Reproduction at the range limits of laminariales at the chilean and european coasts

Luz Valeria Oppliger Zan

THESE DE DOCTORAT DE
L'UNIVERSITE PIERRE ET MARIE CURIE

Spécialité

DIVERSITE DU VIVANT

Présentée par

Mlle. Luz Valeria OPPLIGER ZAN

Pour obtenir le grade de

DOCTEUR de l'UNIVERSITÉ PIERRE ET MARIE CURIE

« Reproduction des grandes algues brunes des côtes Chiliennes et Bretonnes en marge de leur aire de distribution»

Soutenue le 29 Octobre 2010

devant le jury composé de :

Pr. Robert BELLE, Université Pierre et Marie Curie (Président du jury)
Pr. Nicolas PERRIN, University of Lausanne (Rapporteur)
Pr. Bruno de REVIERS, Muséum National d'Histoire Naturelle de Paris (Rapporteur)
Dr. Akira PETERS, Station Biologique de Roscoff (Examinateur)
Pr. Juan A. CORREA, Pontificia Universidad Católica de Chile (Directeur de thèse)
Pr. Christophe DESTOMBE, Université Pierre et Marie Curie (Directeur de thèse)

Université Pierre & Marie Curie - Paris 6
Bureau d'accueil, inscription des doctorants
Esc G, 2ème étage
15 rue de l'école de médecine
75270-PARIS CEDEX 06

Tél. Secrétariat : 01 44 27 28 10
Fax : 01 44 27 23 95
Tél. pour les étudiants de A à EL : 01 44 27 28 07
Tél. pour les étudiants de EM à MON : 01 44 27 28 05
Tél. pour les étudiants de MOO à Z : 01 44 27 28 02
E-mail : scolarite.doctorat@upmc.fr

PONTIFICA UNIVERSIDAD CATOLICA DE CHILE
Facultad de Ciencias Biologicas
Programa de Doctorado en Ciencias Biologicas
Mencion Ecologia

TESIS DOCTORAL:

REPRODUCTION AT THE RANGE LIMITS OF LAMINARIALES AT THE CHILEAN AND EUROPEAN COASTS

Por

Luz Valeria OPPLIGER ZAN

Directores de Tesis: Juan Alberto Correa Maldonado
Christophe Destombe

Comision de Tesis: Nicolas Perrin
Bruno de Reviers
Akira Peters
Robert Belle

A mis abuelas Luz y Rosalba

ACKNOWLEDGEMENTS
AGRADECIMIENTOS

Luego de cinco años en Francia, habituada a funcionar en tres idiomas, retomo mi lengua materna para agradecer a aquellos que permitieron que los tres años de mi tesis doctoral fuera llevada a cabo en sus distintas facetas.

Primero, agradezco a mis directores de tesis, Christophe Destombe y Juan A. Correa, por haberme apoyado a para realizar esta tesis. Agradezco a Christophe por haberme hecho descubrir la evolución, por su dedicación, su tiempo, su positivismo, por haberme acompañado tantas veces a terreno, por su buen humor y por saber estar presente cuantas veces fuera necesario. Agradezco a Juan por haberme acogido a temprana edad en su laboratorio, por haberme dado la oportunidad de venir a Francia y por su apoyo incondicional.

Muchas gracias a Bernard Kloareg por haberme permitido efectuar mi tesis en la Estación Biológica de Roscoff.

Quiero agradecer a Myriam Valero por haberme recibido en su equipo BEDIM, por sus consejos y las agradables comidas en su casa.

Agradezco a los miembros de mi jurado de tesis: Robert Bellé, Akira Peters, Nicolas Perrin y Bruno deReviers por haber aceptado evaluar mi trabajo.

Gracias a Marie Donatien, Céline Manceau, Nicole Sanseau, Héléne Huelvan, Alain Paoli, María Teresa Pino y María Teresa González, por la ayuda logística brindada en los innumerables pasos administrativos que significa hacer un doctorado en co-tutela franco-chilena.

Esta tesis es el producto de una extensa red de colaboración de personas tanto en Francia como en Chile. Sin el apoyo ni la ayuda de estas personas, este trabajo no hubiera podido ser realizado. En la P. Universidad Católica de Chile, estoy profundamente agradecida de: Santiago Andrade, Paula Ayerdi, Jessica Beltrán, Ismael Cáceres, Loreto Contreras, Sylvain Faugeron, Verónica Flores, Gonzalo Gómez, Alejandra González, Marie-Laure Guillemin, Paulina Gyorgy, Luciano Hiriart, Ana Lafuente, Andrés Meynard, Gioconda Peralta, Marco Ramírez, Javier Tapia, Florence Tellier, Daniela Thomas y Attia Zeregallia.

En Roscoff, agradezco a: Catharina Alves de Souza, Sarah Bouchemousse, Xavier Bailly, Gaelle Correc, Margaux Callouet, Matthieu Camusat, Mark Cock, Susana Coelho, Claire Daguin, Dominique Davoult, Gaspard Delebecq, Colin Destombe, Yann Fontana, Alexander Geoffroy, Licinia Gouveia, Claire Gollety, Pauline Gosselin, Daphne Grulois, Jan-Hendrik

Hehemann, Sebastien Henry, Nathalie Kowalczyk, Stacy Krueger, Fiona Lerckx, Laurent Lêveque, Dominique Marie, Stéphane Mauger, Daniella Mella, Laure Noel, Matthieu Oriot, Vincent Ouisse, Tania Pereira, Philippe Potin, Brigitte Riou, Andres Ritter, Marine Robuchon, Denis Roze, Delphine Scornet, Wilfried Thomas, Jihanne Trigui, Frederique Viard and Daniel Vaulot.

Gracias también a Aschwin Engelen, por la importante ayuda brindada en la construcción de los modelos demográficos y en la acogida en Faro.

A los miembros de mis comités de tesis: Solenne Stoeckel, Susana Coelho y Myriam Valero. Por las preciosas criticas que ayudaron a mejorar mi proyecto.

A Anaïs Merck, Bruno Pommard y Kamila Gorna. Por su amistad y acogida en Paris.

Particularmente me gustaría agradecer a Daniella Mella, Catharina Alves, Gaelle Correc, Andrés Ritter, Angela Tonon y Silvana Collao, por su amistad, su constante apoyo y buen humor. Su fraternal cariño queda registrado en mis más dulces recuerdos.

A mi amigo, colega, novio y prometido, Peter Von Dassow, por sus valiosos comentarios científicos, sus innumerables correcciones de ingles, sus lecciones de salsa, su cariño y el maravilloso tiempo juntos.

A mi cariñosa familia. A mis papas por apoyarme en todo tipo de decisiones, por venir a verme cada año y ayudarme en todo lo que estuviera a su alcance. A mis hermanos Pablo y Cristian, por tenerme siempre presente y escribirme a la distancia. A mi nonno, por quererme tanto.

Financiamientos :
Beca doctoral CONICYT-EMBAJADA de FRANCIA 2005-2009
A la cual se agregan los financiamientos siguientes :
Becas VRAI y DIP de la facultad de Ciencias Biológicas de la Universidad Católica de Chile.
FONDECYT 1060493, PÔLE MER BRETAGNE, ANR-ECOKELP (ANR 06 BDIV 012), FONDAP-FONDECYT 1501-0001.
Financiamientos que han favorecido la realización de esta tesis en cotutela: Collège Doctoral Franco-Chilien et programme ARCUS du Ministère des Affaires Etrangères et Européennes, ECOS-CONICYT C03804, Université Pierre et Marie Curie, Universidad Católica de Chile, Laboratoire International Associé « Dispersal and Adaptation in Marine Species » (LIA DIAMS)

INDEX

GLOSSARY OF TERMS

Agamete. Broadly any reproductive propagule which develops into a new individual without syngamy.

Anisogamy. The occurrence of gametes of different size; more broadly, the occurrence of any differences in size, shape, structure or behaviour between gametes.

Apomixis. The absence of both meiosis and syngamy amongst organisms which reproduce by eggs.

Autogamy. Syngamy between gametes (usually) or gametic pronuclei from the same individual. Self-fertilization.

Automixis. Syngamy between meiotically reduced nuclei descending immediately from the same zygote, whether or not distinct gametic cells are formed.

Endomitosis. A mitosis all of whose products are retained within the same nuclear membrane; thus a means of increasing ploidy.

Epistasis. Any interaction with phenotypic effect between nonallelic genes.

Gametophyte. The haploid gamete-producing generation of plants.

Genetic drift. The stochastic change in gene frequency caused by random sampling of a finite population.

Genetic recombination. The change in the relationship between loci on the same chromosome caused by crossing-over.

Inbreeding. Mating between related individuals.

Karyotype. The chromosome complement: the number and appearance of the chromosomes.

Mating type. A gender, especially of isogamous protists with multipolar mating systems.

Sporophyte. The diploid spore-producing generation of plants.

Syngamy. Fertilization. The union of gametes, followed by nuclear fusion.

Zoospore. A motile asexual propagule.

ABSTRACT

Life history theory postulates that the schedule and duration of key events in an organism's lifetime are shaped by natural selection to produce the largest possible number of surviving offspring. These events, notable related to growth, reproduction and survivorship partly depend on the physical and ecological environment of the organism. The relative stability of the environment can lead to selection for certain life history trait (i.e. sexual vs asexual reproduction). Organisms can modify their reproductive strategies face to changing environments having impact in their species geographical distribution, especially at range edges.

Kelps (Phaeophyceae, Laminariales) are key structural components of benthic marine communities. Kelps display complex heteromorphic life histories that include haploid (spores and gametophytes) and diploid (embryonic sporophytes) microscopic life-stages and a diploid (adult sporophyte) macroscopic stage. While numerous studies have examined the ecology and physiology of these microscopic stages, our understanding of the geographic distribution of most kelp species comes primarily from studies on the adult macroscopic stages. Therefore, to fully understand how their range limits are regulated, studies in the diversity of reproduction and how that affects the micro- and early macroscopic life stages is imperative.

The global objective of this thesis is to compare the reproductive systems of Laminarian populations along both the centre of the species' range and in the limits of the species' distribution in order to study the ecological and evolutionary limits of the adaptation of species.

The first objective consisted in study sex ratio in two cryptic species of the *Lessonia nigrescens* complex. These species posses contrasting latitudinal distributions: one located north and the other south of the biogeographic boundary at latitude 29-30° S. Our results demonstrated that sex ratio in two cryptic species seems to be mainly genetically determined and environmental influence can significantly modify it. Second, a latitude effect was revealed but it was mainly explained by an increased variation in sex ratio at the range limits of species. This greater variation at the margins could be due either to differential mortality between sexes or to geographical parthenogenesis (asexual reproduction).

The second objective was to investigate the role of temperature in different developmental stages of the microscopic phases in the two cryptic species of Lessonia in

order to understand the actual distribution of the *Lessonia nigrescens* species complex. Special attention was given to populations in the transition zone to compare to central populations of their respective species. Our results clearly demonstrate that there is differential tolerance to temperature in the two species, with the gametophytes of the Northern species being more tolerant to higher temperatures than gametophytes from the south. Second, the two species exhibit different life history strategies with a shorter haploid phase in the Northern species contrasted with considerable haploid vegetative growth in the Southern species. Third, the local temperature regime was a stronger factor than genotype (Southern or Northern species) in determining the temperature response of marginal populations.

The final objective was to study sexual reproduction in marginal populations of *Laminaria digitata* in the Brittany coast. Results showed that the environmentally unstable populations at the range limit of *L.digitata* displays a decline in genetic diversity compared to central populations. Surprisingly our results showed that sporophytes in these marginal populations gave mainly diploid spores rather than haploid ones suggesting apomeiosis or automixis events. Thes spores developed normally in culture. These results support the existence of geographical parthenogenesis in marine environments.

RESUMÉ

Les hypothèses sur l'évolution des traits d'histoire de vie stipulent que la durée ainsi que la succession des différentes étapes dans les cycles de vie sont le résultat de la sélection naturelle visant à produire le plus grand nombre de descendants possible. Ces étapes, qui sont liées au développement, à la reproduction et à la survie d'un organisme, dépendent principalement de l'environnement physique et/ou écologique de celui-ci. La sélection en faveur de certains traits d'histoire de vie découlerait donc de la stabilité de l'environnement (comme par exemple la sélection en faveur de la reproduction sexuée versus la reproduction asexuée). La stratégie de reproduction d'une espèce est donc susceptible de se modifier en fonction des changements environnementaux pouvant survenir au sein ou plus particulièrement en limite d'aire de distribution d'une espèce où les fluctuations abiotiques sont les plus importantes.

Les grandes algues brunes (Phaeophyceae, Laminariales) sont des espèces structurant les communautés benthiques marines. Ces algues présentent un cycle de reproduction hétéromorphe avec alternance de phase haploïde microscopique (gamétophyte) et de phase diploïde macroscopique (sporophyte). Bien qu'un grand nombre d'études se soit intéressé à l'écologie et à la physiologie de la phase microscopique, les connaissances sur la distribution géographique de la plupart de ces espèces n'est basée que sur la phase macroscopique. Il était donc important, afin de mieux comprendre comment les espèces se maintiennent en limite d'aire, d'étudier leur reproduction ainsi que l'importance relative des phases haploïde et diploïde dans leur cycle.

L'objectif de la thèse était donc de comparer le mode de reproduction de différentes espèces de laminaires en se basant principalement sur la phase haploïde dans les populations au centre et en limite d'aire géographique de distribution afin d'étudier les limites écologiques et évolutives de l'adaptation chez ces espèces.

Le premier objectif concernait l'étude du sexe-ratio chez deux espèces cryptiques du complexe d'espèce *Lessonia nigrescens*. Celles-ci présentent des distributions géographiques disjointes, de part et d'autre de la barrière biogéographique située à 29-30°S de latitude. Nos résultats suggèrent que le déterminisme sexuel de ces espèces est principalement génétique (locus ou chromosomes). Cependant, en conditions expérimentales, nous avons montré que le sex-ratio pouvait être modifié par la température, ce qui suggère une différence de mortalité des différents sexes face à la température à un stade précoce. Enfin, les fortes variations de sexe-ratio observées dans les populations marginales par rapport aux

populations centrales suggèrent d'une part que ces populations ne sont pas en équilibre et qu'elles présenteraient de phénomènes de parthénogenèse géographique.

Le second objectif était de vérifier si la température pouvait expliquer la différence de distribution entre les deux espèces de *Lessonia*, en étudiant les principaux traits d'histoire de vie des gamétophytes. Un intérêt particulier a été porté aux populations situées dans la zone de transition entre espèces afin de comparer les populations centrales et marginales. Par une approche démographique, nous avons montré premièrement qu'il existait une différence de tolérance entre les deux espèces susceptible d'expliquer leur distribution. Les gamétophytes de l'espèce septentrionale résistent mieux aux hautes températures que l'espèce méridionale. Deuxièmement, les deux espèces montrent des stratégies de reproduction différentes, qui se caractérisent pour l'espèce septentrionale par un développement végétatif réduit du gamétophyte et par une reproduction rapide, en opposition avec l'espèce méridionale présentant un développement végétatif marqué et une reproduction différée. Enfin, la variabilité inter-population au sein des espèces suggère l'existence de phénomène d'adaptations locales à la température.

Le troisième objectif consistait à étudier la reproduction sexuée dans les populations marginales de *Laminaria digitata* en sud Bretagne. Nos résultats montrent premièrement que les sporophytes des populations marginales sont moins fertiles que ceux des populations centrales et qu'ils produisent essentiellement des spores diploïdes et non des spores haploïdes comme dans les populations centrales. Ces spores donnent des gamétophytes diploïdes qui se développent normalement en culture. Ces résultats suggèrent l'existence de phénomène d'apomeiose et d'automixie dans les populations marginales correspondant à des phénomènes de parthénogenèse géographique. Ces résultats sont discutés à la lumière des analyses de génétique des populations qui montrent que les populations marginales présentent une diversité génétique plus faible que les populations centrales.

RESÚMEN

La teoría de historias de vida postula que el tiempo y la duración de eventos claves en el tiempo de vida de un organismo son moldeados por selección natural para producir el número mayor posible de descendencia viva. Estos eventos claves, como son el crecimiento, la reproducción y sobrevivencia dependen del medioambiente físico y ecológico de un organismo. La estabilidad relativa del medioambiente puede llevar a la selección de rasgos de vida particulares (i.e. reproducción sexual vs reproducción asexual). Los medioambientes fluctuantes tienen impacto en la distribución geográfica de las especies, especialmente en los márgenes de distribución.

Los Kelps (Phaeophyceae, Laminariales) son componentes estructurales claves de las comunidades bénticas marinas. Ellos poseen historias de vida heteromórficas que incluyen estadios microscópicos haploides (esporas y gametofitos) y diploides (esporas embriónicas) y estadios macroscópicos diploides (esporofitos adultos). Muchos estudios han examinado la ecología y fisiología de los estadios microscópicos, sin embargo, los conocimientos relativos a la distribución de la mayoría de las especies de kelps provienen de estudios de los estadios macroscópicos adultos.

El principal objetivo de esta tesis es comparar los sistemas de reproducción de poblaciones de Laminariales en el centro y los limites de distribución de las especies para comprender la ecología y los limites evolutivos de adaptación de las especies.

El primer objetivo consistió en estudiar el determinismo sexual a través de estimación de la razón macho-hembra en dos especies crípticas del complejo *Lessonia nigrescens*. Estas especies poseen distribución latitudinales contrastantes: una se localiza al norte y la otra al sur de una zona de transición biogeográfica a los 29-30°S. Los resultados muestran que la razón macho-hembra en ambas especies parece estar determinada genéticamente pero que la influencia medioambiental puede modificarla. También se detecto un efecto latitudinal sobre este parámetro, pero este es explicado principalmente por fuertes variaciones de la razón macho-hembra en el límite de distribución de las especies. Estas fluctuaciones en el limite pueden deberse o a mortalidad diferencial entre los sexos o a partenogénesis geográfica (reproducción asexual).

El segundo objetivo trata sobre el rol de la temperatura en diferentes estadios de desarrollo de fases microscópicas en ambas especies crípticas de *L.nigrescens* para mejor entender la distribución actual del complejo *Lessonia nigrescens*. Especial atención fue puesta en poblaciones localizadas en la zona de transición (consideradas poblaciones

marginales) para compararlas con poblaciones centrales de sus especies respectivas. Los resultados muestran claramente que hay una tolerancia diferencial a la temperatura en ambas especies, donde los gametofitos de la especie norte son más tolerantes a alta temperatura que aquellos de la especie sur. Segundo, las especies crípticas exhibieron diferentes estrategias de historia de vida, donde la especie norte muestra una fase haploide reducida contrastada con un gran desarrollo de crecimiento vegetativo en la especie sur. Tercero, los regimenes de temperatura local mostraron ser un factor más fuerte que el genotipo al determinar las respuestas frente a temperatura de las poblaciones marginales (en ambas especies).

El objetivo final consistió en estudiar la reproducción sexual en poblaciones marginales de *Laminaria digitata* en las costas de Bretaña. Los resultados muestran que la población en el limite de área de *L.digitata,* Quiberon, una perdida de diversidad genética al comparar con poblaciones centrales. Sorpresivamente nuestros resultados muestran que los esporofitos de la población marginal de Quiberon generan principalmente esporas diploides en vez de haploides, sugiriendo eventos asexuales de apomeiosis o automixis. Estas esporas se desarrollaron normalmente en cultivos. Estos resultados apoyan la existencia de partenogénesis geográfica en medioambientes marinos.

GENERAL INTRODUCTION

The life history implies the sum of all developmental and reproductive events that occur during life in individuals, populations or species (Caswell, 2001). Life history theory postulates that the schedule and duration of key events in an organism's lifetime are shaped by natural selection to produce the largest possible number of surviving offspring. These events, notably juvenile development, age of sexual maturity, first reproduction, number of offspring and level of parental investment, senescence and death, depend on the physical and ecological environment of the organism (Stearns, 2000). Life history characteristics are traits that affect the life cycle of an organism, and can be imagined as various investments in growth, reproduction (sexual and asexual), and survivorship. The goal of life history theory is to understand the variation in such life history strategies. This knowledge can be used to construct models to predict what kinds of traits will be favoured in different environments. One of the major life history traits studied is reproduction. The cost-of-reproduction hypothesis predicts that higher investment in current reproduction hinders growth and survivorship and reduces future reproduction, while investments in growth will pay off with higher fecundity (Stearns, 1992). Interestingly the costs of reproducing sexually versus asexually are different (detailed in next section) and are key traits to study life cycle evolution.

The life cycle is considered the fundamental unit of description of the organism. Ecology, genetics, evolution, development, and physiology all converge on the study of the life cycle (Caswell, 2001). The rate at which an organism reproduces sexually determines generally how fast a population can colonize a new habitat and how much mortality can be sustained (Begon *et al.*, 2006). The reproductive capacity is generally linked with the sexual reproduction (i.e.: meiosis and syngamy) by production of spores, zygotes or seeds but can also be totally disconnected of sexual reproduction (by production of vegetative propagules: spores, gemma or cutting). In many eukaryotes, life cycle presents an alternation of vegetative growth (mitotis) and of sexual reproduction (meiosis and syngamy). Mitotic growth can occur solely in the haploid phase, or in the diploid phase, or in both phases. All three fundamental life cycle types exist among eukaryotes, with a continuum of variation between fully haplontic to fully diplontic life cycles (Mable and Otto, 1998). Likewise, variation also exists among eukaryotes in the form of different levels of differentiation between life cycle phases and development of haploid and diploid phases.

One of the largest questions in eukaryotic biology is to understand the adaptive significance of these different life cycles and the maintenance of sexual reproduction. Several hypotheses have been proposed (see for review (Otto and Lenormand, 2002)). For example, diplontic cells are expected to accumulate mutations at twice the rate of haploid cells simply

because they include twice the DNA content of the haploid phase. However, recessive mutations will be unmasked in the haploid phase, meaning selection and elimination of deleterious alleles may occur more rapidly if the haploid phase is dominant (Otto and Gerstein, 2008). Alternatively, it has also been proposed that haploid and diploid cells would be inherently different physiologically, as diploid cells automatically carry twice the quantity of DNA than haploid counterparts, and would, in general, be expected to be larger cells (Kondrashov and Crow, 1991).

Sexual reproduction

Sex is a process of genetic reorganization through meiosis and syngamy, usually associated with the formation of several or many reproductive propagules (Bell, 1982). The maintenance of sex is paradoxical because this mode of reproduction is widespread but associated with several costs. Sexual reproduction is generally more time- and energy-consuming than asexual reproduction and, during mating, individuals are typically less able to acquire resources, evade predators and may have higher risks of sexually transmitted diseases and parasitic genetic elements. An intrinsic genetic of sexual reproduction is the cost of producing offspring, such as the twofold cost of sex: In sexual reproduction, two individuals are needed to produce one descendant, whereas only one individual is required in asexual reproduction. Unless the sexually reproducing couple can produce twice as many surviving offspring as the asexual individual, sexual individuals will necessarily have a lower reproductive output per capita. In addition, if sexual couples and asexual individuals produce the same average number of offspring, because one sexual partner does not contribute to the resources to the offspring, the reproductive output per individual for asexual species is twice that for sexual species (twofold cost of sex). Finally, sexual reproduction produces offspring by randomly mixing genes, potentially breaking down beneficial gene combinations (positive epistasis) present in parents.

