrated European biorefineries and experts in seaweed cultivation, genetics and metabolomics to boost the seaweed industry. This project will boost the Blue Biotechnology Economy in Europe by designing high yielding seaweed cultivation systems. The aim of this project is to increase the production and sustainable exploitation of two high-yielding species of European seaweed biomass: the brown alga

Saccharina latissima (also known as Sugar Kelp) and the green algae *Ulva rigida* (often called Sea Lettuce).

More specifically the UMI will be involved to develop a genetic map and QTLs for traits related with growth characteristics, biomolecule content (e.g. lipids, polysaccharides, proteins, amino acids) and stress and disease resistance in the brown algae *Saccharina latissima*. One of the objective of the project is to develop QTLs not only on the F2 sporophytes segregating population but also on the gametophytes segregating population (see Figure 15). The question is to study to what extent phenotypic characters expressed in haploids are also expressed in diploids and reciprocally.

Figure 15: *S. latissima* genetic map and QTL analysis - strategy



GA: gametophytes; SP: Sporophytes

The project will also study the relationships between crops and populations established in the wild in this recently cultivated kelp species. These approaches will be developed specifically for *S. latissima* using data generated by the GENIALG project but will also take advantage of currently available genomic sequence data for this species and the sister species *S. japonica* (Ye et al., 2015, Nature communications, 6), and of additional genomic data that will be generated by the recently funded French “Phaeoexplorer” project. The project will also involve comparative approaches with *Macrocystis pyrifera*, for which a genetic map and other genomic resources will soon be available: draft genome in PHAEOEXPLORER, transcriptomic analyses of approximately 380 haploid individuals from all the Chilean coast in GENEKELP, RADseq map in FONDECYT Macrocystis. In this species, the gametophytic progenies of around 200 sporophytes sampled in 18 populations from Perú to Magellan strait were obtained, from each of which 12 males and 12 females were isolated.

These will constitute the basis for breeding programs in this species.

The establishment of strain collections in both Roscoff and PUC Santiago has more than two decades of history, accumulating different strains from different locations and species used in different experiments. These collections were key to the establishment of the first algal genome sequencing projects. They are now being enriched targetedly by seaweed strains of major biological or commercial interest, as a way of establishing a stock representative of their genetic and phenotypic diversity. They are now being used for phenotyping and genotyping, as well as breeding experiments. Most largely represented seaweed species in the collections include *G. chilensis, M. pyrifera, S. latissima, L. digitata, Ectocarpus sp., Pylaiella littoralis*. We will continue on with the diversification and maintenance of these collections including numerous strains of microalgae species as *E. huxelyi* and *A. minutum*. Concerning breeding strategies, (inbreeding, parthenogenesis, cloning, all knowledge gathered in topics 1 and 2 will be applied to optimize selection efficiency, control for inbreeding or outbreeding depression.

Restocking, restoration and small scale aquaculture

In the marine realm, the concept and science of ecosystem restoration is not well developed, especially in temperate waters. However, it is particularly critical since many populations of harvested marine organisms have reached a level of exploitation that may prevent full recovery, even if protected. As shown above, management and cultivation practices of *G. chilensis* may be unsuitable to ensure long term persistence of the resource. The recent Fisheries Law of Chile has opened the possibility to reintroduce individuals to restore stocks, as well as to conduct small scale aquaculture of native algal species within coastal areas handled (with exclusive territory rights) by local fishermen communities. We will use this opportunity to advance marine restoration science. The most immediate scientific questions we have highlighted (Valero et al. 2017) are related to the prevention of potential negative effects of restocking, such as pests and pathogens introductions, loss of genetic diversity due to inbreeding or outbreeding depression, or the use of locally maladapted genotypes. In the specific case of *G. chilensis*, the possibility of evolutionary rescue has to be addressed and assessed experimentally, as stakeholder's pressure is growing for introducing new genotypes from New Zealand, the source region of the species. The PhD thesis of S. Usandizaga (started 2016) is experimentally assessing the effect of population genetic diversity on population growth and epiphyte prevalence.

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