Considering these costs, it would be expected that any asexual mutant occurring in a population reproducing sexually might rapidly invade and out-compete the population: sex should be an evolutionary dead-end (Maynard-Smith, 1978; Otto, 2009; Otto and Lenormand, 2002; Schwander and Crespi, 2009). Nevertheless, sex is the predominant mode of reproduction in nearly all multicellular taxa. A large body of theory seeks to explain the rarity of asexual reproducing lineages, despite the apparent theoretical advantage of this

mode of reproduction (Kondrashov and Kondrashov, 2001; Maynard-Smith, 1978; Otto, 2009; Schwander and Crespi, 2009). Most hypotheses explain the advantages of sex and recombination as a result of genetic variance reshuffling that accelerates the production of advantageous new genotypes and facilitates adaptation, limits the accumulation of deleterious mutations, or provides both forms of long-term benefit (Kondrashov, 1993; Lynch *et al.*, 1993; Schwander and Crespi, 2009; West and Zuccarello, 1999). It is generally accepted that asexual lineages are of recent origin, because even if initially successful they suffer early extinction (Judson and Normark, 1996). The most ancient asexual lineage correspond to the bdelloid rotifers, that originated more than 35 million years ago, although this case seems to be an exception to the rule (Butlin, 2000). Taken this into consideration, sexual and asexual modes of reproduction persist through time as a result of a dynamic equilibrium between the origin of new asexual lineages and their extinction (Simon *et al.*, 2003).

Parthenogenesis

Parthenogenesis is a form of asexual reproduction in which an unfertilized egg develops into a new individual. Parthenogenetic reproduction occurs in many phyla, and is especially well documented in plants, algae, rotifers, nematodes and arthropods (Simon *et al.*, 2003). In the animal kingdom, 19 out of 34 phyla contain parthenogenic taxa. Approximatelly 70 parthenogenic taxa have been identified in vertebrates, although none have been found in birds and mammals (Vrijenhoek, 1998). Parthenogenesis is associated with a wide variety of cytological mechanisms. The main ones are: apomocitic parthenogenesis, automictic parthenogenesis, gynogenesis and hybridogenesis (Fig. 1) (Simon *et al.*, 2003). Therefore, there are several ways in which parthenogenetic lineages could arise. As already mentioned, depending on the mechanism leading to the loss of sex, newly emerging unisexual lineages may differ greatly in their genomic and phenotypic attributes compared to bisexuals congeners. Thus, it is necessary to modify ideas about the relative costs and benefits of sexual and parthenogenetic reproduction because of the assumption that "all else is equal" between bisexuals and their unisexual descendants does not apply in many cases.

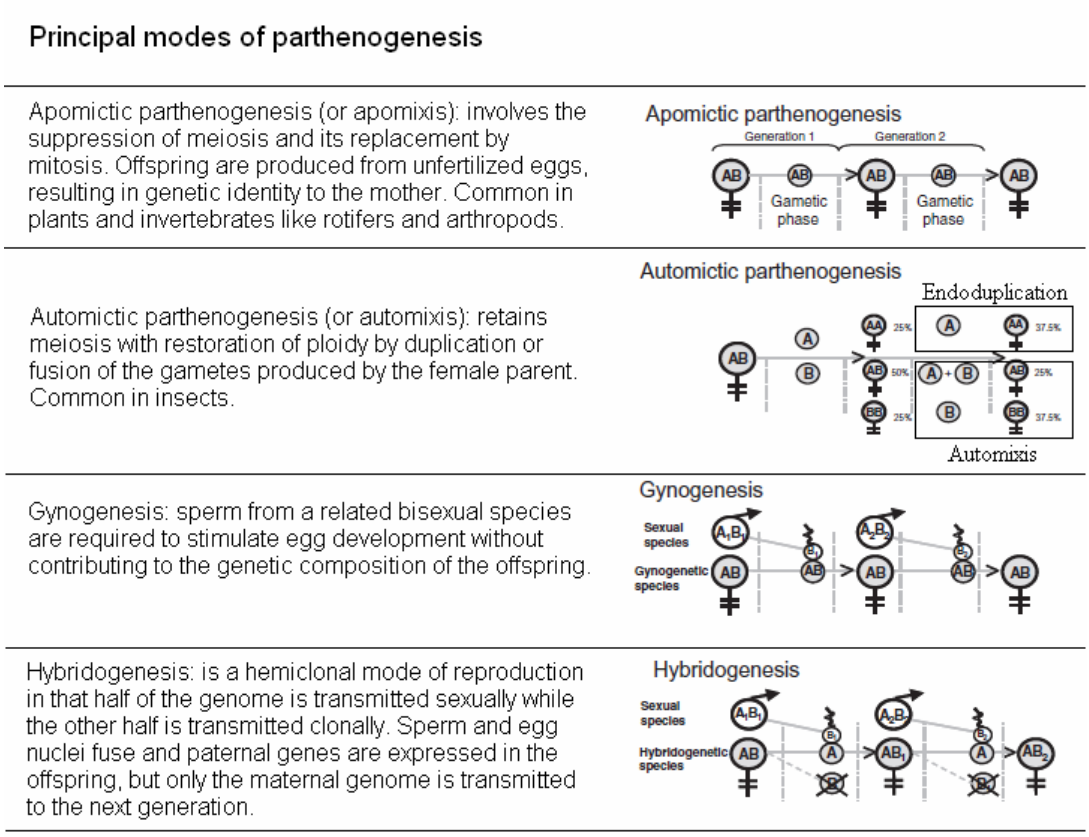


Figure 1. Principal modes of parthenogenesis (from Simon et al. 2003)

The are at least four ways in which parthenogenetic lineages may arise from sexual species (Figure 2)(Schwander and Crespi, 2009; Simon *et al.*, 2003; Vrijenhoek, 1998):

(1) Spontaneous transition to asexuality may occur by mutations in genes underlying sexual forms (Simon *et al.*, 2003) or in genes affecting mating behaviour and successful egg fertilisation. These mutations could result in obligate asexuality or be initially maintained as genetic variation for facultative parthenogenesis in a sexual population. The mechanistic simplest step from sexual to asexual reproduction is apomixis, which is the replacement of meiosis by mitosis (Suomalainen *et al.*, 1987).

(2) Interspecific hybridisation can disrupt meiosis and creates opportunities for the selection of cytological processes that rescue egg production through apomictic parthenogenesis (Vrijenhoek, 1998).

(3) Contagious origin results from the spread of unisexuality genes of pre-existing parthenogenetic lineages as a result of incomplete reproductive isolation between

sexuals and parthenogens. This contagious fashion could convert facultative parthenogens into obligate parthenogens (Simon *et al.*, 2003).

(4) Infectious origin by vertically inherited microorganisms. Induction of parthenogenesis is mainly caused by *Wolbachia*, a member of the Proteobacteria. Females infected with *Wolbachia* reproduce parthenogenetically, but sexual reproduction can be restored by treatment with antibiotics. Unfertilized infected eggs, which would normally develop as haploid males, develop as diploid females. Diploidy is restored either by gamete duplication, that leads to complete homozygosity, or by an unknown cytogenetic mechanism that maintains heterozygosity (Weeks *et al.*, 2002).

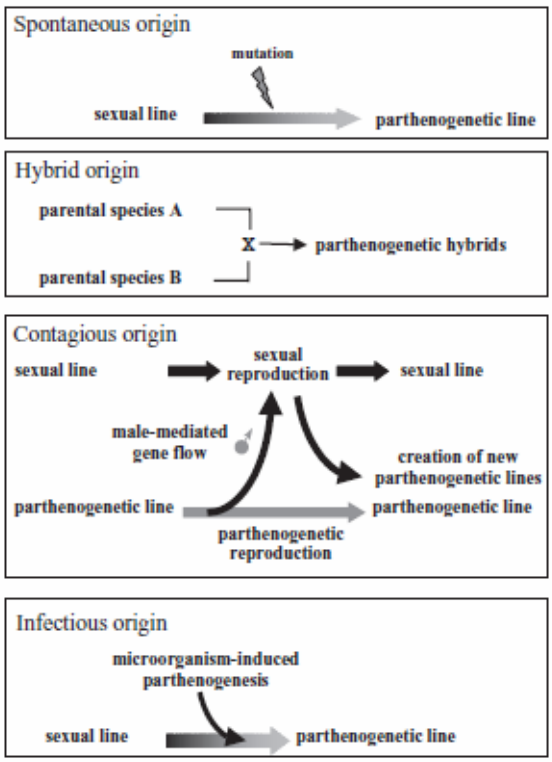


Figure 2 : Origins of parthenogenesis (from Simon et al. 2003)

There are no formal hypotheses concerning the origin of parthenogenetic lineages with the mechanism of automixis. White (1973), Bell (1982) and Suomalainen (1987) suggested that automixis might be the simplest mechanism from sexual to asexual reproduction whereby the diploidy of the zygote is restored secondarily by either fusion (automixis) or duplication (endomitosis) of haploid meiotic products. For the stick insect *Timema shepardi*, the origin of an automictic lineage suggested to be the product of previous hybridization

(Schwander and Crespi, 2009). No cytological details were provided to understand the origin of this type of parthenogenesis.

As discussed above, the explanation for the evolutionary maintenance of sexuality is still unclear because this mode of reproduction is widespread but associated with several costs. Studies in organisms that have closely related evolutionary history and similar ecological functions but vary in life cycle or reproductive mode provide the best way to answer the potential advantages of sexuality and asexuality.

Brown algae are an especially important group of organisms for studying the ecology and evolution of reproductive systems. They are key structural and primary producers of benthic marine ecosystems (Dayton, 1985). Also, a large range of life cycle variation exists among the brown algae. Further, in the order Laminariales there is a high flexibility of the life cycle even within a species (Bartsch *et al.*, 2008). The objective of my thesis was to study the phenotypic variability of some reproductive traits of three species of Laminariales (*Laminaria digitata*, and two cryptic species of *Lessonia nigrescens*) in order to understand the evolutionary and ecological significance of their life history.

Models: Laminariales within brown macroalgae

General characteristics

The Order Laminariales are composed of brown macroalgae and together with the order Fucales are commonly called kelps. Laminariales are found in temperate and sub-polar zones and represent some of the world's most productive and dynamic ecosystems, facilitating local diversity through diverse trophic and habitat associations (Graham *et al.*, 2007). Laminariales are particularly important as producers of structural habitat allowing the settlement and development of a great variety of invertebrates and fishes, offering refuge from environmental disturbances (Lobban and Harrison, 1994; Tala and Edding, 2005). They are of economic importance, given the presence in their cell walls of alginic acid, a commercially valuable polysaccharide (Lobban and Harrison, 1994). The kelps are also model organisms to investigate the effects of environmental stress on species' range limits (Matson and Edwards, 2007). These brown algae are dependent on a suite of physical factors related to habitat (Dayton, 1985). Kelp growth depends on interactions among nutrient availability, temperature and light. Kelps dominate cold-water coastal zones but can become physiologically stressed at high sea temperatures, particularly when nutrient availability is

low (Gerard, 1997; Tegner *et al.*, 1996). In some regions without upwelling, periods of low nutrient concentrations correspond with warm summer temperatures when the water is stratified. The combined effects of low nutrients and high rates of respiration result in kelp plants that erode more rapidly than they grow (Gagné *et al.*, 1982). In kelp forests driven by the upwelling of new nitrogen, such as those of southern California, warm surface water temperature is a surrogate for low nutrient availability (Tegner *et al.*, 1996). In this system when El Niño events disrupt coastal upwelling, kelp becomes nutrient starved and dies back (Tegner and Dayton, 1991). As a result, the distribution, abundance and size of kelp plants decline as sea surface temperatures increase (Dayton *et al.*, 1999). Furthermore, new biotic stresses can result from the introduction of exotic species and increased harvesting pressure by humans. These changing environmental pressures may modify kelps' perennity, their distribution and also the diversity associated with kelp forests (Steneck *et al.*, 2002).
Kelp forests are vulnerable to environmental changes. Temperature is a key abiotic factor determining the distribution of marine intertidal species (Helmuth *et al.*, 2002). We studied temperature tolerance in two Chilean species of Laminariales that are distributed in a latitudinal gradient coupled with a temperature gradient, and they are also affected by the episodicaly phenomenon of El Niño.

The *Lessonia nigrescens* complex and *Laminaria digitata*

Two cryptic species of the *Lessonia nigrescens* species-complex (family Lessoniaceae) and the species *Laminaria digitata* (Fig. 3) were chosen as models in this thesis. These species occupy the upper sub-tidal zone of rocky shores, are important components of their ecosystems, and have well-defined and well-studied ecological distributions, making them particular suitable for study.

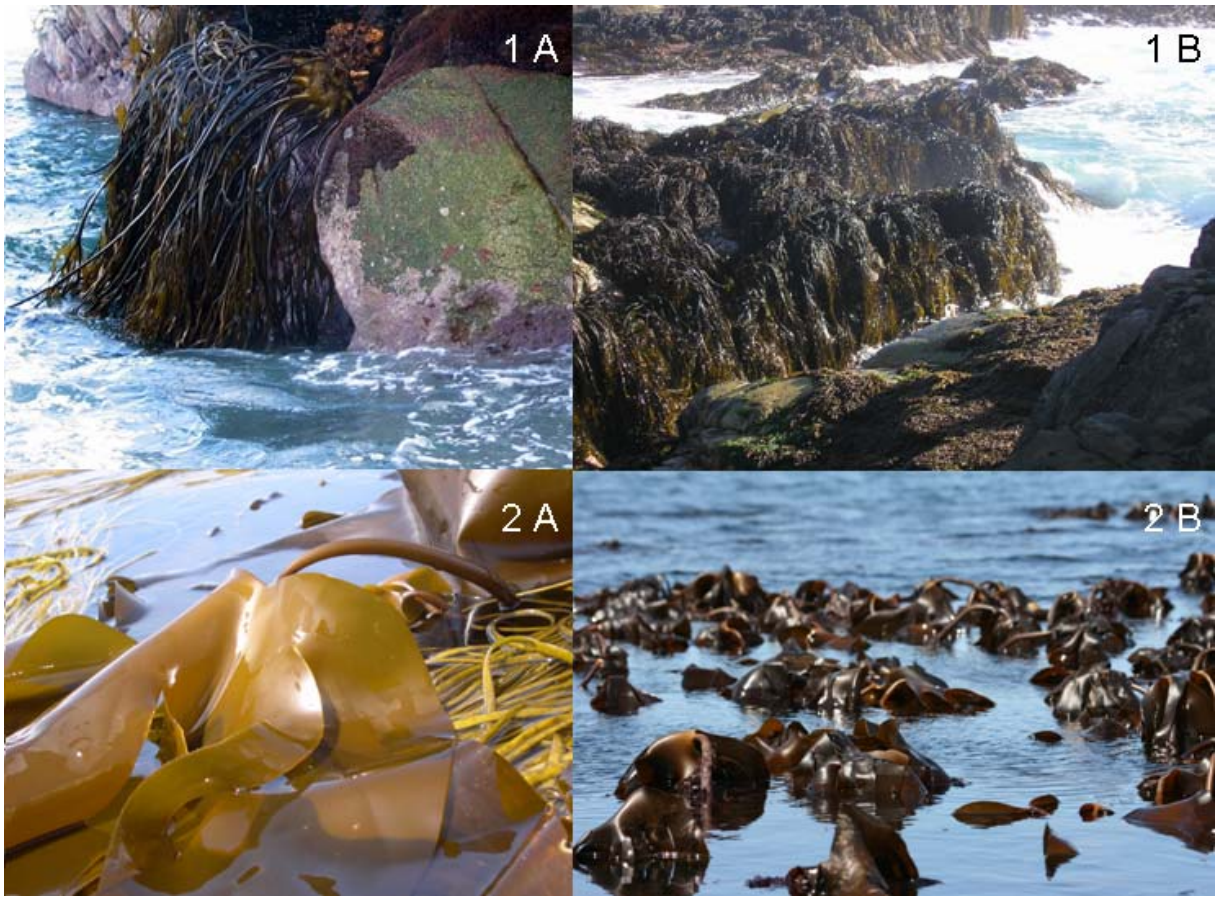


Figure 3: Biological models. 1) A-B, *Lessonia nigrescens* 2) A-B, *Laminaria digitata*

The *L. nigrescens* complex occurs along the west coast of South America from 12°S (Peru) to 56°S (Cape Horn) (Ramirez and Santelices, 1991)in the low intertidal of exposed and semi-exposed rocky shores (Villouta and Santelices, 1984). It is considered an ecologically and economically important component of the benthic communities from the Eastern South Pacific (Santelices and Ojeda, 1984; Vásquez and Tala, 1995; Villouta and Santelices, 1986). There is strong evidence that *L. nigrescens* is highly susceptible to environmental changes such as high temperatures occurring during El Niño events (Castilla and Camus, 1992; Vásquez and Vega, 2004) or heavy metal pollution (Correa *et al.*, 1996; Correa *et al.*, 2000), resulting in population extinction over large coastal areas (Martínez *et al.*, 2003).

The recent phylogeographical study in *Lessonia nigrescens*, which compared three kinds of molecular markers (mitochondrial, chloroplastic and nuclear), showed the existence of two differentiated clades (Tellier *et al.*, 2009)(Fig. 4). These two taxons or clades posses a strong genetic discontinuity at the zone of 30°S and appear to represent a northern and southern

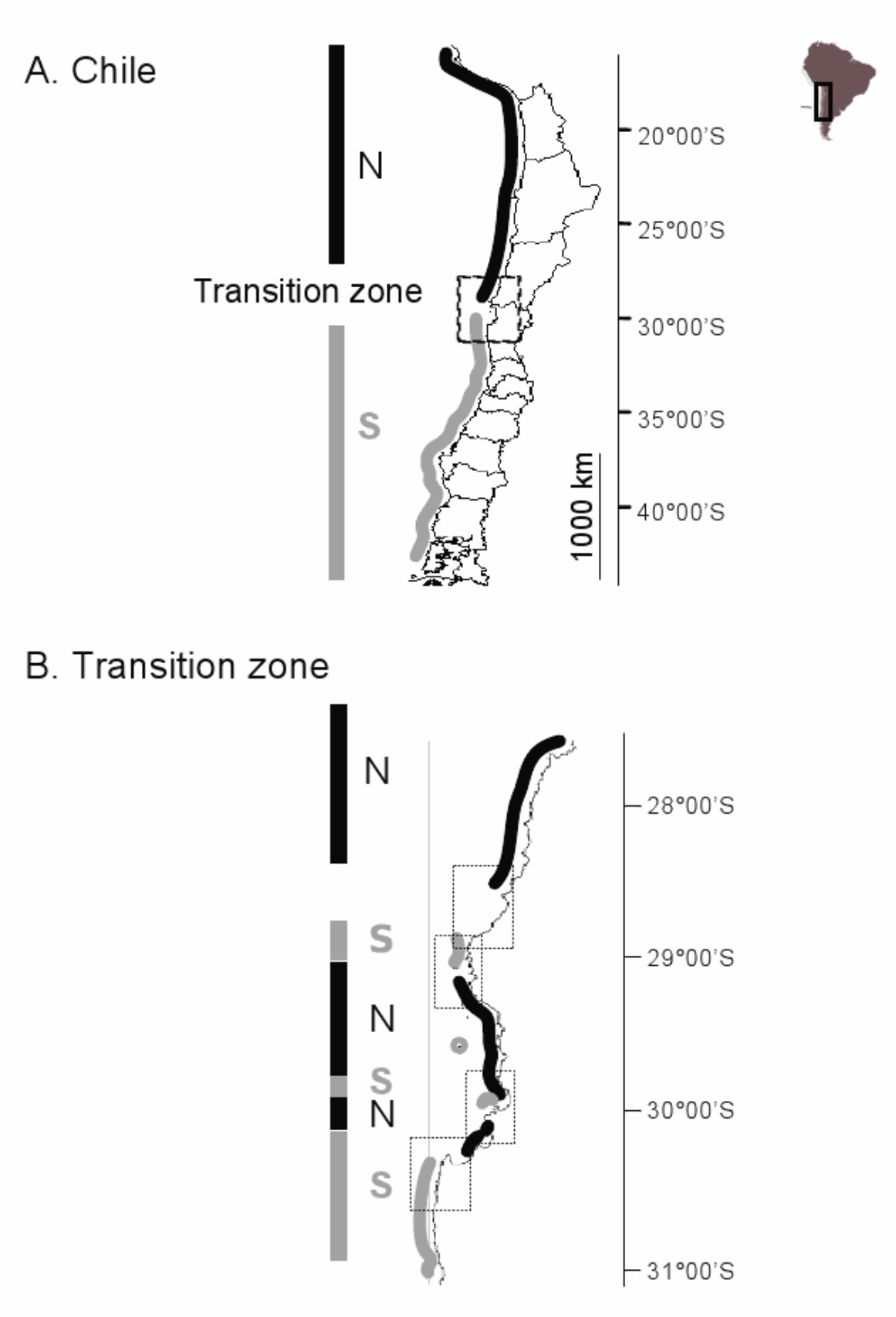


Figure 4: Geographic distribution of the two cryptic species of the *Lessonia nigrescens* complex (from Tellier et al. 2009).

cryptic species. The 30°S zone corresponds to a biogeographical barrier for a large number of marine taxons (Camus, 2001). This barrier has been attributed to a modification in temperature of coastal waters under the influence of El Niño. The 30°S zone thus may be a possible hybridizing zone for the two *Lessonia* species.

Laminaria digitata is a perennial species found in the upper subtidal of rocky shores and is located from Southern Brittany to Spitzberg in Northern Europe (Figure 5). Because of the large distribution this species inhabits, it may include a cold-temperate species (Boreal zone), a transition zone species, and warm-temperate species (Lusitanian zone)(Lüning, 1990). These geographic distributions are apparently associated to winter and summer isotherms that define their limits of reproduction (Lüning, 1990). Following this rationale, the southern distribution of *L. digitata* might be constrained by overheating of North-Atlantic waters (Arzel, 1998; Birkett *et al.*, 1998). This may be the cause of the current regression of southern populations of *L. digitata* in Brittany (Arzel, 1998). Brittany, as a biogeographical transition zone, is a region where alterations in life history parameters and such as survival and reproduction can be found.

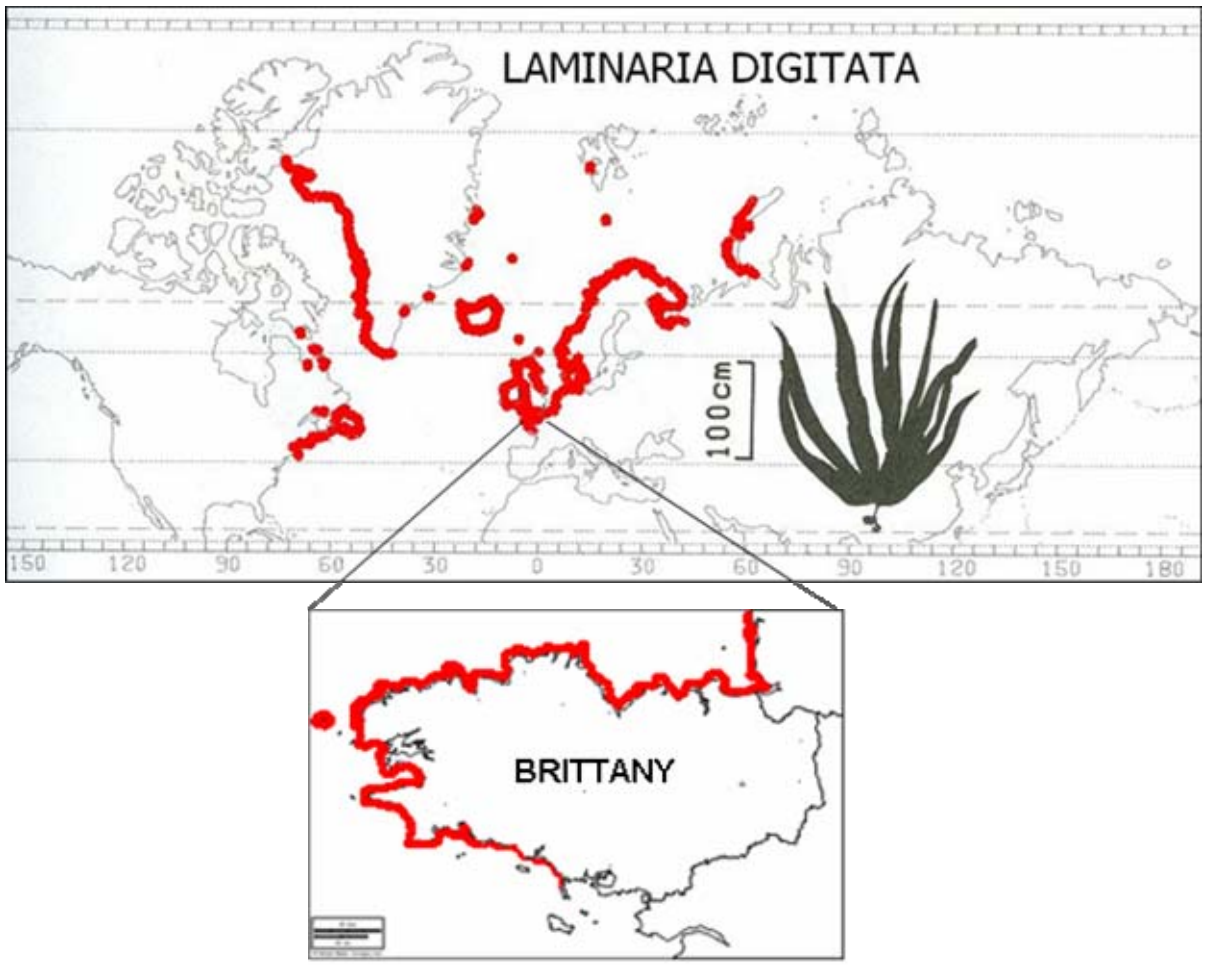


Figure 5: Geographical distribution of *Laminaria digitata* (from Lüining 1990)

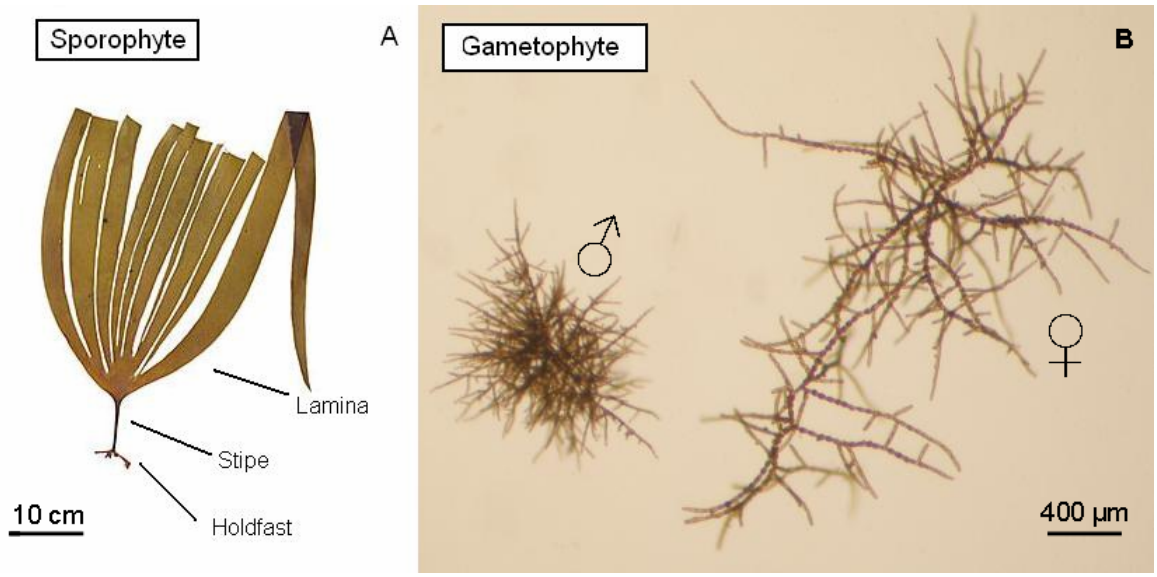


Figure 6 : Macroscopic and microscopic phases of Laminariales. A) sporophytes (2n) and B) dimorphic gametophytes (n).

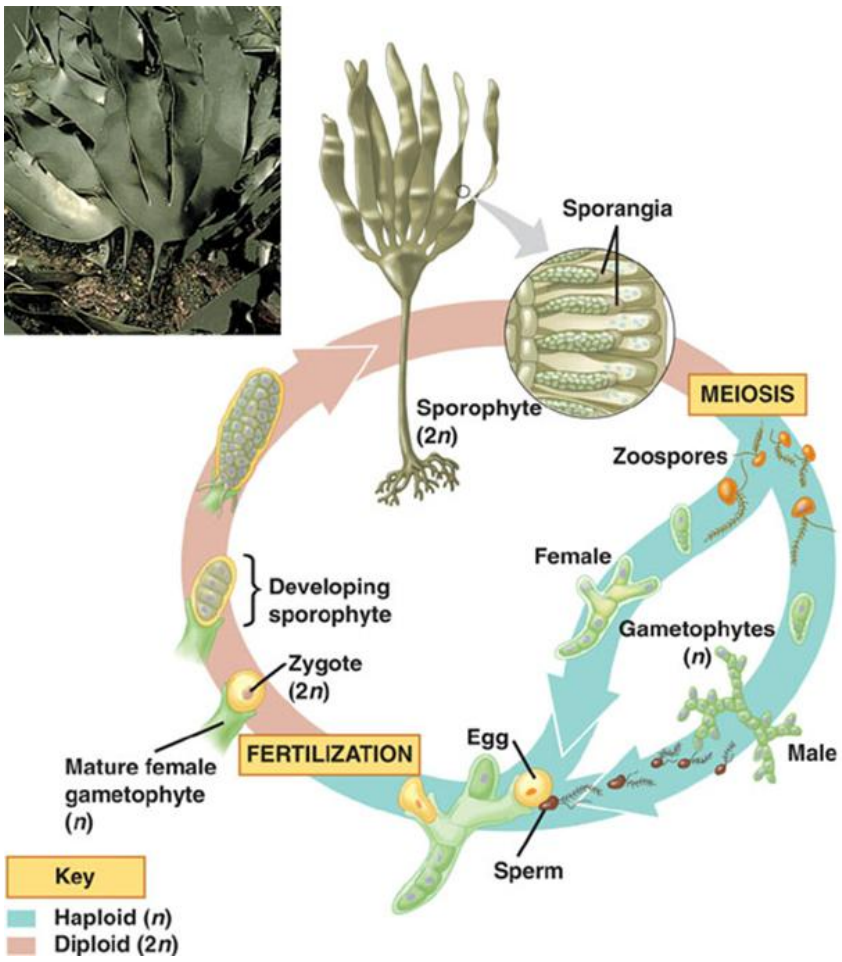


Figure 7: Typical life cycle of a Laminarial (from kentsimmons.uwinnipeg.ca).

Life cycles in Laminariales

Reproduction in Laminariales is mainly sexual. The life cycle of Laminariales is haplo-diplontic, alternating between diploid sporophytes and haploid gametophytes. The sporophytes grow and differentiate into rhizomatous holdfast (which is usually branched, but can also be disc-like), a stipe, and a blade. The stipe can be cylindrical or compressed, solid or sometimes hollow, but generally terminate into a single blade (Figure 6 A). Annual growth rings are formed in some species. Most species with perennial sporophytes (2-18 years old) produce a blade that is regenerated from the meristematic region between blade and stipe. Unilocular sporangia as sori arise on both surfaces of blades. Each sporangium is joined by an unicellular paraphysis and produces 32 haploid zoospores. Motile zoospores contain a single chloroplast, without eyespot and flagellar swelling. After settling, zoospores develop into dioecious heteromorphous microscopic gametophytes with oogamous reproduction (Fig. 6 B). Male and female gametophytes range from one-celled, few-celled to filamentous individuals of up to hundreds of cells. Males bear clusters of colorless one-celled antheridia at tips of branches, each producing a single biflagellate spermatozoid. Genetic studies show that the order Laminariales posses an apparently poor capacity for dispersal by meiotic spores and by gametes (Billot *et al.*, 2003; Tellier *et al.*, 2009). Each female gametophyte cell is able to develop into a one-celled oogonium producing a single egg (with remnants of an apparently vestigial flagellum), that is extruded from the oogonium but remains attached to it. Release appears to be controlled by a circadian rhythm, occurring mostly during the first 30 minutes of darkness (Lüning, 1981). At release of eggs the sexual pheromone lamoxirene is secreted causing ejection of spermatozoids from antheridia and attraction to the eggs where fertilization takes place (Boland, 1995; Lüning and Müller, 1978). Zygotes germinate to form young sporophytes (Sauvageau, 1918) (Figure 7).

In seaweeds, sex determination is poorly known. For example, sexual chromosomes have never been observed in red algae (Rhodophyta) with an alternation of isomorphic haploid and diploid individuals. However, the development in culture of equal numbers of haploid male and female gametophytes from meiotic spores suggested that primary control of sex determination is through a single pair of Mendelian factors (Martínez et al., 1999; VanderMeer, 1990). In brown algae (Phaeophyceae), genetic determination of sex (GDS) was demonstrated in the filamentous *Ectocarpus* sp. using crossing experiments (Müller, 1967).

In kelps (Laminariales), a large chromosome was reported in female gametophytes of different species of Laminariaceae, including *Laminaria digitata*, *L. ochroleuca*, *L. saccharina*, *Alaria esculenta* and *Chorda filum* (Evans, 1963), *L. hyperborea* and *Saccorhiza polyschides* (Evans, 1965) and *L. yendoana* (Yasui, 1992), suggesting the possible existence of sexual chromosomes in these species.

Sex determinism has never been clearly demonstrated in Laminariales. Some studies of sex ratio in culture suggested a similar proportion of males and females (Cosson, 1978; Sauvageau, 1918; Schreiber, 1930). However, it has also been reported that sex ratio can be modified by abiotic stress such as salinity or temperature (for review see Bartsch *et al.*, 2008). For example, high temperatures in culture result in a greater number of males in *Laminaria saccharina* (Lee and Brinkhuis, 1988) and in *L. digitata* (Cosson, 1978). In *L. religiosa*, on the other hand, both high and low temperatures resulted in a decrease of males (Funano, 1983). Recently, Nelson (2005) demonstrated in *Lessonia variegata* that high temperature and long days resulted in sex ratio biased towards females, suggesting that males were less resistant to stressful conditions. In this context, is it possible to imagine that Laminariales are part of organisms where abiotic stresses, such as temperature can induce differential mortality in sexes or over-ride the genetic determination of sex. The influence of temperature on the sex ratio of Laminariales must be an ecological important factor in setting the margins of the species' distribution.

Interestingly, it has been described several alternatives to the sexual life cycle of some Laminarian species. Between them we can find apomixis (development of unfused gametes), apogamy (development of sporophytes without the production of gametes) and apospory (development of gametophytes without the production of spores)(Ar-Gall *et al.*, 1996; Lewis, 1996; Nakahara, 1984; Oppliger *et al.*, 2007; Yabu and Sanbonsuga, 1990). These asexual alternatives questions the potentials of sexuality in Laminariales, but at the same time show the high phenotypic flexibility of the life cycle inside this order. However, all this variants of the life cycle have been observed *in vitro* studies and naturally raises the question of whether environmental conditions will favor these asexualities in nature.

Study sites: Chilean and Brittany coasts

Chilean coast

The Chilean coast is oriented from the North to the South, between 18.4-56°S, a distance equivalent to 4200 km. The coast is continuous and linear in the northern half of Chile, from the limits with Peru, at 18.4˚S, to the big island of Chiloé, at 40˚S, whereas the coast is strongly fragmented and composed of a large number of fjords, channels, and islands from Chiloé to Cape Horn at 56°S (Camus, 2001). The coast of Chile presents a great geographic climate variability mainly due to latitude: An arid climate with precipitation nearly absent occurs in the north; A Mediterranean climate with seasonal precipitation occurs in the central region; and an oceanic climate with regular strong precipitation occurs in the south. These regions are also distinguished by oceanographic characteristics. Year-round upwellings of deep cold water masses rich in nutrients occur in the northern zone 18.4-30°S. Between 30-40°S, the upwellings are seasonal and of different intensities (Camus, 2001). Another important oceanographic characteristic of these coasts is the influence of the subantarctic water mass on the surface of circulation. The third most important characteristic of the Chilean coast is the inter-annual variability in the north due to El Niño Southern Oscillation (ENSO) events. This climatic phenomenon can be presented in two contrasted forms: El Niño and La Niña (Trenberth, 1997). On the coasts of South America, El Niño is characterized by the arrival of hot waters poor in nutrients, upwelling diminution, and precipitation enhancement on the continent whereas La Niña is characterized by upwelling enhancement and an arid climate over the continent. The frequency of ENSO events may have increased in recent decades (Villa-Martinez et al., 2003). The climatic changes during ENSO events have strong consequences over marine and terrestrial organisms (Castilla and Camus, 1992), and the limits' of the distribution of a specie can be drastically modified (e.g. *Macrocystis pyrifera* (Hernandez-Carmona et al., 2001)). The Chilean coast is composed by three major biogeographical zones: 1) Peruvian Province (PP) at the north, 2) Magellan Province (PM) at the south, and 3) Intermediate Area (AI) at the centre. These biogeographical zones correspond to the climatological, oceanographic zones defined above. Two main biogeographical barriers have been described separating the already mentioned zones: one at 42°S and other at 30°S (Camus, 2001).

Brittany coast

The European, North Atlantic, English Channel, and North Sea coasts, are characterized by a very diverse geomorphology. This diversity is the product of its substrate nature and enhanced by the variety of reliefs (bays, gulfs, estuaries, abers…). This assortment in topology, and therefore in habitats, is the main reason of the richness of biocenoses in these coasts. These coasts are subjected to strong tides, with amplitudes depending on geographic position. For example, the amplitude of the spring tide is of 5 m. in front of Flandre, of 8 m. in front of Somme bay and 12 m. in Saint Michel bay (Castel et al., 1997). Another oceanographic characteristic in this zone is the presence of a summer thermocline from June to September which divides the English Channel in west/east, with stratifies and mix waters respectively. The principal water masses that characterize temperature variations in time and space in this zone are: 1) the summer continental thermal wave, 2) the winter continental thermal wave, and 3) the open sea homothermal wave. The Northeast Atlantic coasts are composed of three major biogeographical zones: 1) Lusitania (warm-temperate), 2) Boreal (cold-temperate) and 3) Artic/Subartic (cold)(Castel et al., 1997). The limits between zones are considered transition zones with characteristics of both zones at the edges. The Northwest coast of Brittany is a transition zone between Lusitanian and Boreal species (Cabioch, 1968).

Contrasting globally the Atlantic and Chilean zones, we can find that they are submitted to different intensities respect to their tidal regimes. Also in Chile there are coastal upwellings, absents in the North Atlantic coasts. On the other side, the analogies of these coasts are that they are both located on inter-tropical zones, both exhibit a large range of climates, posses similar thermal variations and are both affected by cyclic climatic-oceanographic events (El Niño in Chile and NAO in the North Atlantic). From an ecological point of view both coasts posses similar zonation patterns. Finally, from a geographical view, both coasts have three major biogeographical zones with transition zones characterized by high ecological complexity, because there is overlapping in the limits' of the distribution of species allowing reproductive isolation, the maintenance of hybridizing zones, or the coexistence of cryptic species.

Unstable environments and ecology of life cycles

The life history of a species is a continuous interaction between the individuals and their biotic and abiotic environments. In plants, successful growth and reproduction are possible over quite wide ranges of forms, size, and relative proportion of the parts but size is normally constrained by the environment (Motomura, 1991). The switch from vegetative growth to reproduction often depends on environmental factors such as temperature and light. The timing for reproduction is also influenced by the photoperiod but other environmental factors like nutrient availability and UV irradiance seem to strongly influence the life history of plants (Begon *et al.*, 2006).

The long-term survival of individuals will depend on their physiological capacity to survive, grow and reproduce in a fluctuating environment (Crawford, 2008). Theoretically, life history strategies that yield the highest population growth rate in a particular environment will be selected in species and populations (Sibley and Antonovics, 1992). For example, plant life history traits such as rapid reproduction, annuality and asexual reproduction might be selected in unstable marginal populations, whereas vegetative growth, resource storage and perennity might be selected in more stable central populations (Eckert *et al.*, 2008).

Studies of populations at the boundaries of the distribution of a species often find that alterations of life history traits occur in such marginal populations. The most studied trait is reproduction, such as number of flowers per individual or seed production in the case of plants (for review see Gaston, 2009). These studies have found reductions or failure of reproduction at the range margin and were typically linked to higher levels of environmental stress. Marginal populations may also experience higher temporal variability in environmental conditions resulting in higher fluctuations in fecundity and other parameters (Kluth and Bruelheide, 2005). These changes in marginal populations can be local adjustments to unfavourable or highly variable local conditions. For instance, life history theory predicts that size at first reproduction should decrease in stressful environments as mortality increases in adults (Stearns and Koella, 1986). Selection for high investment in reproduction is possible in this situation (Karlsson, 2005).

Temperature represents one of the main environmental factors influencing life history traits (Begon *et al.*, 2006). Seasonal temperatures (heat or cold) can influence demographic parameters in populations, adversely affecting recruitment (when temperatures are not optimal for reproduction or completion of life cycles), or increasing mortality (when temperatures become too extreme for survival)(Hutchins, 1947). In this context,

temperature tolerance of a species is in part responsible for the patterns of the geographic distribution. Distribution boundaries of several species are correlated with winter or summer seasonal conditions (Hutchins, 1947). In the contemporary context of global warming, a growing number of studies have asserted a possible link between recent changes in climate and observed changes in species (McCarty, 2001; Wood and McDonald, 1996; Woodward, 1987). Between these changes, variations in the geographic range have been described for many species. Northward movements of species' range boundaries consistent with climate warming have been observed for fish (Perry *et al.*, 2005), birds (Thomas and Lennon, 1999), mammals (Hersteinsson and Macdonald, 1992) and butterflies (Dennis, 1993; Hill *et al.*, 1999; Parmesan, 1996; Parmesan *et al.*, 1999). The prediction that species distributions will shift poleward as global warming continues depends on the idea that thermal stresses are always higher at their more-equatorial ends (Helmuth *et al.*, 2002).

Climate change and El NIÑO lead to changes in the levels and timing of abiotic stresses directly affecting kelp forests. The flexibility in the life cycle of Laminariales can allow them to adapt to this thermic pressure maintaining geographical ranges or moving boundaries creating a new geographical distribution. In this context raises the question: can temperature be use as a predictor of a species distribution range? And for complex organism with haplo-diplontic life cycles, which are the key sensitive traits that face to stressful temperature limit the completion of life cycles?

Range limits of species are partly determined by environmental stress gradients (Sexton *et al.*, 2009). These determinant environmental conditions raise the question if they have an effect on sex ratios at the margins of distribution. In this context, increased asexual reproduction has been often observed in marginal populations (Eckert, 2001), including parthenogenesis, which can modify sex ratio. Additionally, marginal habitats may cause unbalanced sex ratios as a result of i) selective mortality of one sex, ii) evolutionary responses of the mating system or iii) a combination of both. In dioecious plants, females usually expend more resources in reproduction than males, and a recurrent pattern observed in this group is the presence of male-biased sex ratios in marginal populations experiencing higher levels of environmental stress (Delph, 1999).

Geographical parthenogenesis

Where sexual and asexual modes of reproduction coexist within a species, the asexual mode prevails in marginal habitats, in particular those whose marginal nature is due

to abiotic factors. This phenomenon has been largely documented in terrestrial plants and animals (Hörandl, 2006; Kearney, 2005). This geographically distinct distribution of sexuals and asexuals is called geographical parthenogenesis (Vandel, 1928).

Most asexuals in both plants and animals are polyploid or hybrid in origin, or both, and hybridization and polyploidy appear to be mechanistically involved in the origin of asexuality in many cases (Hörandl, 2006; Simon *et al.*, 2003). Polyploidization (and aneuploidization) also appear to be a mechanistic by-product frequently arising from hybridization, resulting from the failure of homologous chromosomes from the two parents to successfully pair at the first division Meiosis I (Kawecki, 2008; Ramsey and Schemske, 1998). Recent studies suggest that hybrid origin may be crucial for the success of asexuals in marginal habitats, presumably owing to the resulting high heterozygosity. Following this idea, asexual reproduction and polyploidy would play a secondary role in protecting the hybrid genotypes from loss of heterozygosity, preventing recombination with locally maladapted immigrants and escaping problems associated with meiosis (Hörandl, 2006; Kearney, 2005).

Asexual lineages are evolutionary limited by the lack of recombination capacity that makes them prone to accumulate deleterious mutations. Genetic diversity of asexual lineages is generated through recurrent origin of asexual clones from the parental species rather than diversification of existing asexual lineages. Asexuals are a sub-group of the genetic pool of the parental species, and thus for the parental species they represent an evolutionary dead end. On the other hand, hybridisation not associated with asexuality may enrich the genetic pool through introgression of alleles from a related species. Through hybridization a species may acquire a specific adaptation from a relative that already has it, and recombination of hybrid genotypes creates a large amount of genetic variation, often exceeding the range of variation in the parental species (Rieseberg *et al.*, 2007). Horizontal gene transfer, though involving different mechanisms, is in some ways an analogous phenomenon, playing an important role in the evolution of ecological niches of prokaryotes (Koonin *et al.*, 2001).

In some cases, adaptation to marginal habitats assisted by hybridisation is accompanied by the evolution of reproductive isolation, leading to the origin of new species (recombinational or homoploid hybrid speciation)(Bierzychudek, 1985; Kawecki, 2008; Rieseberg *et al.*, 2007).

Several authors have discussed that geographical parthenogenesis is probably caused by a combination of factors (Bierzychudek, 1985; Haag and Ebert, 2004; Hörandl, 2006; Kearney, 2005). There are five non-exclusive hypotheses proposed to explain geographic parthenogenesis:

(1) Asexuality eliminates the risk of not finding a sexual partner in low-density marginal populations and makes it possible for a single individual to colonize an empty habitat (Cuellar, 1977; Gerritsen, 1980).
(2) Asexuality allows a genotype that happens to be locally well adapted to breed true. Sexual organisms living in marginal habitats may be permanently maladapted because of gene-flow from core-habitat, whereas any well-adapted asexual genotypes that may exist are isolated from non-adapted immigrants and thus can maintain adaptation (Peck *et al.*, 1998).
(3) Sexual genotypes may be more advantageous in habitats that involve many biotic interactions because co-evolutionary arms races with parasites, predators, and competitors favour sexual reproduction (Hamilton, 1980; Lively *et al.*, 1990). Asexuals, on the other hand, would occur more often in sparsely inhabited habitat, where these interactions are rare and where environmental factors are dominating (Glesener and Tilman, 1978; Hamilton *et al.*, 1990).
(4) Among asexual species, they may occupy a broader range of environments because of selection for generalist clones (Lynch, 1984), because of their hybrid origin (Bulger and Schultz, 1979), or because they may in fact represent many differentially adapted clonal "microspecies" (Pound *et al.*, 2004).
(5) Marginal populations are often small and subject to frequent bottlenecks, and thus prone to inbreeding, but clonal reproduction preserves the original heterozygosity (Haag and Ebert, 2004).

As previously commented, geographical parthenogenesis has been largely studied in terrestrial environments where asexuality is prevalent in stressful or marginal populations. The classification of marginality has been very broad concerning deserts, high altitudes, latitudes, resource poor habitats, between others. The first natural question that raises studying reproduction in a marine macroalgae is, if parthenogenesis happens in natural environments, where is it suppose to happen? In other words, what can we consider a marginal population?, and given the diversity of asexual mechanisms described for Laminariales, how should we study it?, further, which methods should we use?

Objectives

The general objective of this thesis is to compare the reproductive systems of Laminarian populations along both the centre of the species' range and in the limits of the species' distribution in order to study the ecological and evolutionary limits of the adaptation of species.

The specific objectives are:

1) To study the sex-determinism through sex ratio estimations.

2) To determine how temperature tolerance varies among different microscopic life history stages in laboratory studies.

3) To study parthenogenesis *in vitro* and in natural populations through culturing, genetic characterisation and flow cytometry analyses.

Thesis structure

Chapter N° 1 presents the study of sex ratio in the two cryptic species of the *Lessonia nigrescens* complex, along 2000 km of lineal coast, comprising central and marginal populations (total of 13) of both species. Culturing techniques were used to obtain sex ratio estimates with temperature variation, in order to obtain information of the sex determination system of Laminariales. This work shows that the sex determination system in these species seems to be genetically determined but can be modulated by temperature. Marginal populations displayed biased sex ratios compared to central populations of both species. This chapter has been accepted for publication in Journal of Phycology the 23rd of April 2010.

Chapter N°2 examines the effect of temperature on 7 life history traits of microscopic stages from the two cryptic species of the *L.nigrescens* complex. This study comprises central and marginal populations of both species (total of 8) along 2000 km of chilean coast. Culturing techniques and microscopy were used to estimate the selected traits. In addition a periodic model was developed to calculate total population growth and elasticity analyses were performed to detect key steps in gametophytes' demography. This work shows that the cryptic species posses differential tolerance to temperature, being the northern species more tolerant than the southern one. Also different life history strategies were detected between the species. Local adaptation was found in marginal populations of both species.

Chapter N°3 explores sexuality variations at the southern range limit of *Laminaria digitata* comparing with 2 central populations. Genetic, cytological, culturing and microscopy techniques were used to described and understand the lost of sex at the range limit. This work shows the existence of geographical parthenogenesis in *L.digitata*, that involves several life cycle modifications.

Literature cited

Ar-Gall E, Asensi A, Marie D, Kloareg B (1996) Parthenogenesis and apospory in the Laminariales: a flow cytometry analysis. *European Journal of Phycology* **31**, 369-380.

Arzel P (1998) *Les laminaires sur les côtes bretonnes. Evolution de l'exploitation et de la flottille de pêche, état actuel et perspectives*, Plouzané, France.

Bartsch I, Wiencke C, Bischof K*, et al.* (2008) The genus *Laminaria sensu lato*: recent insights and developments. *European Journal of Phycology* **43**, 1-86.

Begon M, Townsend CR, Harper JL (2006) *Ecology: From Individuals to Ecosystems*, Fourth edition edn. Blackwell Publishing, Oxford.

Bell G (1982) *The masterpiece of nature: the evolution and genetics of sexuality* University of California Press, California.

Bierzychudek P (1985) Patterns in plant parthenogenesis. *Experientia* **41**, 1255-1264.

Billot C, Engel CR, Rousvoal S, Kloareg B, Valero M (2003) Current patterns, habitat discontinuities and population genetic structure: the case of the kelp *Laminaria digitata* in the English Channel. *Marine Ecology Progress Series* **253**, 111-121.

Birkett DA, Maggs CA, Dring MJ, Boaden PJS (1998) Infralittoral reef biotopes with kelp species: an overview of dynamic and sensitivity characteristics for conservation management of marine SACs In: *Natura 2000* Scottish Association of Marine Science (SAMS) for the UK Marine SACs Project.

Boland W (1995) The chemistry of gamete attraction: chemical structures, biosynthesis, and (a) biotic degradation of algal pheromones. *PNAS* **92**, 37-42.

Bulger AL, Schultz RJ (1979) Heterosis and interclonal variation in thermal tolerance in unisexual fishes. *Evolution* **33**, 848-859.

Butlin RK (2000) Virgin rotifers. *Trends in Ecology and Evolution* **15**, 389-390.

Cabioch L (1968) Contribution à la connaissance des peuplements benthiques de la Manche Occidentale. *Cahiers de Biologie Marine* **9**, 493-720.

Camus P (2001) Biogeographía marina de Chile continental. *Revista Chilena de Historia Natural* **74**, 587-617.

Castel J, Dauvin J-C, Glémarec M (1997) Le conditions générales en Atlantique, Manche et Mer du Nord. In: *Les Biocénoses Marines et Littorales Françaises des côtes Atlantique, Manche et Mer du Nord* (ed. Dauvin J-C), pp. p 9-19. Museum National d'Histoire Naturelle, Paris.

Castilla JC, Camus PA (1992) *The humboldt-El Nino scenario: coastal benthic ressources and anthropogenic influences, with particular reference to the 1982783 ENSO* Benguela trophic functioning.

Caswell H (2001) *Matrix population models: construction, analysis, and interpretation*, Second Edition edn. Sinauer Associates, Sunderland, Massachusetts.

Correa JA, Gonzalez P, Sanchez P, Muñoz J, Orellana MC (1996) Copper-algae interactions: inheritance or adaptation? *Environmental Monitoring and Assessment* **40**, 41-54.

Correa JA, Ramirez M, delaHarpe J-P, Roman D, Rivera L (2000) Copper, copper mining effluents and grazing as potential determinants of algal abundance and diversity in northern chile. *Environmental Monitoring and Assessment* **61**, 265-281.

Cosson J (1978) *Recherches morphogenetiques et ecophysiologiques sur la pheophycee Laminaria digitata (L.) Lamouroux*, Université de Caen.

Crawford RMM (2008) *Plants at the Margin: Ecological Limits and Climate Change* Cambridge University Press, New York.

Cuellar O (1977) Animal Parthenogenesis. *Sience* **197**, 837-843.

Dayton PK (1985) Ecology of kelp communities. *Annual Review of Ecology and Systematics* **16**, 215-245.

Dayton PK, Tegner MJ, Edwards PB, Riser KL (1999) Temporal and spatial scales of kelp demography: the role of oceanographic climate. *Ecological Monographs* **69**, 219-250.

Delph LF (1999) *Sexual dimorphism in life history* Springer-Verlag, Berlin.

Dennis RLH (1993) *Butterflies and climate change* Manchester University Press, New York.

Eckert CG (2001) The loss of sex in clonal plants. *Evolutionary Ecology* **15**, 501-520.

Eckert CG, Samis E, Lougheed C (2008) Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology* **17**, 1170-1188.

Evans L (1963) A large chromosome in the laminarian nucleus. *Nature* **198**, 215.

Evans L (1965) Cytological studies in the Laminariales. *Annals of Botany* **116**, 541-562.

Funano T (1983) The ecology of *Laminaria religiosa* Miyabe. I. The life history and alternation of nuclear phases of *Laminaria religiosa*, and the physiological ecology of the gametophytes and the embryonal sporophytes. *Hokusui-Shiho* **25**, 61-109.

Gagné JA, Mann KH, Chapman ARO (1982) Seasonal patterns of growth and storage in *Laminaria longicruris*; in relation to differing patterns of availability of nitrogen in the water. *Marine Biology* **69**, 91-101.

Gaston KJ (2009) Geographic range limits: achieving synthesis. *Proceedings of the Royal Society B: Biological Sciences* **276**, 1395-1406.

Gerard VA (1997) Environmental stress during early development of kelp sporophytes (Laminaria saccharina): how long do effects persist? *Journal of Applied Phycology* **9**, 5-9.

Gerritsen J (1980) Sex and parthenogenesis in sparse populations. *American Naturalist* **115**, 718-742.

Glesener RR, Tilman D (1978) Sexuality and the components of environmental uncertainty: clues from geographical parthenogenesis in terrestrial animals. *American Naturalist* **112**, 659-673.

Graham MH, Kinlan BP, Druehl LD, Garske LE, Banks S (2007) Deep-water kelp refugia as potential hotspots of tropical marine diversity and productivity. *Proceedings of the National Academy of Sciences* **104**, 16576-16580.

Haag CR, Ebert D (2004) A new hypothesis to explain geographic parthenogenesis. *Annales Zoologici Fennici* **41**, 539-544.

Hamilton WD (1980) Sex versus non-sex versus parasite. *OIKOS* **35**, 282-290.

Hamilton WD, Axelrod R, Tanese R (1990) Sexual reproduction as an adaptation to resist parasites (a review). *Proceedings of the National Academy of Sciences of the United States of America* **87**, 3566-3573.

Helmuth B, Harley C, Halpin P*, et al.* (2002) Climate change and latitudinal patterns of intertidal thermal stress. *Science* **298**, 1015-1017.

Hernandez-Carmona G, Robledo D, Serviere-Zaragoza E (2001) Effects of nutrient availability on Macrocystis pyrifera recruitment and survival near its southern limit off Baja California. *Botanica Marina* **44**, 221-229.

Hersteinsson P, Macdonald DW (1992) Interspecifc competition and the geographical distribution of red and arctic foxes *Vulpes vulpes* and *Alopex lagopus*. *Oikos* **64**, 505-515.

Hill JK, Thomas CD, Huntley B (1999) Climate and habitat availability determine 20th century changes in butterfly's range margin. *Proceedings of the Royal Society of London* **266**, 1197-1206.

Hörandl E (2006) The complex causality of geographical parthenogenesis. *New Phytologist* **171**, 525-538.

Hutchins LW (1947) The Bases for Temperature Zonation in Geographical Distribution. *Ecological Monographs* **17**, 325-335.

Judson OP, Normark BB (1996) Ancient asexual scandals. *TRENDS in Ecology and Evolution* **11**, 41-46.

Karlsson PS (2005) The Ressource Economy of Plant Reproduction. In: *Reproductive allocation in plants* (eds. Reekie EG, Bazzaz FA), pp. 1-40. Elsevier, London, UK.

Kawecki TJ (2008) Adaptation to Marginal Habitats. *Annual Review of Ecology and Systematics* **39**, 321-342.

Kearney M (2005) Hybridization, glaciation and geographical parthenogenesis. *TRENDS in Ecology and Evolution* **20**, 495-502.

Kluth C, Bruelheide H (2005) Central and peripheral Hornungia petraea populations: patterns and dynamics. *Journal of Ecology* **93**, 584-595.

Kondrashov AS (1993) Classification of hypotheses on the advantage of amphimixis. *Journal of Heredity* **84**, 372-387.

Kondrashov AS, Crow JF (1991) Haploidy or Diploidy - Which Is Better. *Nature* **351**, 314-315.

Kondrashov FA, Kondrashov AS (2001) Multidimensional epistasis and the disadvantage of sex. *PNAS* **98**, 12089-12092.

Koonin EV, Makarova KS, Aravind L (2001) Horizontal gene transfer in prokaryotes: Quantification and classification. *Annual Review of Microbiology* **55**, 709-742.

Lamouroux JVF (1813) Essai sur les genres de la famille des thalassiophytes non articulées. *Annales du Muséum d'Histoire Naturelle de Paris* **20**, 21-47, 115-139, 267-293.

Lee JA, Brinkhuis BH (1988) Seasonal light and temperature interaction effects on development of Laminaria saccharina (Phaeophyta) gametophytes and juvenile sporophytes. *Journal of Phycology* **24**, 181–191.

Lewis RJ (1996) Chromosomes of the brown algae. *Phycologia* **35**, 19-40.

Lively CM, Craddock C, Vrijenhoek RC (1990) Red Queen hypothesis supported by parasitism in sexual and clonal fish. *Nature* **344**, 864-866.

Lobban C, Harrison P (1994) *Seaweeds Ecology and Physiology* Cambridge University Press, New York, USA.

Lüning K (1981) Egg release in gametophytes of *Laminaria saccharina*: Induction by darkness and inhibition by blue light and u.v. *British Phycological Journal* **16**, 379 - 393.

Lüning K (1990) *Seaweeds: Their environment, biogeography and ecophysiology* John Wiley & sons, New York.

Lüning K, Müller DG (1978) Chemical interaction in sexual reproduction of several Laminariales: release and attraction of spermatozoids. *Zeitschrift für Pflanzenernährung und Bodenkunde* **89**, 33-41.

Lynch M (1984) Destabilizing hybridization, general purpose genotypes and geographic parthenogenesis. *The Quarterly Review of Biology* **59**, 257-290.

Lynch M, Burger R, Butcher D, Gabriel W (1993) The mutational meltdown in asexual populations. *Journal of Heredity* **84**, 339-344.

Mable BK, Otto SP (1998) The evolution of life cycles with haploid and diploid phases. *BioEssays* **20**, 453-462.

Martinez, E. A., C. Destombe, M. C. Quillet, and M. Valero. 1999. Identification of random amplified polymorphic DNA (RAPD) markers highly linked to sex determination in the red alga Gracilaria gracilis. Molecular Ecology **8**:1533-1538.

Martínez EA (1999) Latitudinal differences in thermal tolerance among microscopic sporophytes of the kelp *Lessonia nigrescens* (Phaeophyta: Laminariales). *Pacific Science* **53**, 74-81.

Martínez EA, Cárdenas L, Pinto R (2003) Recovery and genetic diversity of the intertidal kelp *Lessonia nigrescens* (Phaeophyceae) 20 years after El Niño 1982/83. *Journal of Phycology* **39**, 504-508.

Matson PG, Edwards MS (2007) Effects of ocean temperature on the southern range limits of two understory kelps, *Pterygophora californica* and *Eisenia arborea*, at multiple life-stages. *Marine Biology* **151**, 1941-1949.

Maynard-Smith J (1978) *The evolution of sex* Cambridge University Press, Cambridge.

McCarty JP (2001) Ecological Consequences of Recent Climate Change. *Conservation Biology* **15**, 320-331.

Motomura T (1991) Inmunofluorescence microscopy of fertilization and parthenogenesis in *Laminaria angustata* (Phaeophyta). *Journal of Phycology* **27**, 248-257.

Müller D (1967) Generationswechsel, Kernphasenwechsel und Sexualität der Braunalgae *Ectocarpus siliculosus* im kulturversuch. *Planta Berl* **75**, 39-54.

Nakahara H (1984) Alternation of generations of some brown algae in unialgal and axenic cultures. *Scientific Papers of the Institute of Algological Research. Faculty of Science. Hokkaido University.* **7**, 77-292.

Oppliger LV, Peters A, Correa JA (2007) Parthenogenesis in the brown algae *Lessonia nigrescens* (Phaeophyceae) from Central Chile. *Journal of Phycology* **43**, 1295-1301.

Otto SP (2009) The Evolutionary Enigma of Sex. *The American Naturalist* **174**, S1-S14.

Otto SP, Gerstein AC (2008) The evolution of haploidy and diploidy. *Current Biology* **18**, 1121-1124.

Otto SP, Lenormand T (2002) Resolving the paradox of sex and recombination. *Nature* **3**, 252-260.

Parmesan C (1996) Climate and species' range. *Nature* **382**, 765-766.

Parmesan C, Ryrholm N, Stefanescus C*, et al.* (1999) Poleward shifts in geographical ranges and butterfly species associated with regional warming *Nature* **399**, 579-583.

Peck JR, Yearsley JM, Waxman D (1998) Explaining the geographic distributions of sexual and asexual populations. *Nature* **391**, 889-892.

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) A Changing Climate for Fish and Chips. *Science* **308**, 1837a-.

Pound GE, Cox SJ, Doncaster CP (2004) The accumulation of deleterious mutations within the frozen niche variation hypothesis. *Journal of Evolutionary Biology* **17**, 651-662.

Ramirez ME, Santelices B (1991) *Catalogo de las algas marinas bentonicas de la costa temperado del Pacifico de Sudamerica. Monografia Biologicas n°5*, Pontificia Universidad Catolica de Chile.

Ramsey J, Schemske DW (1998) Pathways, mechanisms, and rates of polyploidy formation in flowering plants. *Annual Review of Ecology and Systematics* **29**, 467-501.

Rieseberg LH, Kim SC, Randell RA, Whitney KD, Gross BL (2007) Hybridization and the colonization of novel habitats by annual sunflowers. *Genetica* **129**, 149-165.

Santelices B, Ojeda P (1984) Recruitment, growth and survival of *Lessonia nigrescens* (Phaeophyta) at various tidal levels in exposed habitats of central Chile. *Marine Ecology Progress Series* **19**, 73-84.

Sauvageau C (1918) Recherches sur les Laminaires des côtes de France. *Mémoires de l'Académie des Sciences de Paris* **56**, 1-240.

Schreiber E (1930) Untersuchungen über Parthenogenesis, Geschlechtsbestimmung und Bastardisierungsvermögen bei Laminarien. *Planta* **12**, 331-353.

Schwander T, Crespi BJ (2009) Multiple direct transitions from sexual reproduction to apomictic parthenogenesis in Timema stick insects. *Evolution* **63**, 84-103.

Sexton JP, McIntyre PJ, Angert AL, Rice KJ (2009) Evolution and Ecology of Species Range Limits. *Annual Review of Ecology, Evolution and Systematics* **40**, 415-436.

Sibley R, Antonovics J (1992) Life-history Evolution. In: *Genes in Ecology* (eds. Berry RJ, Crawford TJ, Hewitt GM), pp. 87-122. Blackwell, Oxford.

Simon JC, Delmotte F, Rispe C, Crease T (2003) Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. *Biological Journal of the Linnean Society* **79**, 151-163.

Stearns S (1992) *The Evolution of Life Histories* Oxford University Press, Oxford, England.

Stearns S (2000) Life history evolution: successes, limitations and prospects. *Naturwissenschaften* **87**, 476-486.

Stearns SC, Koella JC (1986) The Evolution of Phenotypic Plasticity in Life-History Traits: Predictions of Reaction Norms for Age and Size at Maturity. *Evolution* **40**, 893-913.

Steneck RS, Graham MH, Bourque BJ*, et al.* (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation* **29**, 436-459.

Suomalainen E, Saura A, Lokki J (1987) *Cytology and evolution in parthenogenesis* CRC Press, Boca Raton.

Tala F, Edding M (2005) Growth and loss of distal tissue in blades of *Lessonia nigrescens* and *Lessonia trabeculata* (Laminariales). *Aquatic Botany* **82**, 39-54.

Tegner MJ, Dayton PK (1991) Sea urchins, El Nino, and the long term stability of Southern California kelp forest communities. *Marine Ecology Progress Series* **77**, 49-63.

Tegner MJ, Dayton PK, Edwards PB, Riser KL (1996) Is there evidence for long-term climate change in Southern California kelp forests? *California Cooperative Oceanic Fisheries Investigations Reports*, 111-126.

Tellier F, Meynard AP, Correa JA, Faugeron S, Valero M (2009) Phylogeographic analyses of the 30°S south-east Pacific biogeographic transition zone establish the occurence of a sharp genetic discontinuity in the kelp *Lessonia nigrescens*: vicariance or parapatry? *Molecular Phylogenetics and Evolution* **53**, 679-693.

Thomas CD, Lennon JJ (1999) Birds extend their ranges northwards. *Nature* **399**, 213.

Trenberth K (1997) The definition of El Nino. *Bulletin of American Meteorological Society* **78**, 2771-2777.

Vandel A (1928) La parthénogenese geographique. Contribution a l'étude biologique et cytologique de la parthénogenese naturelle. *Bulletin Biologique de la France et de la Belgique* **62**, 164-182.

VanderMeer J (1990) Biology of the red Algae. In: *Genetics* (eds. Cole K, Sheath R), pp. 103-122. Cambridge University Press, New York.

Vásquez JA, Tala F (1995) Repopulation of intertidal areas with *Lessonia nigrescens* in northern Chile. *Journal of Applied Phycology* **7**, 347-349.

Vásquez JA, Vega JM (2004) *El Nino 1997-98 en el norte de Chile: efectos en la estructura y en la organizacion de comunidades submareales dominadas por algas pardas*, CONA, Valparaiso.

Villa-Martinez R, Villagran C, Jenny B (2003) The last 7500 cal yr B.P. of westerly rainfall in Central Chile inferred from a high-resolution pollen record from Laguna Aculeo (34°S). *Quaternary Research* **60**, 284-293.

Villouta E, Santelices B (1984) Estructura de la comunidad submareal de *Lessonia* (Phaeophyta, Laminariales) en Chile norte y central. *Revista Chilena de Historia Natural* **57**, 111-122.

Villouta E, Santelices B (1986) *Lessonia trabeculata* sp. nov. (Laminariales, Phaeophyta) a new kelp from Chile. *Phycologia* **25**, 81-86.

Vrijenhoek RC (1998) Animal clones and diversity. *Biosciences* **48**, 617-628.

Weeks AR, Reynolds KT, Hoffman AA (2002) *Wolbachia* dynamics and host effects: what has (and has not) been demonstrated. *Trends in Ecology and Evolution* **17**, 257-262.

West JA, Zuccarello GC (1999) Biogeography of sexual and asexual populations in *Bostrychia moritziana* (Rhodomelaceae, Rhodophyta). *Phycological Research* **7**, 115-123.

Wood CM, McDonald CG (1996) *Global warming: implications for freshwater and marine fish* Cambridge University Press, New York.

Woodward FI (1987) *Climate and plant distribution* Cambridge University Press, New York.

Yabu H, Sanbonsuga Y (1990) Mitosis in the female gametophytes and young sporophytes of *Laminaria diabolica* f. *longipes* Miyabe et Tokida. *Bulletin of the Faculty of Fisheries, Hokkaido University.* **41**, 8-12.

Yasui H (1992) Chromosome numbers and a sex chromosome of *Laminaria yendoana* Miyabe (Phaeophyta). *Nippon Suisan Gakkaishi* **58**, 1385.

CHAPTER 1

SEX RATIO VARIATION IN THE *LESSONIA NIGRESCENS* COMPLEX (LAMINARIALES, PHAEOPHYCEAE): EFFECT OF LATITUDE, TEMPERATURE AND MARGINALITY

(Article in press, Journal of Phycology)

L. Valeria Oppliger[1,2,3], Juan A. Correa[1], Sylvain Faugeron[1], Florence Tellier[1,2], Jessica Beltrán[1], Myriam Valero[3] and Christophe Destombe[2]

(1) Departamento de Ecología, Center for Advanced Studies in Ecology and Biodiversity, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Post-code 6513677, Santiago, Chile.
(2) UPMC Univ. Paris 06, UMR 7144, Equipe "BEDIM", Station Biologique de Roscoff, 29682 Roscoff, France.
(3) CNRS, UMR 7144, Station Biologique de Roscoff, Place Georges Teissier, 29682 Roscoff, France.

ABSTRACT

Little is known on variation of sex ratio, the proportion of males to females, in natural populations of seaweed while it is a major determinant of the mating system. In this paper, the variation of sex ratio, was studied in the kelp *Lessonia nigrescens* species complex recently identified as two cryptic species occurring along the Chilean coast: one located north and the other south of the biogeographic boundary at latitude 29-30° S. *L. nigrescens* is characterised by an alternation of microscopic haploid gametophytic individuals and large macroscopic fronds of diploid sporophytes. Sex ratio was recorded in 241 gametophytic progenies from sporophytic individuals collected from 13 populations distributed along the Chilean coast in order (1) to examine the effect of an environmental gradient coupled with latitude and (2) compare marginal populations to central populations of the two species. In addition, we specifically tested the hypothesis that the sex ratios of the two cryptic species would be affected differently by temperature. First, our results demonstrate that sex ratio seems to be mainly genetically determined and temperature can significantly modify it. Populations of the northern species showed a lower frequency of males at 14°C than at 10°C, whereas populations of the southern species showed the opposite pattern. Second, a latitude effect was revealed but it was mainly explained by an increased variation in sex ratio at the range limits for both species. This greater variation at the margins could be either due to differential mortality between sexes or to geographical parthenogenesis (asexual reproduction).

Key index words: Cryptic species; gametophyte; latitude; *Lessonia nigrescens*; marginal populations; Phaeophyceae; sex determination; sex ratio; temperature.

INTRODUCTION

The expression of a given sexual phenotype is defined by genetic or environmental factors, or a combination of both (Nakamura 2009). The most studied type of genetic sex determination (GSD) involves sex chromosomes. Sex determination in diploid mammals and some diploid plants depends on the combination of sexual chromosomes, where an individual with XX becomes female (i.e. homogametic sex), while an individual with XY becomes male (i.e. heterogametic sex). In some other groups including birds, butterflies and some fishes, females are heterogametic (ZW) and males are homogametic (ZZ). Alternatively, one or more mating-type loci determine mating compatibility in organisms such as *Chlamydomonas* and some fungi where two incompatibility types are known (Charlesworth et al. 2005). In haploid or haplo-diploid life cycles, sex determination could directly rely on sexual chromosomes (e.g. gametophytes bearing X are females and those bearing Y are males), whereas diploid individuals with both sexual chromosomes (XY) produce meiotic spores. This was clearly demonstrated in the dioecious liverwort *Marchantia polymorpha,* where male (bearing Y chromosome) and female (bearing X chromosome) are morphologically identical until the male and female organs differentiate. Thus, segregation of sexual chromosomes during meiosis of the diploid phase ensures equal proportions of female and male gametophytes. Whatever the mechanism of GSD, sex ratios are often close to 0.5 in natural populations (i.e. 1 male: 1 female) (Uller et al. 2007).

However, the sexual phenotypes of species with GDS can be ultimately defined by environmental conditions (environmental sex determination: ESD) (Kraak and Pen 2002). It is well known that incubation temperature of eggs in some reptiles (Sarre 2006), and water temperatures during larval development in amphibians modify sex ratios (for review see Nakamura, 2009) . A case of complete ESD (i.e. no evidence of GSD) was reported in *Equisetum* sp., a horsetail with haplo-diploid life cycle. Indeed, it was shown that haploid gametophytes were potentially bisexual (Duckett 1977), and that sex expression was, at least partly, determined by environmental factors (Guillon and Fievet 2003). Other mechanisms involved in sex determination evolved in the fern *Ceratopteris richardii*, where male:hermaphrodite ratios are determined epigenetically by an antheridiogen pheromone; thus, male frequencies increased with the density of the population (Tanurdzic and Banks 2004). In this context, evolutionary theory predicts that ESD is an evolutionarily stable strategy, particularly in patchy environments where some patches are more valuable to females and others to males (Charnov and Bull 1977, Bull 1981). Finally, the effect of the environment on sex ratios has been re-examined in the context of global change. Studies in

amphibians (Blaustein and Wake 1990), birds (Thomas and Lennon 1999), mammals (Hersteinsson and Macdonald 1992) and butterflies (Parmesan et al. 1999) have highlighted that drastic changes in sex ratio due to a temperature effect could lead to population extinctions (due to the loss of one of the sexes), and thus to changes in the range of species' distribution. These findings raise the question of whether environmental conditions, occurring in habitats coinciding with the distribution limit for a given species, have an effect on sex ratios. In this context, increased asexual reproduction has been often observed in marginal populations (Eckert 2002), including parthenogenesis, which can modify sex ratio. Additionally, marginal habitats may cause unbalanced sex ratios as a result of i) selective mortality of one sex, ii) evolutionary responses of the mating system or iii) a combination of both.

Sex determination is poorly understood in seaweeds. In brown algae (Phaeophyceae), GDS was demonstrated in *Ectocarpus* sp. using crossing experiments (Müller 1967). Sexual reproduction in *Ectocarpus* is isogamous and involves an alternation between diploid sporophytes and haploid, dioecious male and female gametophytes. Sporophytes and gametophytes have a similar morphology, both consisting of branched, uniseriate filaments. The life cycle of kelps (Laminariales), on the other hand, is heteromorphic with an alternation of microscopic haploid dioecious dimorphic gametophytes, and macroscopic diploid sporophytes that produce haploid spores by meiosis. Sexual reproduction is anisogamous. Female gametophytes produce eggs that, after fertilized by sperms, produce new diploid sporophytes. A large chromosome was reported in female gametophytes of several kelp species of Laminariaceae, including *Laminaria digitata*, *L. ochroleuca*, *Saccharina latissima* as *L. saccharina*, *Alaria esculenta* and *Chorda filum* (Evans 1963), *L. hyperborea* and *Saccorhiza polyschides* (Evans 1965) and *L. yendoana* (Yasui 1992), suggesting the existence of sexual chromosomes in these species. Recently a putative sex-determining region was identified in Laminaria using AFLP molecular tool (Yang et al. 2009). Few studies have reported on sex ratio in culture, most suggesting a similar proportion of males and females (Sauvageau 1918, Schreiber 1930, Cosson 1978). However, it has also been reported that sex ratio can be modified by abiotic stresses such as salinity or temperature (for review see Bartsch et al. 2008). For example, high temperatures in culture result in a higher proportion of males in *Saccharina latissima* as *Laminaria saccharina* (Lee and Brinkhuis 1988) and in *L. digitata* (Cosson 1978). In contrast, both high and low temperatures resulted in a decrease of males in *L. religiosa* (Funano 1983). More recently, it was demonstrated that high temperature and long days resulted in sex ratio biased towards females in *Lessonia variegata*, suggesting that males were less resistant to stressful

conditions (Nelson 2005). The above evidence suggests that the effect of temperature on sex ratio is variable and species-dependent in kelps. Unfortunately, the effects of temperature on sex ratio in the field are unknown because gametophytes are microscopic and impossible to observe in nature.

This study focused on the variation of sex ratio in two cryptic species of the *Lessonia nigrescens* complex (Tellier et al. 2009) by comparing individual progenies at three different levels (i.e. within populations, between populations of each species separately and between species). These two cryptic species were described recently by Tellier et al. (2009), who showed that individuals of this complex were arranged in two highly differentiated lineages with contrasting latitudinal distributions and a contact area at the 29°-30°S biogeographic transition zone. The Northern species is often exposed to extended periods of warmer waters from the north as a result of El Niño Southern Oscillation (ENSO) events which, during the 1980s were associated with massive local population extinctions of this kelp (Castilla and Camus 1992, Martínez et al. 2003). Experimental support to a temperature-related mortality was reported by Martínez (1999), who studied thermal tolerance of young sporophytes of *Lessonia nigrescens* and detected differences between Northern and Southern populations in both survival and growth rate. Thus, we specifically tested the hypothesis that the sex ratios of the two cryptic species would be affected differently by temperature.

MATERIAL AND METHODS

Material.

The two cryptic species of *L. nigrescens* Bory de Saint-Vincent are dominant in the intertidal and shallow-subtidal wave-exposed areas. They are distributed along the temperate Pacific coasts of South America between 17 and 42°S. One of them is located in the northern region of the Chilean coast, in the biogeographic Peruvian Province (17°37'S - 30°14'S) whereas the other is located in the Intermediate Area (29°03'S - 41°48'S) (Camus 2001). The two species (respectively designated as PP and AI species in Tellier et al. 2009 and called here Northern and Southern species) were never found co-existing in the same location, even within the transition zone between the two range distributions (from 27°S to 30°14'S), where a mosaic of pure populations of the Northern or Southern species was observed (Tellier et al. 2009).

Sampling and culture conditions.

Reproductive fronds from 6 to 30 mature sporophytic individuals were sampled in 13 locations distributed along the Chilean coast between 20°25'S and 39°46°S (Table 1). Seven and six locations were chosen for the Northern and Southern species respectively, and of these, two populations of the Northern species and three populations of the Southern species were sampled within the transition zone. These latter populations were considered as marginal because, geographically they are at the distribution limits of the species. Sea Surface Temperature (SST), estimated for each site from long time survey (11 years, 1996-2007) data of the Advanced Very-High Resolution Radiometer (AVHRR) satellite (Casey and Cornillon 1999), ranged from 11.0ºC (mean monthly minima) to 15.2ºC (mean monthly maxima) in the Valdivia, and from 16.2 to 23.0ºC in Los Verdes (Table 1). Temperatures in the transition zone ranged from 13.3 to 17.7ºC.

From each frond, a fragment of vegetative thallus was excised and stored in silica gel for molecular identification and two fragments of reproductive thallus of equal size (3,8 cm^2) with mature sori were washed with running tap water and sterile seawater. These fragments were incubated in darkness, at 16°C, in 50mL Falcon tubes containing sterile seawater and a glass slide. Spores released from the sori attached to the glass slide after 12 to 24 hours of incubation. Sterile seawater was replaced with SFC enriched seawater (Correa et al. 1988) and tubes were placed horizontally in culture chambers under standard culture conditions (10°C, 12:12 light:dark, 25-35 µmol photons $m^{-2}s^{-1}$) . Culture medium was changed once a week and observations were done every three days.

Molecular analyses.

In order to verify that sampled plants belonged to either the Northern or Southern species, five individuals of each population from the northern and southern regions, and ten individuals from each population within the transition zone, were sequenced and/or analyzed using the Single Stranded Conformation Polymorphism (SSCP) method as described elsewhere (Tellier et al. 2009). Total DNA was extracted from ca. 10 mg of dried material from the meristematic zone of each sporophytic thallus using an extraction buffer that combined a standard CTAB extraction with the addition of PVP to extract polyphenols (Martínez et al. 2003). Individuals were analysed using the mitochondrial intergenic markers atp8/trnS (Engel et al. 2008) and the PCR conditions were as in Voisin et al. (2005). PCR products were purified and sequenced in an ABI PRISM® 3100 Automated DNA Sequencer (Applied Biosystems, Foster City, California).

Sex ratio estimates.

Sex ratio was estimated for each progeny after 15 days in culture. Male and female gametophytes were identified according to their morphological characteristics using a Nikon Eclipse TE300 inverted microscope (Nikon Corp., Tokyo, Japan). Female gametophytes are characterized by large cells and filaments with few branches whereas male gametophytes are smaller and display highly branched filaments formed by small cells. These morphological differences make them unambiguously identifiable under the light microscope. The numbers of male and female gametophytes were determined by counting their occurrence in three visual fields per slide using the 10x objective. Sex ratio was expressed as the frequency of males per progeny (i.e. males/(males+females).

Effect of temperature.

In order to test the effect of temperature on sex ratio, additional cultures of gametophytes were established at 14°C using spores from 18-30 sporophytic individuals of two Northern populations (Los Verdes and Pan de Azúcar) and three populations of the Southern species (El Quisco, Las Cruces and Valdivia) (Table 1).

Statistical Analyses.

The binomial law was used to estimate the probability of detecting sex ratio deviation from p= 0.5 and the Bonferoni correction was applied to the tests. In order to have robust estimates, only replicates with at least 50 gametophytes per progeny were considered. The significance of the differences in sex ratios in progenies from the various populations was then analyzed within each species and between species using one-way ANOVAs. We excluded marginal populations located within the transition zone to analyze differences between the two cryptic species in order to obtain an average pattern representative of each species. Sex ratio variations in marginal populations were then compared to the average for the respective species.

In addition, we examined the effect of environmental factors on sex ratio of both cryptic species: we tested whether there was an effect of an environmental gradient coupled with latitude using regression analyses, and the effect of temperature was tested using a two-way ANOVA with species and temperature as fixed factors. General linear model procedures were used and Tukey's student range tests were performed for multiple comparisons. Homogeneity of variance was verified for all analyses of variance. All statistical analyses were done with MINITAB version 13.2.

RESULTS

SSCP and sequencing.

All 90 parental individuals were unambiguously assigned to either Northern or Southern species using SSCP and sequencing. Results (summarized in Table 1) verified that individuals from Los Verdes, Pan de Azucar, Cerro Elefante, Piqueros and Soldado belonged to the Northern species and those individuals from El Quisco, Las Cruces and Valdivia corresponded to the Southern species. In the contact zone (29°03'S - 30°14'S), individuals from Chañaral de Aceituno, Aceituno Playa Sur and Ermitaño belonged to the Southern species and the individuals from the populations of Apolillado and Choros Camping to the Northern species.

Sex ratio.

A total of 241 progenies, 119 from the northern species and 122 from southern species (corresponding to about 59,000 gametophytes) were included in the analyses. Sex ratio varied between 0.137 and 0.673 in populations of the Northern species and between 0.300 and 0.793 in those of the Southern species.

When all populations of each species were compared separately, a total of 108 progenies (90.7%) of the Northern species showed sex ratios not significantly different from 0.5 whereas 7 (5.9%) presented a significant male bias and 4 (3.4%) a significant female bias (Fig. 1). The same patterns were observed in the Southern species, where 108 progenies (88.5%) presented sex ratios not different from 0.5, whereas 13 (10.7%) displayed a significant excess of males and 1 (0.8%) a significant excess of females (Fig. 1). Overall, and with all marginal populations excluded, sex ratios of the progenies belonging to the Northern species (0.499 ± 0.09 SD) were not significantly different from those displayed by the progenies from the Southern species (0.515 ± 0.07) ($F= 1.99$, $P= 0.160$).

In the northern species, mean proportions of males varied from 0.343 (± 0.14) in Choros Camping to 0.545 (± 0.08) in Apolillado (Fig. 1) and between-populations differences in sex ratio were significant ($F=12.73$, $P< 0.0001$) due to the Choros Camping population which is the only one that departs from all the others. In the southern species, mean values of males varied from 0.548 (± 0.07) in El Quisco to 0.450 (±0.09) in El Ermitaño and between-populations differences in sex ratio were significant ($F=6.39$, $P<0.0001$) due to the Ermitaño, population which departs from all but from the Chañaral de Aceituno population.

Sex ratio varied with latitude. Whereas sex ratio diminished from the north towards the transition zone ($y=0.660-0.00639x$; $F=5.56$; $P=0.020$) in the progenies of the northern species, it gradually increased from the transition zone towards the south in those from

southern species (y=0.345+0.00517x; F=7.32; P=0.008) (Fig. 2). However, the regressions were not significant when the marginal populations of Choros Camping and Ermitaño were excluded, indicating that the significance of both regressions was determined by the female excess within these populations. Marginal populations of the Southern species within the transition zone showed a mean frequency of male gametophytes of 0.488 ± 0.09, significantly lower (F= 12.42; P=0.001) than male frequency calculated for central populations of the same species. Similarly, marginal populations of the Northern species displayed a mean frequency of males of 0.469 ± 0.141, significantly lower (F= 4.89; P=0.029) than male frequency estimated for central populations of the same species. Interestingly, most of the biased values were located in the transition zone where variance in sex ratio was significantly higher than in central populations (F=0.301; P=0.001).

Temperature effects on sex ratio

Sex ratios in populations of the Northern species (Los Verdes and Pan de Azúcar) increased with temperature (mean 0.526 ± 0.05 at 10°C and 0.551 ± 0.07 at 14°C, respectively, Fig. 3). By contrast, sex ratio of Southern species populations (El Quisco, Las Cruces and Valdivia) decreased with increasing temperature (mean 0.535 ± 0.06 at 10°C and 0.518 ± 0.05 at 14°C; Fig. 3). Even though there was no significant effect of temperature and species on sex ratio (Table 2), the interaction of these factors was highly significant (Table 2, Fig. 4). It was also clear that the main deviation from a sex ratio of 0.5 occurred in the northern species at 14°C (Fig. 4).

DISCUSSION

In this study, we first established that males and females generally occurred in equal proportions in natural populations of both species of *Lessonia*. These results suggest that sex determination is likely controlled by one or few genetic loci (i.e. GSD) as shown by Yang et al. 2009 in *Laminaria*. Then, it was also shown that temperature modulates sex ratio in both species, advocating that there is an interaction between genetic (GSD) and environmental factors (ESD) during the expression of sex. It was further observed that marginal populations of both species displayed significant female excesses as well as the largest variances in sex ratios. Finally, significant differences in sex ratio were revealed between the two cryptic species when exposed to diverse temperature conditions, demonstrating that these two phylogenetic species also correspond to ecological species.

One locus or chromosomal sex determination generally leads to sex ratios close to 0.5 because of the random segregation of sex alleles (or chromosomes) during meiosis (Bull and Charnov 1988). However, deviation from these expected 0.5 values have been often reported in natural populations and explained by interactions with environmental factors (Guillon and Fievet 2003, Nakamura 2009, Zaborski et al. 1988). Many examples of difference in mortality between males and females during their life span have been documented in plants (Delph 1999). Therefore, the resulting estimates of sex ratio have been usually biased in either direction depending on the period of the life history considered to make the observations (DeJong and Klinkhamer 2002). For example, in papayas the use of molecular genetic markers to distinguish male from female seeds showed that their sex ratio was biased towards males and then changed to equilibrated sex ratios at the adult stage (Parasnis et al. 1999).

In the present study, sex determination was monitored shortly after spore germination, based on the clear dimorphism of the gametophytes. Our results indicate that sex ratio in the two cryptic species of *L. nigrescens* is close to 0.5, and confirms that sex determination is probably governed by a major genetic factor (i.e. sex chromosome) as reported in other kelp species (Yasui 1992). However, our results also show that sex ratio can be modified by the effect of temperature. The lower frequency of males observed at higher temperature in populations from the southern species is consistent with the observations of *L. variegata* reported by Nelson (2005), showing an increase in female frequency at high temperature. Temperature-dependent sex ratio was also reported in *Laminaria religiosa* (Funano 1983), where values of 0.5 at optimum temperature shifted toward male-biased sex ratio at both higher and lower temperatures. Similarly, our results showed that an increase in temperature has two opposite effects, depending on the cryptic species of *L. nigrescens*. In populations of the northern species (Los Verdes and Pan de Azúcar) the proportion of males increased with temperature, whereas in populations of the southern species (El Quisco, Las Cruces and Valdivia) the proportion of males decreased with temperature. These results suggest the occurrence of different temperature optima for each species, probably related to their geographic origin. Indeed, the two temperatures selected for the experimental approach corresponded roughly to the minima and maxima of the monthly mean values to which the Southern species is exposed, both being clearly lower than the minimum temperatures encountered by the northernmost population (Los Verdes). Paradoxically, the northern species displayed an equilibrated sex ratio at the lowest temperature whereas the southern had an equilibrated sex ratio at the highest temperature. To better understanding these results, higher temperatures and additional populations must

be included in future work. Nevertheless, the differences in responses to temperature observed between the two cryptic species indicate environment-genotype interaction for the expression of this quantitative trait, and thus validates that the two genetic entities are ecologically differentiated.

We found that sex ratios differed greatly among progenies of the same population, and among populations of the same cryptic species, suggesting the occurrence of epigenetic factors inducing either segregation distortion during meiosis, or differential mortality between female and male spores, or differences in phenotypic expression or a combination of these mechanisms. Indeed, most of the studied populations (70%) displayed at least one progeny with a sex ratio significantly different from 0.5. Furthermore, sex ratios were more often biased toward males (22.8% of the progenies) than females (5.8% of the progenies), a phenomenon still in need of explanation.

Interestingly, two populations located in the transition zone (one from the northern species and one from the southern species) presented progenies with an excess of females. The highest proportion of females (85%) was recorded in the progeny from Choros Camping (Northern species) and Ermitaño (Southern species), located at the southern range limit of the northern species. More broadly, we observed that male frequency was significantly lower in marginal than in central populations in both species. Sporophytes from these populations released fewer spores (i.e. lower fertility), suggesting that they might be poorly adapted to the conditions prevailing at their range limits causing chronic stress (data not shown). In addition, 33% of the progenies of the Choros Camping population showed a significant female bias. This major change in sex ratio, in populations located at the species range limit, indicates an ESD due to the specific characteristics of the marginal habitat. Marginal populations are generally more fragmented and more prone to local extinctions due to environment fluctuations, demographic stochasticity and edge effects (Kawecki 2008). As a result, changes in the mating system as a response to low fertility success are often observed, in particular an increase of asexual reproduction (Eckert 2002, Kearney 2005). If, we had been able to observe progenies exhibiting 100% of females, then we would have been able to prove the occurrence of parthenogenesis (as previously observed in this complex species by Oppliger et al. 2007) in range limit. However, such progenies were not observed in the present study.

In conclusion, the sex ratio of gametophytes in *L. nigrescens* seems to be governed first by a major genetic factor (sex chromosomes or a sex locus) and secondarily modulated by environmental factors such as temperature. The two closely related species of *L. nigrescens* present different temperature optima for reproduction. It would be valuable to

compare sex ratio under a broader spectrum of temperature conditions to determine the optimum for each species in order to, at least in part, explain the geographical distribution of these two sibling species. Optimum reproduction at range limits could influence the survival of local populations and thus the preservation of a species.

ACKNOWLEDGEMENTS

We deeply thank M. Ramirez, G. Peralta, C. Figueroa, C. Chianale, D. Thomas, M-L. Guillemin, L. Prado, A. Gonzalez, P. von Dassow and B. Broitman, for their help during various stages of the research. V. Oppliger was supported by a CONICYT-FRENCH EMBASSY PhD grant and F. Tellier by a doctoral grant from the Ministry of Education and Research (MENRT). This study is part of the research programs FONDAP 1501-0001 Program 7, ANR ECOKELP (ANR 06 BDIV 012), "ARCUS" of the French Ministry of Foreign Affairs, and the Laboratoire International Associé "Dispersal and Adaptation of Marine Species (LIA DIAMS).

Table 1. Geographical location and number of studied progenies obtained from sporophytic individuals of each cryptic species sampled in different sites along the Chilean coast.

Site	Code	Species	Latitude (South)	Longitude (West)	Sampling date	Number of studied progenies		Monthly average Sea Surface Temperature	
						10°C	14°C	Min	Max
LOS VERDES	IQ	Northern	20°25'62	70°12'48	09/04/2007	27	29	16.2	23.0
PAN DE AZÚCAR	PAN	Northern	26°07'58	70°39'14	13/08/2007	33	21	14.4	19.9
CERRO ELEFANTE	CE	Northern	26°08'37	70°40'09	22/01/2007	7	----	14.4	19.9
PIQUEROS	PIQ	Northern	26°09'12	70°40'09	22/01/2007	6	----	14.4	19.9
SOLDADO	SOL	Northern	26°09'57	70°40'17	23/01/2007	13	----	14.4	19.9
CHAÑARAL DE ACEITUNO	ACE	Southern	29°04'02	71°29'21	20/02/2007	20	----	13.4	17.9
ACEITUNO PLAYA SUR	ACES	Southern	29°06'92	71°28'25	20/02/2007	15	----	13.4	17.7
EL ERMITAÑO	ER	Southern	29°08'74	71°30'16	20/02/2007	18	----	13.4	17.7
APOLILLADO	AP	Northern	29°11'03	71°29'80	21/02/2007	21	----	13.3	17.7
CHOROS CAMPING	CHC	Northern	29°15'26	71°27'90	21/02/2007	12	----	13.2	17.4
EL QUISCO	Q	Southern	33°25’27	71°42’31	20/03/2007	24	23	12.5	17.3
LAS CRUCES	LCR	Southern	33°30'09	71°38'01	20/03/2007	27	25	12.4	16.7
VALDIVIA	V	Southern	39°46'75	73°23'49	20/03/2007	18	23	11.0	15.2

Table 2. Effect of the temperature on the sex ratio in the two species: two-way factorial ANOVA (temperature and species fixed factors). M.S.: mean squares, d.f.: degree of freedom.

Source	d.f.	MS	F	P value
Temperature	1	0.000104	0.03	0.863
Species	1	0.011195	3.20	0.075
Species x Temperature	1	0.028814	8.22	0.005
Error	234	0.003503		

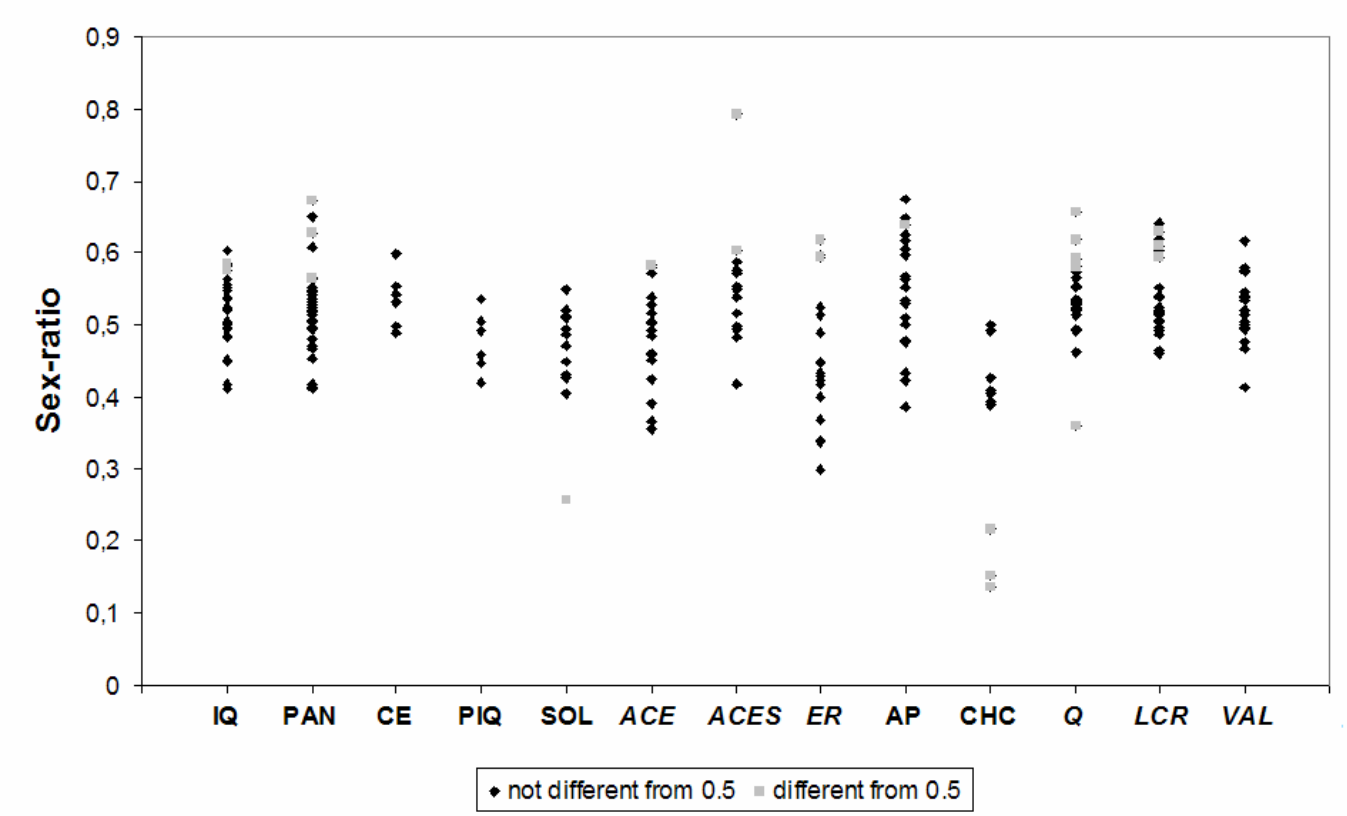


Figure 1. Oppliger et al.

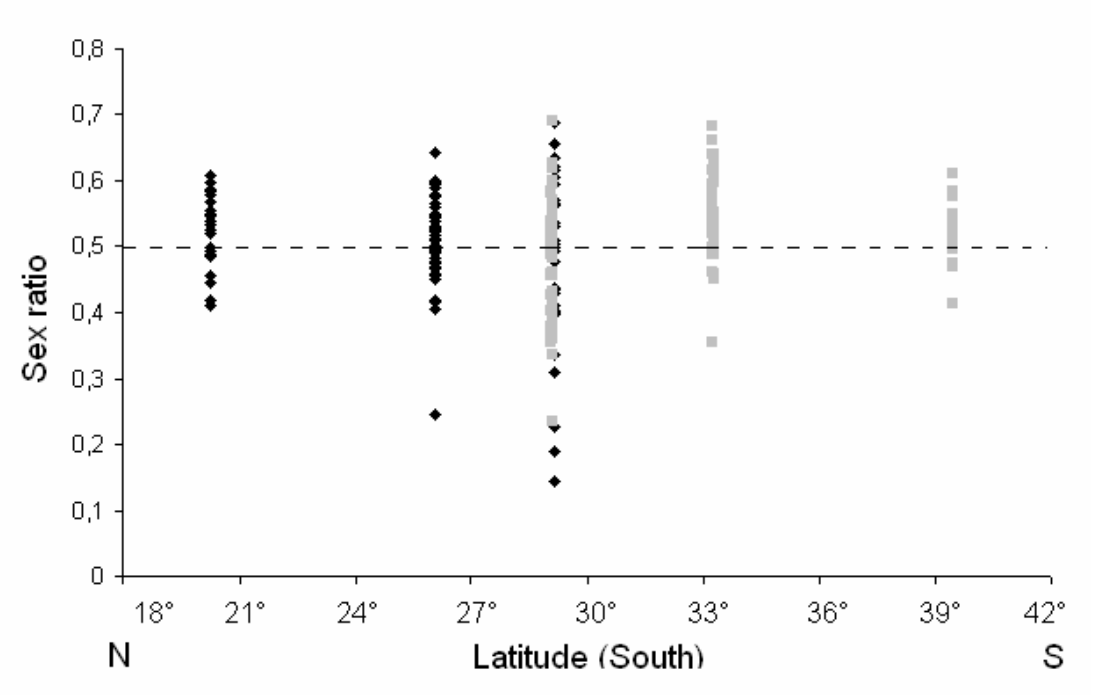


Figure 2. Oppliger et al.

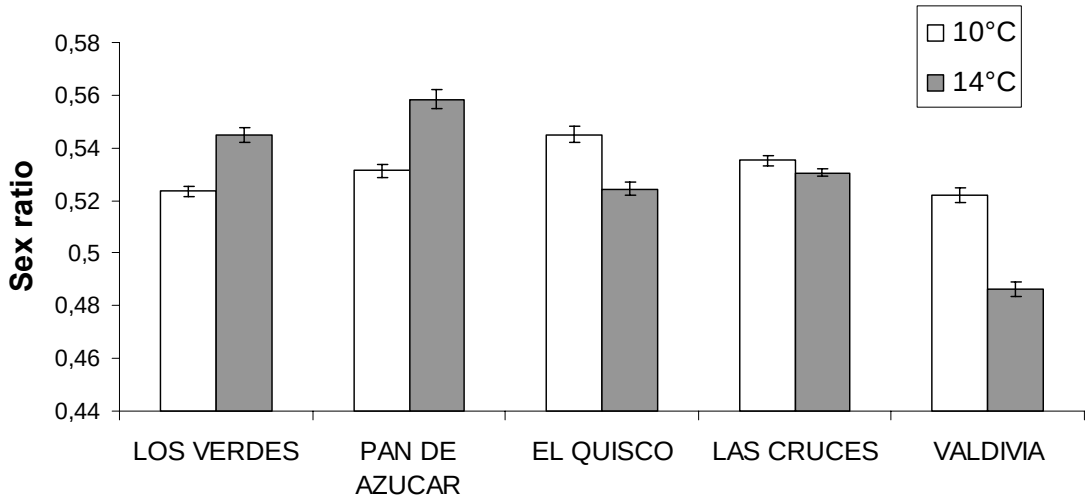


Figure 3. Oppliger et al.

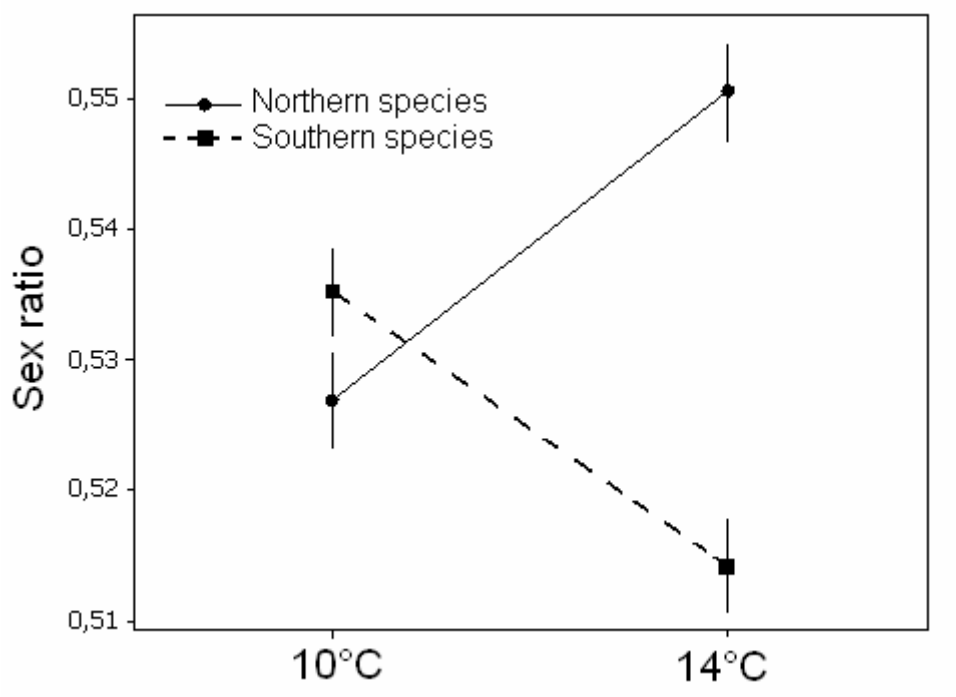


Figure 4. Oppliger et al.

Figure captions

Figure 1. Sex ratio of *Lessonia nigrescens* gametophytes using laboratory cultures of spores from sporophytes collected in 7 populations from the Northern cryptic species and 6 populations from the Southern cryptic species (italics).

Figure 2. Effect of latitude on *Lessonia nigrescens* sex ratio using laboratory cultures of spores from sporophytes collected in 13 populations. Each symbol represents one progeny. Northern species are represented in black symbols and Southern species in gray symbols.

Figure 3. Effect of temperature on *Lessonia nigrescens* sex ratio using laboratory cultures of spores from sporophytes collected in 5 selected populations. Bars represent mean values and dispersal corresponds to 1 SE.

Figure 4. Interaction plot (fitted means) for frequency of male gametophytes in the two species of *Lessonia nigrescens* for the two temperatures. Temperature and species are the interactive factors.

REFERENCES

Bartsch, I., Wiencke, C., Bischof, K., Buchholz, C., Buck, B. H., Eggert, A., Feuerpfeil, P., Hanelt, D., Jacobsen, S., Karez, R., Karsten, U., Molis, M., Roleda, M., Schubert, H., Schumann, R., Valentin, K., Weinberger, F. & Wiese, J. 2008. The genus *Laminaria sensu lato*: recent insights and developments. *Eur. J. Phycol*. 43:1-86.

Blaustein, A. R. & Wake, D. B. 1990. Declining amphibian populations: a global phenomenon? *Trends Ecol. Evol.* 5:203-204.

Bull, J. J. 1981. Evolution of environmental sex determination from genotypic sex determination. *Heredity* 47:173-184.

Bull, J. J. & Charnov, E. L. 1988. How fundamentals are Fisherian sex ratios?. *Oxf. Surv. Evol. Biol.* 5:96-135.

Camus, P. 2001. Biogeografía marina de Chile continental. *Rev. Chil. Hist. Nat.* 74:587-617.

Casey, K. S. & Cornillon, P. 1999. A comparison of satellite and in situ-based sea surface temperature climatologies. *J. Climate* 12:1848-1863.

Castilla, J. C. & Camus, P. A. 1992. The Humboldt-El-Nino Scenario - Coastal Benthic Resources and Anthropogenic Influences, with Particular Reference to the 1982/83 Enso. *South Afr. J. Mar. Sci.* 12:703-712.

Charlesworth, D., Charlesworth, B. & Marais, G. 2005. Steps in the evolution of heteromorphic sex chromosomes. *Heredity* 95:118-128.

Charnov, E. L.& Bull, J. J. 1977. When is sex environmentally determined?. *Nature* 266:828–830.

Correa, J., Nielsen, R. & Grund, D. W. 1988. Endophytic algae of *Chondrus crispus* (Rhodophyta). II. *Achrochaete heteroclada* sp.nv., and *Phaeophila dendroides* (Chlorophyta). *J. Phycol.* 24:528-539.

Cosson, J. 1978. *Recherches morphogenetiques et ecophysiologiques sur la pheophycee Laminaria digitata* (*L.) Lamouroux*. Ph.D. Thesis, Université de Caen, Caen, 203 pp.

De Jong, T. J. & Klinkhamer, P. G. L. 2002. Sex ratios in dioecious plants. *In* Hardy, I. C. W. [Ed.] *Sex Ratios: Concepts and Research Methods*. Cambridge University Press, Cambridge, UK, pp. 349-364.

Delph, L.F. 1999. Sexual dimorphism in life history. *In*: Gender and sexual dimorphism in flowering plants. Geber, M. A., Dawson, T. E. & Delph, L. F. [Eds.] Springer-Verlag, Berlin, Germany, pp. 149-173.

Duckett, J. G. 1977. Towards an understanding of sex determination in *Equisetum*: an analysis of regeneration in gametophytes of the subgenus *Equisetum*. *Bot. J. Linn. Soc*. 74:215-242.

Eckert, C. G. 2002. The loss of sex in clonal plants. *Evol .Ecol.* 15: 501-520.

Engel, C. R., Billard, E., Voisin, M. & Viard, F. 2008. Conservation and polymorphism of mitochondrial intergenic sequences in brown algae. *Eur. J. Phycol.* 43:195-205.

Evans, L. 1963. A large chromosome in the laminarian nucleus. *Nature* 198:215.

Evans, L. 1965. Cytological studies in the Laminariales. *Ann. Botany* 116:541-562.

Funano, T. 1983. The ecology of *Laminaria religiosa* Miyabe. I. The life history and alternation of nuclear phases of *Laminaria religiosa*, and the physiological ecology of the gametophytes and the embryonal sporophytes. *Hokusui-Shiho* 25:61-109.

Guillon, J-M. & Fievet, D. 2003. Environmental sex determination in response to light and biased sex ratios in Equisetum gametophytes. *J. Ecol.* 91:49-57.

Hersteinsson, P. & Macdonald, D. W. 1992. Interspecific competition and the geographical distribution of red and arctic foxes *Vulpes vulpes* and *Alopex lagopus*. *Oikos* 64:505-515.

Kawecki, T. J. 2008. Adaptation to marginal habitats. *Annu. Rev. Ecol. Syst.* 39: 321-342.

Kraak, S. B. M. & Pen, I. 2002. Sex-determining mechanisms in vertebrates. *In* Hardy, I. C. W. [Ed.] *Sex Ratios: Concepts and Research Methods*. Cambridge University Press, Cambridge, UK, pp. 158-177.

Lee, J. A. & Brinkhuis, B. H. 1988. Seasonal light and temperature interaction effects on development of Laminaria saccharina (Phaeophyta) gametophytes and juvenile sporophytes. *J. Phycol*. 24:181–191.

Martínez, E. A. 1999. Latitudinal differences in thermal tolerance among microscopic sporophytes of the kelp *Lessonia nigrescens* (Phaeophyta: Laminariales). *Pac. Sci.* 53:74-81.

Martínez, E. A., Cárdenas, L. & Pinto, R. 2003. Recovery and genetic diversity of the intertidal kelp *Lessonia nigrescens* (Phaeophyceae) 20 years after El Niño 1982/83. *J. Phycol*. 39:504-508.

Müller, D. 1967. Generationswechsel, Kernphasenwechsel und Sexualität der Braunalgae *Ectocarpus siliculosus* im kulturversuch. *Planta Berl.* 75:39-54.

Nakamura, M. 2009. Sex determination in amphibians. *Sem. Cell. Dev. Biol.* 20:271-282.

Nelson, W. A. 2005. Life history and growth in culture of the endemic New Zealand kelp *Lessonia variegata* J. Agardh in response to differing regimes of temperature, photoperiod and light. *J. Appl. Phycol.* 17:23-28.

Noyes, R. D. 2005. Inheritance of apomeiosis (diplospory) in fleabanes (*Erigeron*, Asteraceae). *Heredity* 94:193-198.

Oppliger, L. V., Peters, A. & Correa, J. A. 2007. Parthenogenesis in the brown algae *Lessonia nigrescens* (Phaeophyceae) from Central Chile. *J. Phycol.* 43:1295-1301.

Parasnis, A. S., Ramakrishna, W., Chowdari, K. V., Gupta, V. S. & Ranjekar, P. K. 1999. Microsatelite (GATA)(n) reveals sex-specific differences in Papaya. *Theor. Appl. Genet.* 99:1047-1052.

Parmesan, C., Ryrholm, N., Stefanescus, C., Hill, J. K., Thomas, C. D., Descimon, H., Huntley, B., Kaila, L., Killberg, J., Tammaru, T., Tenent, W. J., Thomas, J. A. & Warren, M. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* 399:579-583.

Sarre, S. 2006. Getting the temperature right for sex. *Heredity* 96:273-274.

Sauvageau, C. 1918. Recherches sur les Laminaires des côtes de France. *Mém. Acad. Sci. Paris.* 56:1-240.

Schreiber, E. 1930. Untersuchungen über Parthenogenesis, Geschlechtsbestimmung und Bastardisierungsvermögen bei Laminarien. *Planta* 12:331-353.

Tanurdzic, M. & Banks, J. A. 2004. Sex-Determining Mechanisms in land Plants. *Plant Cell* 16:61-71.

Tellier, F., Meynard, A. P., Correa, J. A., Faugeron, S. & Valero, M. 2009. Phylogeographic analyses of the 30°S south-east Pacific biogeographic transition zone establish the occurence of a sharp genetic discontinuity in the kelp *Lessonia nigrescens*: vicariance or parapatry? *Mol.Phyl.Evol. In press, doi:10.1016/j.ympev.2009.07.030.*

Thomas, C. D. & Lennon, J. J. 1999. Birds extend their ranges north-wards. *Nature* 399:213.

Uller, T., Pen, I., Wapstra, E., Beukeboom, L. W. & Komdeur, J. 2007. The evolution of sex ratios and sex-determining systems. *Trends Ecol. Evol.* 22:292-297.

Voisin, M., Engel, C. & Viard, F. 2005. Differential shuffling of native genetic diversity across introduced regions in a brown alga: aquaculture vs. maritime traffic effects. *Proc. Natl. Acad. Sci. U.S.A.* 102:5432-5437.

Yang, G., Sun, Y., Shi, Y., Zhang, L., Guo, S., Li, B., Li, X., Li, Z.,Cong, Y., Zhao, Y. & Wang ,W. 2009. Construction and characterization of a tentative amplified fragment length polymorphism; simple sequence repeat linkage map of *Laminaria* (Laminariales, Phaeophyta). *J. Phycol.* 45(4):873-878.

Yasui, H. 1992. Chromosome numbers and a sex chromosome of *Laminaria yendoana* Miyabe (Phaeophyta). *Nippon Suis. Gak.* 58:1385.

Zaborski, P., Dorizzi, M. & Pieau, C. 1988. Temperature-dependent gonadal differentiation in the turtle *Emys orbicularis*: concordance between sexual phenotype and serological H-Y antigen expression at threshold temperature. *Differentiation* 38:17-20.

CHAPTER 2

TEMPERATURE EFFECTS ON LIFE-HISTORY TRAITS AND GEOGRAPHIC DISTRIBUTION OF THE *LESSONIA NIGRESCENS* COMPLEX

(Article in preparation, to be submitted to Ecology)

L. Valeria Oppliger[1,2,3], Florence Tellier[1,2], Aschwin H. Engelen[4], Myriam Valero[3], Juan A. Correa[1], Sylvain Faugeron[1], Paula Ayerdi[1], Gonzalo Gomez[1], Vasco Vieira[4] and Christophe Destombe [2]

(1) Departamento de Ecología, Center for Advanced Studies in Ecology and Biodiversity, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Post-code 6513677, Santiago, Chile.
(2) UPMC Univ. Paris 06, UMR 7144, Equipe "BEDIM", Station Biologique de Roscoff, 29682 Roscoff, France.
(3) CNRS, UMR 7144, Station Biologique de Roscoff, Place Georges Teissier, 29682 Roscoff, France.
(4) CCMAR, CIMAR-Laboratório Associado, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

ABSTRACT

Temperature has long been shown to influence species distributions, and the range of a species often corresponds to the geographic extent of temperature regimes the organism can physiologically tolerate. Temperature can affect differentially stages in species with multiple life-history stages. In this study, the role of mid to high temperature was studied in the kelp species complex *Lessonia nigrescens,* recently identified as two cryptic species occurring along the Chilean coast: one located north and the other south of a biogeographic boundary at latitude 29-30°S. *L. nigrescens* is characterized by an alternation of microscopic haploid gametophytes and large macroscopic fronds of diploid sporophytes. Six life history traits from microscopic stages were identified and estimated under five treatments of temperature in eight populations distributed along the Chilean coast in order to (1) estimate the role of temperature in the present distribution of the *Lessonia nigrescens* species complex, (2) compare marginal populations to central populations of the two cryptic species. In addition, we developped a periodic matrix model to estimate the population growth rate (λ) at the five temperature treatments. Our results demonstrate that there is differential tolerance to temperature in the two species, with the gametophytes of the Northern species being more tolerant to higher temperatures than gametophytes from the south. Second, the two species exhibit different life history strategies with a shorter haploid phase in the Northern species contrasted with considerable frequency of haploid vegetative growth in the Southern species. Third, marginal populations of both species showed marked differences from central populations of the same species. These results provide strong ecological evidence for the differentiation process of the two cryptic species and show local adaptation cases at the range limits of the distribution and have ecological and evolutionary implications.

INTRODUCTION

Understanding the mechanisms that limit geographical distributions of species has long been a key question in both ecology and evolutionary biology (Darwin 1859, MacArthur 1972, for review see Sexton et al. 2009) and it is generally accepted that both biotic and abiotic multiple causes can be interacting. Variations of geographic species range resulting from current climatic change have been widely demonstrated (Woodward 1987, Wood and McDonald 1996, McCarty 2001), including, for example, poleward movements of species' range boundaries in fish (Perry et al. 2005), mammals (Hersteinsson and Macdonald 1992), birds (Hill et al. 1999) and butterflies (Dennis 1993). Predictions of range shift are generally based on statistical relationships between the current species distribution and selected environmental variables, the so called "climatic envelope" (Pearson and Dawson 2003). Such predictions, however, may be unrealistic unless information on physiological limitations is taken into account, as it may be the key in constraining the distributions and abundance of organisms. Thus, there is a clear need for improved understanding on how the variation of environmental factors in space and time affect critical fitness components such as survival and reproduction (Kearney and Porter 2009, Sexton et al. 2009).

To study the mechanisms that constrain a species' distribution, a highly informative zone is the edge of the range itself (Brown et al. 1996, Holt and Keitt 2005, Sagarin et al. 2006), particularly to study evolutionary processes. First, because marginal populations tend to occur in patches, genetic drift is expected to be stronger than in central populations where distribution is continuous (Kawecki 2008). As a consequence, marginal populations are expected to be genetically deprived, and therefore more sensitive to environmental changes in comparison with central populations. Furthermore, when dispersal is too low, even small abiotic changes in space and/or in time may have a large impact on the persistence of these local populations (Kawecki 2008, Sexton et al. 2009). On the other hand, marginal populations may also be a place where local adaptation occurs, due to the particular environmental conditions (Kawecki 2008). However, the occurrence of this process depends on the relative genetic isolation of the marginal populations and on the dispersal capacity of the species.

Temperature is considered as the most important factor determining the geographic distribution of numerous species, as it affects survival, reproduction and/or growth (Hutchins 1947). This is particularly true for benthic marine macroalgae (Breeman 1988, Pakker and Breeman 1996, Adey and Steneck 2001). Kelps (Laminariales, Phaeophyceae) are cold-temperate species occurring from polar to inter-tropical zones that play a major ecological role by structuring the ecosystem and are commercially exploited for alginate extraction

(Steneck et al. 2002, Vásquez 2008). At low latitudes, their range edge is generally determined by warm temperatures and nutrients (see for review Steneck et al. 2002). To understand the mechanisms that define the range limit, a quantitative estimate of multiple fitness components across the life cycle is necessary (Purves 2009). Because of their complex life cycle, kelps are a challenging model: these species display a heteromorphic life history with an alternation of microscopic haploid gametophytes and diploid sporophytes (Sauvageau 1918). Therefore, in geographic range studies, there is a need to integrate the survival and reproduction of both phases (Schiel and Foster 2006). Thermal responses of macroscopic sporophytes and microscopic stages are generally consistent with the geographical distribution of species and strongly dependent of the species studied (macroscopic stage: Bolton and Lüning 1982, Breeman 1988; microscopic stages: Lüning and Müller 1978, Muñoz et al. 2004, Schiel and Foster 2006; for review see Bartsch et al. 2008 and references therein). However, the number of studies that have addressed the temperature responses of kelps across their entire geographic range is limited and, when available, these studies only include sporophytes (Matson and Edwards 2007).

The South Eastern Pacific temperate coast is particularly interesting to study the effects of temperature fluctuation on kelps, because Sea Surface Temperature (SST) shows a complex pattern of both spatial and temporal variability. While a general trend of increasing temperatures with decreasing latitude is described in the Humboldt Current System (Fernandez et al. 2000, Thiel et al. 2007), a patchy structure of thermal conditions along the coast is created by upwelling centers where cold, nutrient-rich subsurface waters are upwelled by equatorward winds (for review see Thiel et al. 2007). In addition, temporal fluctuations of SST occur: (i) at interannual scale due predominantly to El Niño Southern Oscillation (ENSO) events, (ii) at the seasonal scale, and (iii) at the synoptic scale (several days), the scale of variation in upwelling conditions (Faugeron et al. 2009, Tapia et al. 2009, Castillo in prep.). In comparison to southern regions, the northern part of the Humboldt Current System is much more affected by ENSO events (Thiel et al. 2007). At a smaller scale, the marine biogeographic transition zone described around 30°S of latitude in the Chilean coast seems to have unpredictable but high temperature fluctuations at inter-annual scales at the north of 30°S, in contrast to predictable and limited temperature fluctuations at intra-annual scales south of 30°S (Faugeron et al. 2009, Tapia et al. 2009, Castillo in prep.). Consequently, fluctuating temperatures and their duration of exposure to temperature must be considered along with mean temperatures present in the coast.

We chose to study two cryptic species of *Lessonia nigrescens* distributed along the Chilean coast. These two kelp species have contrasting geographic ranges: the 'Northern species' occurs between 16°S and 30°S and the 'Southern species' stretches between 29°S

and 41°S (Fig. 1)(Tellier et al. 2009). Previous studies have shown that these two species are reproductively isolated (Faugeron et al. 2009, Oppliger et al. 2010 in press) and were never found co-existing in the same location, even within the transition zone between the two range distributions (from 29°S to 30°14'S), where a mosaic of pure populations either of the Northern or Southern species was observed (Tellier et al. 2009, Oppliger et al. 2010 in press). Because of the contrasting distribution ranges, the species are differentially exposed to environmental disturbances. For example, during the El Niño event of 1982/83, a massive mortality affected individuals from the northernmost populations of the Northern species (Tomicic 1985), although some populations survived, such as in Iquique (20°S, Soto 1985). It was hypothesized that this survival could have been the result of local adaptation to high temperatures (Martínez 1999).

Using these two cryptic species of the *L. nigrescens* complex, we aimed to test the following hypotheses regarding the tolerance to temperature of microscopic stages: (i) the Northern species is expected to be more tolerant to high temperature than the Southern species, and (ii) local adaptation within each species is expected as a differential thermal tolerance among populations across geographic range. Particularly, we expected that marginal populations, located in the transition zone, would present singular responses to temperature.

MATERIAL AND METHODS

Site characterization and sampling.

Fragments of 15 fertile sporophytic thalli were sampled in eight locations, three occupied by the Northern species and five by the Southern species. Sampling covered the Chilean coast from 20°25'S (Iquique) to 39°46'S (Valdivia, Fig. 1 & Table 1) during April 2008. Sampling effort was especially focused near and within the transition zone (28-31°S) where both cryptic species are found in neighboring patches. Locations in this area were considered as marginal habitat because they are on the overlapping transition zone of the two cryptic species, which also corresponds to the geographical range margin of each species. SST were estimated from long time survey data (25 years, 1982-2007) of the Advanced Very-High Resolution Radiometer (AVHRR) satellite (Casey and Cornillon 1999). Raw SST corresponded to the 7-d temperature record at each site interpolated to monthly resolution. At the spatial scale of resolution (4 km), all sampling sites were located in different pixels. SST ranged from 11.0°C (monthly mean minima) to 15.2°C (monthly mean

maxima in Valdivia) and from 16.2°C to 23.0°C in Iquique. Temperature in the transition zone ranged from 13.2 to 17.9°C.

Experimental design and spore release.

The 15 sporophytic individuals from each location were grouped in three sub-sets of five individuals each. This grouping strategy was used to diminish the effect of individual variation within location. The mature fragments of thalli were rinsed with running tap water and sterile seawater, immediately after collection to induce spore release. For each subset of individuals and each treatment condition, we initiated cultures in two replicate 50mL Falcon tubes (BD Biosciences, San Jose, CA, USA), each containing one mature fragment of equal size (1.9 cm^2) of each of the five individuals of the subset, a marked glass slide and sterile seawater.

Culture conditions.

Tubes with mature tissue were carried immediately to the laboratory in darkness and low temperature (ca 10°C). After 12 to 24 hours of incubation, spores were settled on the glass slides and sterile seawater was replaced with SFC enriched seawater (Correa et al. 1988). Falcon tubes were placed horizontally in culture chambers (12:12 light:dark, 25-35 $\mu mol \cdot photons \cdot m^{-2} \cdot s^{-1}$) under each temperature treatment, and culture medium was changed every five days. The five temperature treatments were chosen to include the temperature range experienced by the species in natural conditions (see above), with three fixed temperature conditions: 10°C, 15°C and 20°C. In addition, to consider the temporal variation of the environmental conditions, we included two variable temperature regimes: 10-15°C and 15-20°C, where the culture temperature was changed every three days (initiating the culture at the lowest temperature, i.e. 10°C and 15°C respectively). This protocol resulted in a total of 240 culture tubes: 5 temperature treatments applied to gametophytes from 8 locations, each composed of 3 individual subsets, with two replicate tubes.

At the beginning of the experiment, a total of 46,303 spores were counted: 30,847 spores from central populations (11,258 and 19,589 spores from Northern and Southern species respectively) and 15,456 from marginal populations (2,124 and 13,332 spores from Northern and Southern species respectively).

Characteristics of the life cycle and criteria for life history stages determination

The genus *Lessonia* exhibits a heteromorphic haploid-diploid life cycle with an alternation of microscopic haploid dioecious gametophytes and macroscopic diploid sporophytes (up to >6 m long) that produce haploid spores by meiosis. Male and female gametophytes show clear sexual dimorphism. Female gametophytes produce eggs that, after fertilization by sperms, produce new diploid sporophytes. Previous studies have shown that the dispersal capacity of the two cryptic species is limited (Parada 2001, Camus et al. 2005, Oppliger et al. 2010 in press).

Seven microscopic life-history stages were defined (Fig. 2), using a combination of developmental characteristics and reproductive structures of the gametophytes (Sauvageau 1918, Hoffman and Santelices 1982): 1) settled meiospores, 2) germinated spores, identified by the formation of a protuberance that becomes a germination tube, 3) immature female gametophytes of 1-2 cells, 4) immature female gametophytes > 2 cells, 5) mature female gametophytes bearing oogonia (female fecundity), 6) fertilised female gametophytes bearing microscopic sporophytes (female fertility), 7) male gametophytes. Since formation of oogonia has been observed in one-celled, two-celled and multicellular gametophytes of *Lessonia* (Hoffman and Santelices 1982), we distinguished two types of female gametophytes: 1-2 cells and >2 cells, to estimate the effect of culture conditions on the frequency of vegetative growth before reaching maturity. Male and female gametophytes were unambiguously identified because of their sexual dimorphism: male gametophytes are narrower than female gametophytes and display highly branched filaments formed by small cells (Fig. 2). Only well-colored individuals (gametophytes and sporophytes) were considered; bleached individuals were considered dead (Contreras et al. 2007).

Life history traits estimations.

Observations were made on days 2, 5, 13, 15, 17, 24, 25 of incubation. All life history stages (as previously defined, see also Fig. 2) were counted on three marked visual fields per slide using a Nikon Eclipse TE300 inverted microscope (Nikon Corp., Tokyo, Japan) using a 10X objective, except for the germination estimates (20X objective). For each observation date, survivorship was estimated by counting the number of well-pigmented gametophytes over the initial count (day 2 of culture) on the same area. The germination rate was estimated at day 2 and 5 of culture by counting the number of germinated spores over the total number of spores alive. At days 13, 17 and 24 of culture, the frequencies of female gametophytes of 1-2 cells and of female gametophytes of >2 cells were determined to

characterize vegetative development. Sex ratio was estimated after 15 days in culture and was expressed as the frequency of males, i.e. males/(males+females). Finally, reproduction was estimated at days 13, 17 and 24 of culture: 1) the frequency of mature females was estimated over 30 females in each visual field, and 2) female fecundity was estimated indirectly using the frequency of females bearing juvenile sporophytes in each visual field.

Statistical analyses.

The response variables were the life history estimates: rates of germination, survival, vegetative development, sex ratio, female maturity and female fecundity. Data was transformed (arc sin transformated, as recommended for proportion data, (Sokal and Rohlf 1981)) and examined for normality and homogeneity of variances . All statistical analyses were done with MINITAB version 13.2 (State College, PA, USA), by performing general linear models and Tukey's tests for *a posteriori* multiple comparisons.
Hypothesis 1 (comparison between species and between temperature regimes) was tested through comparison of central populations of both species, i.e. the populations located within the continuous range of each species (2 locations for Northern species and 3 locations for Southern species, Fig. 1). The fixed model used was: species, treatment and treatment*species. Also λ comparisons between species for each treatment of temperature were realized with Kruskal-Wallis tests. Hypothesis 2 (comparison between marginal and central populations within species) was tested separately for each species (Northern species: 2 central vs 1 marginal location, Southern species: 2 marginal vs 3 central locations, Fig. 1). The fixed model used was: marginality, treatment and marginality*treatment. Also λ comparisons between marginal and central populations within species for each treatment of temperature were realized with Kruskal-Wallis tests.

Periodic matrix model

The matrix model was structured using the six stage classes as previously defined, but without the male gametophytes for stages 5 and 6 because of difficulties in recognizing male reproductive structures (Fig. 2). As a consequence we assume that male densities were always sufficient to fertilise all oogonia produced. Transition probabilities among stage classes were estimated by calculating the proportion of individuals of each stage that transited to another stage for selected observation intervals, resulting in periodic matrices for each census intervals (days 0-2, 2-5, 5-13, 13-17 and 17-24). As the purpose of the experiment was to elucidate temperature dependent effects on the development of

microscopic stages, the entire sporophytic diploid part of the life cycle was reduced to a fecundity vector describing the spore production of each microscopic stage over its entire life: [0 1 10 100 1,000 10,000]. It was necessary to assign a fecundity vector to all microstages as in some combinations of locations x temperatures gametophytes did not all reach the microscopic sporophyte phase by the end of the experiment.

For each location and temperature treatment, the population dynamics was described by a non-linear projection matrix **A**, where the element a_{ij} represents the probability of transition from stage *j* to stage *i* over one census period. The growth of each population was projected by multiplying the transition matrix with a column vector $\mathbf{n}(t)$, which includes the number of individuals in each stage class at time *t*:

$$\mathbf{n}_{t+1} = \mathbf{A}_n * \mathbf{n}(t) \qquad \text{eqn 1}$$

The dynamics of each population over the cycle was described by the periodic matrix produced by multiplying all matrices (**B**) of a location x temperature combination, sequentially (Caswell 2001, Engelen and Santos 2009):

$$\mathbf{n}_{(t+1)} = [\mathbf{B}_{(m)}\mathbf{B}_{(m-1)} \ldots \mathbf{B}_{(h)}]n_{(t)}$$

$$\mathbf{n}_{(t+1)} = \mathbf{A}_{(h)}\mathbf{n}_{(t)} \qquad \text{eqn 2}$$

where the periodic cycle starts at period *h* and ends at period *m*.

In this way, for each combination of location and temperature, a model was created which consisted of a data set of five matrices (one for each observation interval) containing transitions. The population growth rate, or population fitness, was calculated as the dominant eigen value (λ) of the product of each set of matrices. Due to the cyclic arrangement λ is the same for all census periods.

$$\ln \lambda_s(i) = \ln \mathbf{n}(i+1) \ldots \ln \mathbf{n}(i) \qquad \text{eqn 3}$$

We estimated the population growth rate for each population * treatment condition. Uncertainties in the population growth rate were estimated from bootstrap confidence intervals (95%) by using the percentiles of the distribution of 1,000 bootstrap estimates. No bias adjustment or bias estimation was implemented, because these only reduce certain kinds of bias while greatly reducing the precision of the resulting estimates (Efron and Tibshirani 1993). Significant differences between pairwise combinations of population * treatment and time were tested using two-tailed t-test with unequal variances using the last 100 λ estimates from the 1,000 bootstrap estimates.

Using non-parametric tests (Kruskal-Wallis), we tested for differences of population growth (λ) (i) among species, for each temperature treatment, and (ii) among central and marginal populations for each temperature treatment and each species.

Elasticities identify the most vulnerable or important transitions of a life history, that is the transitions which have a greater effect on the population fitness (or growth rate, (de Kroon

et al. 1986, Mills et al. 1999)). The elasticity values of each observation interval were estimated by calculating 5 periodic **A**P(*h*)P matrices, for the entire cycle beginning in each observation interval (Caswell and Trevisan 1994). Since elasticities sum to one, each elasticity value may also be interpreted as the contribution of each matrix element to the population fitness (de Kroon et al. 1986, Caswell 2001). Thus, elasticities may be summed across selected regions of a matrix, corresponding to different demographic stages or processes. In this case it was used to asses the relative importance of each gametophyte stage.

RESULTS

Germination (Fig. 3a) of the central populations of the Northern species (hereafter referred to as the "Northern species") was high at all temperatures whereas germination of the central populations of the Southern species (hereafter referred to as "Southern species") was significantly lower than the Norththern species (results of 2-way ANOVA given in Table 2, *p*-value threshold of post-hoc test: 0.05). Germination of the marginal populations of the Southern species (hereafter referred to as "Marginal Southern populations") was lower than the Southern species at all but the 20˚C temperature treatment, and increased with temperature. No clear trend in germination *versus* temperature was seen for the Marginal Northern populations.

Survival of the Southern species was low (<0.3) at the 20˚C treatment, and was high (≤0.7) at 10˚, 10-15˚, and 15˚C treatments (Fig. 3b). In contrast, survival of the Northern species was consistently high (≥0.8) at all but the lowest temperature treatment (10˚C), where it dropped below 0.6. In contrast to the Southern species central populations, the Marginal Southern populations maintained high survival (≥0.7) at all temperature treatments. No clear trend was seen between survivorship and temperature for the Marginal Northern population.

Sex ratio (Fig. 3c) was slightly less than 0.5 at all temperatures for all but for the Southern species. In the Southern species, the sex ratio dropped to 0.22 (± 0.04) in the 20˚C treatment (representing either an over-production of female gametophytes, or a diminished survival of male relative to female gametophytes under these conditions). This effect was judged to be significant and was not exhibited by the Marginal Southern populations (Table 2 for 2-way ANOVA results, post-hoc threshold at 0.05).

The frequency of vegetative growth of the Southern species was highest (>0.37) at 15-20° and 20°C but was low (≤0.16) at the other treatments (Fig. 3d). The most striking finding was that the Northern species showed very little frequency of vegetative growth at all but the highest temperature treatment. At 10°, 10-15°, and 15°C the Northern species exhibited frequencies of vegetative growth consistently <0.05, reaching 0.10 ± 0.03 at 15-20°C. At 20°C, it was (0.34 ± 0.02) nearly the same as that of the Southern species. In contrast, the Marginal Southern populations showed a high frequency of vegetative development (near 0.4) at all temperature treatments. The Marginal Northern populations showed an inconsistent pattern of vegetative development with temperature, showing moderately high vegetative development (close to 0.3) both at low (10° and 10-15°C) treatments and at the highest temperature (20°C) but dropping to 0 (15-20°C) or near 0 (at 15°C).

The frequencies of female maturity (Fig. 3e) and female fertility (Fig. 3f) were dramatically diminished in all populations at the higher temperature treatments tested (15-20° and 20°C). Only the Northern species was able to produce mature females at 20°C, although at greatly reduced frequencies (0.09 ± 0.03 for female maturity, 0.03 ± 0.01 for female fertility). Over all temperatures tested, the Southern species showed lower rates of female maturity and female fertility than the Northern species (post-hoc tests at α=0.05). The Marginal Southern population exhibited consistently the lowest frequencies of female maturity and female fertility. Likewise, the Marginal Northern population exhibited consistently lower frequencies of female maturity and female fertility than the Northern species central populations.

The population growth rate calculated for the Southern species was consistently ca. 2-fold greater than that of the Northern species for all but the highest temperature treatments tested, where it dropped to nearly 0 (Fig. 4). In contrast, the population growth rate calculated for the Northern species did not show as strong variation with temperature, remaining moderate (≤0.39) at all treatments and reaching a maximum of 0.79 ±0.07 at 15°C. The Marginal Southern population consistently exhibited a substantially lower population growth rate than the Southern species central populations at all but the highest temperatures. At 15-20°C, the Marginal Southern populations exhibited a population growth rate approximately double than that of the Southern species central populations, while at 20°C growth rates were near 0 for both. The Marginal Northern population exhibited population growth rates approximately 3-fold greater than the Northern species central populations at 10°C and 10-15°C treatments, but were much lower than the Northern species central populations at 15-20°C and dropped to 0 at 20°C, a temperature at which

the Northern species central populations still exhibited moderate population growth rate of 0.49 ± 0.07.

Elasticity analyses revealed that stages 1 and 2 (spore and germination) contributed the most to the fitness of all populations (data not shown). In more advanced stages (stage 3 to stage 6) it is possible to observe differences between species (Fig. 1). The Northern species displayed an almost inexistent stage 4 (frequency of vegetative growth) and considerable elasticities for stages 5 and 6 (female fecundity and female fertility) for all tested treatments of temperature (except for 20ºC), whereas the Southern species displayed important elasticities for stages 3 and 4 (frequency of vegetative growth), and less for 5 and 6 (female fecundity and female fertility). These results suggest differential life-history strategies between species. The marginal Northern and Southern populations exhibited considerable contribution of stage 4 compared to their respective central populations.

DISCUSSION

This study demonstrates that the microscopic stages of the two species of the *Lessonia nigrescens* complex show different (i) thermal responses and (ii) life history strategies to cope with temperature extremes. Furthermore, there were differences between central and marginal populations within each species in their responses to temperature, which suggests the occurrence of local adaptation. These three main results are discussed below.

Regarding the differential thermal tolerance observed between the two cryptic species of *L. nigrescens,* our results showed that, in contrast to the Northern species, the Southern species is not able to survive or to reproduce at high temperatures. These results suggest that, as expected, the Northern species is more tolerant to high temperature than the Southern one, and that the geographic distribution of these two species seems to be related to the environmental conditions. Relationship between thermal responses and location of distribution range boundaries have been previously shown in different macroalgal species (Breeman 1988, Peters and Breeman 1993). Van den Hoek (1982) demonstrated a strong association between temperature and seaweed range distribution and suggested that the southern and the northern boundaries of each species were dependent on three main effects: the temperature effect on survival (corresponding to the 'lethal boundary'), on growth ('growth boundary'), and on reproduction ('reproductive boundary'). For example, Van den Hoeck (1982) explained the difference of distribution between the two kelps *Laminaria digitata* and *Saccharina latissima* (as *Laminaria saccharina*) by the fact that *L.*

digitata is limited to the south by the 'southern reproductive boundary' of the gametophyte (corresponding to the 10°C February isotherm), while *S. latissima* could be limited by the 'southern lethal boundary' of the sporophyte (corresponding to the 19°C August isotherm). Our study suggests the occurrence of a clear 'reproductive northern boundary' for the gametophyte of the Southern species that was unable to produce gametes at 20°C. This phenomenon could explain the absence of the Southern species in the Northern region. Temperature could also explain the absence of the Northern species in the Southern area. The Northern species showed the ability to growth and reproduce at low and high temperature treatments, whereas at 10°C, 10-15°C and 15°C treatments the Southern species displayed higher populations growth rate. This might result in a better performance of the Southern species in the south, where it could outcompete the Northern species. These characteristics further suggest that the Northern species came from the southern region of the country. Therefore, according to the hypothesis developed by Breeman (Breeman 1988), the Northern species would have conserved its ancestral capacity to growth and reproduce under low temperatures. Interestingly, our results support the previous phylogenetic study (Faugeron et al. 2009), which also suggested that the Northern species was derived from the Southern one. Thus, the colonization of the Northern region could have resulted from an adaptation to warmer conditions (Faugeron et al. 2009). In conclusion, the clear cut distribution of these two species observed along the Chilean coast can be explained by the response of the gametophytes.

Moreover, the Northern species tolerated fluctuations (treatment 15-20°C) of temperature better than Southern species. For example, the Northern species was able to survive and produce reproductive female gametophytes under fluctuating condition (15-20ºC) whereas the Southern species was able to survive but not reproduce. These results suggest that, in addition to its better tolerance to high temperature, the Northern species showed a better resistance to variations in temperature. Different hypotheses can be proposed to explain this difference: (i) the better resistance of the Northern species could be due to an adaptation to a particular environmental (i.e. temperature) drastic but rare fluctuation (i.e. ENSO events) as compared to the Southern species (Tomicic 1985, Martínez et al. 2003, Thiel et al. 2007), or (ii) the reproduction of the Southern species is inhibited by higher temperatures (20°C) whatever the duration of the exposure.

The Northern and Southern species showed different traits of life history. Gametophytes of the Northern species were generally small and usually consisting of one cell that immediately formes oogonia, while gametophytes of the Southern species exhibited a stronger vegetative development (generally of multiple cells) and then underwent gametogenesis to develop egg cells inside oogonia. Consequently, female gametophytes

from Northern species reproduce rapidly and give very few oogonia per gametophyte, whereas female gametophytes from Southern species delay their reproduction, grow vegetatively to then produce numerous oogonia per individual. These different patterns of development were observed in *L. nigrescens* (Avila et al. 1985) under different culture conditions and were attributed to different reproductive strategies in relation to different seasons. In our study, the results obtained for different populations under the same environmental conditions suggest that this difference of the gametophytic life span correspond to an adaptation to environmental conditions.

The influence of disturbance and risk of changes in climate on the evolution of life span have been previously described in different angiosperms (Till-Bottraud et al. 1990, Hautekèete et al. 2002, Hill et al. 2002, Van Kleunen and Johnson 2007) and support the earlier cited existing theoretical literature (Charlesworth 1980, Young 1981, Reznick et al. 1990, Reznick et al. 1996). For example, Hautekèete et al (2009) suggested that life span of the sea beet (*Beta vulgaris* ssp. *maritima*) may be explained by several climatic factors and by habitat stability. Indeed, in this species, difference of life history traits (life span and age at first reproduction) has been observed between populations. Populations with the longest-lived individuals occurred in the most stable habitats (Northern Europe) whereas populations with the shortest-lived individuals in the most disturbed locations (Southern Europe). In disturbed locations, a strong association between earlier reproduction and a short life span was demonstrated (Hautekèete et al. 2002, Hautekèete et al. 2009). Similarly, in our study, we hypothesized that gametophytes of the Northern species could avoid unpredictable environmental conditions (e.g. ENSO events) by earlier reproduction producing rapidly a larger sporophyte, whereas in the Southern species that are located in a more stable and colder environment, the reproduction could be delayed, favouring a long life span by allowing reproduction when the plant is more vigorous. Consequently, more time can then be spent on juvenile growth, which may be advantageous because larger gametophytes can produce more offspring.

While temperature tolerance has commonly been regarded as a conservative trait in many seaweeds (see Lüning 1990), some studies have demonstrated ecotypic genetic variation within species for this trait (e.g. Bolton 1983, Breeman 1988). In our study, differences in temperature tolerance were detected within species, mostly between marginal and central populations. For example, whatever the temperature tested in both species, female maturity and fertility were higher in central than in marginal populations. Similarly, for each species, population growth rates were different between marginal and central populations. Interestingly, similar population growth rates were observed between geographically close populations (i.e. Chañaral de Aceituno and Choros Ventana), suggesting

the occurrence of local adaptation in populations at the range limits (Fig. 1). Ecotypic variation in tolerance to high and/or low temperatures has been shown in kelps. For example, high-temperature tolerance observed in the sporophytes of *Saccharina latissima* (as *Laminaria saccharina*) in the southern marginal population in the North-East Atlantic was interpreted as genetic ecotype in response to high-temperature (Gerard and Du Bois 1988). Similarly, Martinez (1999) pointed out the existence of different ecotypes in *L. nigrescens* by studying thermal tolerance in the early sporophytic progeny from plants of three different Chilean localities: north, central and south. At the higher temperature tested, plants from the central and northern localities had higher survival and growth rates than those from the south. At lower incubation temperatures, the growth trend was opposite. Finally, a detailed study (Hay 1990) demonstrated that *M. pyrifera* does not persist at its geographical limit in New Zealand in areas where maximum summer temperatures exceed 18°-19°C for several days, whereas in southern North America, *M. pyrifera* extends to areas where the sea surface maxima reach 27°C during several days. Also, a temperature ecotype of a sporophyte from Japan has been described (North 1972), which indicates that more southern locations of *M. pyrifera* may also survive temperatures higher than 25°C (Bartsch 1993).

Marginal populations in this study showed a higher diversity of responses compared to central populations (Fig. 1). We can hypothesize that this differential pattern of response could be the result of independent evolutionary processes because of the relative geographic isolation. The low dispersion of these species (Faugeron et al. 2009, Tellier et al. 2009, Oppliger et al. 2010 in press) is an attribute that favors genetic isolation and local adaptation (Kawecki 2008). Theoretically, marginal populations are expected to be more fragmented and more affected by genetic drift processes, leading to a higher differentiation between them than between central populations (Kawecki 2008, Sexton et al. 2009), enabling the evolution of differential temperature responses.

CONCLUSIONS

Our study of the microscopic stages of the two cryptic species of the *Lessonia nigrescens* complex revealed different thermal tolerances between the two species. A strong species-specific response was observed, with the Northern species being more tolerant to high temperatures than the Southern species. These results are congruent with the geographic distribution of the cryptic species, where higher temperature conditions exist in the northern region compared to the southern region of the country. However, this study

comprised only the microscopic stages, and these results can only partially explain the current distribution of the cryptic species. Thus, it is necessary to assess thermal tolerance in sporophytes of both species prior to fully understand the present distribution of the *L. nigrescens* complex.

In addition, the cryptic species showed different reproductive strategies that could be related to the stability of the environment. Shortening of the development period prior to reaching maturity of the gametophytic phase in the Northern species opens new questions about the demographic characteristics of the alternation of generations. In particular, it would be interesting to determine the life history traits of sporophyte for the Northern and the Southern species.

Finally, our results suggest local adaptation in marginal populations (from the range edge). This adaptation is characterized by different thermal tolerances between central and marginal populations of the same species. As about 70% of the harvest of the *L. nigrescens* resources for the alginate industry is done at the range margins of the two cryptic species (29-31°S), the peculiar adaptation observed in these marginal populations would need further study to preserve this adaptive diversity to diverse environmental conditions.

ACKNOWLEDGEMENTS

We deeply thank Bernardo Broitman, Ismael Cáceres, Alejandra González, Ana Lafuente, Pablo Oppliger, Marco Ramírez, Constanza Ruiz, Vincent Tellier and Daniela Thomas for their help during various stages of the research. L.V. Oppliger was supported by a CONICYT-FRENCH EMBASSY PhD grant and F. Tellier by a doctoral grant from the Ministry of Education and Research (MENRT). Additional support from the programs "Collège Doctoral Franco-Chilien" and "ARCUS" of the French Ministry of Foreign Affairs is also acknowledged. This study is part of the research programs FONDAP 1501-0001 6 Program 7, ANR ECOKELP (ANR BDIV 012), Pôle Mer Bretagne, and the Laboratoire International Associé "Dispersal and Adaptation of Marine Species" (LIA DIAMS).

REFERENCES

Adey, W. H., and R. S. Steneck. 2001. Thermogeography over time creates biogeographic regions: a temperature/space/time-integrated model and an abundance-weighted test for benthic marine algae. Journal of Phycology **37**:677-698.

Avila, M., A. J. Hoffman, and B. Santelices. 1985. Interacciones de temperature, densidad de flujo fotonico y fotoperiodo sobre el desarrollo de etapas microscopicas de *Lessonia nigrescens* (Phaeophyta, Laminariales). Revista Chilena de Historia Natural **58**:71-82.

Bartsch, I. 1993. Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. Marine Ecology Progress Series **100**:253-264.

Bartsch, I., C. Wiencke, K. Bischof, C. Buchholz, B. H. Buck, A. Eggert, P. Feuerpfeil, D. Hanelt, S. Jacobsen, R. Karez, U. Karsten, M. Molis, M. y. Roleda, H. Schubert, R. Schumann, K. Valentin, F. Weinberger, and J. Wiese. 2008. The genus *Laminaria sensu lato*: recent insights and developments. European Journal of Phycology **43**:1-86.

Bolton, J. J. 1983. Ecoclinal variation in *Ectocarpus siliculous* (Phaeophyceae) with respect to temperature optima and survival limits. Marine Biology **73**:131-138.

Bolton, J. J., and K. Lüning. 1982. Optimal growth and maximal survival temperatures of Atlantic Laminaria species in culture. Marine Biology **66**:89-94.

Breeman, A. 1988. Relative importance of temperature and other factors in determining geographic boundaries of seaweeds: Experimental and phenological evidence. Helgoland Marine Research **42**:199-241.

Brown, J. H., G. C. Stevens, and D. M. Kaufman. 1996. The geographic range: size, shape, boundaries, and internal structure. Ann. Rev. Ecol. Syst. **27**:597-623.

Camus, C., A. Meynard, S. Faugeron, K. Kogame, and J. Correa. 2005. Differential life history phase expression in two coexisting species of *Scytosiphon* (Phaeophyceae) in northern Chile. Journal of Phycology **41**:931-941.

Casey, K. S., and P. Cornillon. 1999. A comparison of satellite and in situ-based sea surface temperature climatologies. J. Climate **12**:1848-1863.

Castillo, M., Tapia, F., Largier, J., Navarrete, S.A. . in prep. Latitudinal discontinuities in the inner-shelf environment along the eastern Pacific coast.

Caswell, H. 2001. Matrix population models: Construction, Analysis, and Interpretation. Sinauer Associates, Inc., Sunderland, Massachusetts.

Caswell, H., and M. C. Trevisan. 1994. Sensitivity analysis of periodic matrix models. Ecology **75**:1299-1303.

Charlesworth, B. 1980. Evolution of Age-structured populations. Cambridge University Press, Cambridge.

Contreras, L., M. H. Medina, S. Andrade, V. Oppliger, and J. A. Correa. 2007. Effects of copper on early developmental stages of *Lessonia nigrescens* Bory (Phaeophyceae). Environmental Pollution **147**:75-83.

Correa, J., R. Nielsen, and D. W. Grund. 1988. Endophytic algae of *Chondrus crispus* (Rhodophyta). II. *Achrochaete heteroclada* sp.nv., and *Phaeophila dendroides* (Chlorophyta). Journal of Phycology **24**:528-539.

Darwin, C. R. 1859. On the origins of species by means of natural selection, or the preservation of favoured races in the struggle for life. London: John Murray.

de Kroon, H., A. Plaisier, J. van Groenendael, and H. Caswell. 1986. Elasticity: the relative contribution of demographic parameters to population growth rate. Ecology **67**:1427-1431.

Dennis, R. L. H. 1993. Butterflies and climate change. Manchester University Press, New York.

Efron, B., and R. J. Tibshirani. 1993. Introduction to the bootstrap. Monographs on Statistics & Applied Probability, vol. 57, Chapman & Hall/CRC, Taylor & Francis, Inc.

Engelen, A., and R. Santos. 2009. Which demographic traits determine population growth in the invasive brown seaweed *Sargassum muticum*? Journal of Ecology **94**:675-684.

Faugeron, S., D. Veliz, G. Peralta, J. Tapia, F. Tellier, C. Billot, and E. Martinez. 2009. Development and characterization of nine polymorphic microsatellite markers in the Chilean kelp *Lessonia nigrescens*. Molecular Ecology Resources **9**:937-939.

Fernandez, M., E. Jaramillo, P. A. Marquet, C. A. Moreno, S. Navarrete, P. Ojeda, C. R. Valdovinos, and J. A. Vásquez. 2000. Diversity, dynamics and biogeography of Chilean benthic nearshore ecosystems: an overview and guidelines for conservation. Revista Chilena de Historia Natural **73**.

Gerard, V. A., and K. R. Du Bois. 1988. Temperature ecotypes near the southern boundary of the kelp *Laminaria saccharina*. Marine Biology **97**:575-580.

Hautekèete, N. C., Y. Piquot, and H. Van Dijk. 2002. Life span in Beta vulgaris ssp. maritima: the effects of age at first reproduction and disturbance. Journal of Ecology **90**:508-516.

Hautekèete, N. C., H. VanDijk, Y. Piquot, and A. Teriokhin. 2009 Evolutionary optimization of life-history traits in the sea beet *Beta vulgaris* subsp. maritima: Comparing model to data. Acta Oecologica:1-13.

Hay, C. H. 1990. The distribution of Macrocystis (Phaeophyta: Laminariales) as a biological indicator of cool sea surface temperature, with special reference to New Zealand waters. Journal of the Royal Society of New Zealand **20**:313-336.

Hersteinsson, P., and D. W. Macdonald. 1992. Interspecific competition and the geographical distribution of red and arctic foxes *Vulpes vulpes* and *Alopex lagopus*. Oikos **64**:505-515.

Hill, J. K., C. D. Thomas, and B. Huntley. 1999. Climate and habitat availability determine 20th century changes in butterfly's range margin. Proceedings of the Royal Society of London B **266**.

Hill, M. O., D. B. Roy, and K. Thompson. 2002. Hemeroby, urbanity and ruderality: bioindicators of disturbance and human impact. Journal of Applied Phycology **39**:708-720.

Hoffman, A. J., and B. Santelices. 1982. Effects of light intensity and nutrients on gametophytes and gametogenesis of *Lessonia nigrescens* Bory (Phaeophyta). Journal of Experimental Marine Biology and Ecology **60**:77-89.

Holt, R. D., and T. H. Keitt. 2005. Species' borders: a unifying theme in ecology. Oikos **108**:3-6.

Hutchins, L. W. 1947. The bases for temperature zonation in geographical distribution. Ecological Monographs **17**:325-335.

Kawecki, T. J. 2008. Adaptation to marginal habitats. Annual Review of Ecology, Evolution and Systematics **39**:321-342.

Kearney, M., and W. Porter. 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. Ecology Letters **12**:334-350.

Lüning, K. 1990. Seaweeds: Their environment, biogeography and ecophysiology.

Lüning, K., and D. G. Müller. 1978. Chemical interaction in sexual reproduction of several Laminariales: release and attraction of spermatozoids. Z. Pflanzenphysiol. **89**:33-41.

MacArthur, R. H. 1972. Geographical ecology. New York: Harper and Row.

Martínez, E. A. 1999. Latitudinal differences in thermal tolerance among microscopic sporophytes of the kelp *Lessonia nigrescens* (Phaeophyta: Laminariales). Pacific Science **53**:74-81.

Martínez, E. A., L. Cárdenas, and R. Pinto. 2003. Recovery and genetic diversity of the intertidal kelp *Lessonia nigrescens* (Phaeophyceae) 20 years after El Niño 1982/83. Journal of Phycology **39**:504-508.

Matson, P. G., and M. S. Edwards. 2007. Effects of ocean temperature on the southern range limits of two understory kelps, *Pterygophora californica* and *Eisenia arborea* at multiple life-stages. Marine Biology **151**:1941-1949.

McCarty, J. P. 2001. Ecological consequences of recent climate change. Conservation Biology **15**:320-331.

Mills, L. S., D. F. Doak, and M. J. Widson. 1999. The reliability of conservation actions based on elasticity analysis of matrix models. Conservation Biology **13**:815-829.

Muñoz, V., M. C. Hernández-González, A. H. Buschmann, M. H. Graham, and J. A. Vásquez. 2004. Variability in per capita oogonia and sporophyte production from giant kelp gametophytes (*Macrocystis pyrifera*, Phaeophyceae). Revista Chilena de Historia Natural **77**:639-647.

North, W. J. 1972. Observations on populations of *Macrocystis*. Pages 75-92 in I. A. Abott and M. Kurogi, editors. Contributions to the systematics of benthic marine algae of the North Pacific Japanese Society of Phycology, Kobe.

Oppliger, L. V., J. A. Correa, S. Faugeron, F. Tellier, J. Beltran, M. Valero, and C. Destombe. 2010 in press. Sex ratio variation in the *Lessonia nigrescens* complex (Laminariales, Phaeophyceae): effect of latitude, temperature and marginality. Journal of Phycology.

Pakker, H., and A. M. Breeman. 1996. Temperature responses of tropical to warm-temperate Atlantic seaweeds. II. Evidence for ecotypic differentiation in amphi-Atlantic tropical-Mediterranean species. European Journal of Phycology **31**:133-141.

Parada, G. 2001. Factores inhibitorios y facilitadores en el reclutamiento de *Lessonia nigrescens*, Bory 1826.

Pearson, R. G., and T. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Global Ecology and Biogeography **12**:361-371.

Perry, A. L., P. J. Low, J. R. Ellis, and J. D. Reynolds. 2005. Climate change and distribution shifts in marine fishes. Science **308**:1912-1919.

Peters, A. F., and A. M. Breeman. 1993. Temperature tolerance and latitudinal range of brown algae from temperate Pacific South America. Marine Biology **115**:143-150.

Purves, D. W. 2009. The demography of range boundaries versus range cores in eastern US tree species. Proceedings of the Royal Society of London B **276**:1477-1484.

Reznick, D. A., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. Nature **346**:357-359.

Reznick, D. N., M. J. Butler, F. H. Rodd, and P. Ross. 1996. Life-history evolution in Guppies (Poeciliata reticulata): Differential mortality as a mechanism for natural selection. :. Evolution **50**:1651-1660.

Sagarin, R. D., S. D. Gaines, and B. Gaylord. 2006. Moving beyond assumptions to understand abundance distributions across the ranges of species. Trends in Ecology and Evolution **21**:524-530.

Sauvageau, C. 1918. Recherches sur les laminaires des côtes de France. Mémoires de l'Académie des Sciences de France **56**:1-240.

Schiel, D. R., and M. S. Foster. 2006. The population biology of large brown seaweeds: Ecological consequences of multiphase life histories in dynamic coastal environments. Annual Review of Ecology and Systematics **37**:343-372.

Sexton, J. P., P. J. McIntyre, A. L. Angert, and K. J. Rice. 2009. Evolution and Ecology of Species Range Limits. Annual Review of Ecology, Evolution and Systematics **40**:415-436.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry: The principles and practice of statistics in biological research. W. H. Freemand and Company, New York.

Soto, R. 1985. Efectos del fenomeno El Niño 1982-83 en ecosistemas de la I Region. Investigacion Pesquera **31**:196-205.

Steneck, R. S., M. H. Graham, B. J. Bourque, D. Corbett, J. M. Eralndson, J. A. Estes, and M. J. Tegner. 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. Environmental Conservation **29**:436-459.

Tapia, F., S. A. Navarrete, M. Castillo, B. Menge, J. C. Castilla, J. Largier, E. A. Wieters, B. L. Broitman, and J. A. Barth. 2009. Thermal indices of upwelling effects on inner-shelf habitats. Progress in Oceanography **83**:278-287.

Tellier, F., Meynard, A. P., Correa, J. A., Faugeron, S. & Valero, M. 2009. Phylogeographic analyses of the 30°S south-east Pacific biogeographic transition zone establish the occurence of a sharp genetic discontinuity in the kelp *Lessonia nigrescens*: vicariance or parapatry? *Mol.Phyl.Evol. In press, doi:10.1016/j.ympev.2009.07.030.*

Thiel, M., E. C. Macaya, E. Acuña, W. E. Arntz, H. Bastias, K. Brokordt, P. A. Camus, J. C. Castilla, L. R. Castro, M. Cortés, C. P. Dumont, R. Escribano, M. Fernandez, J. A. Gajardo, C. F. Gaymer, I. Gomez, A. E. González, H. E. González, P. A. Haye, J. E. Illanes, J. L. Iriarte, D. A. Lancellotti, G. Luna-Jorquera, C. Luxoro, P. H. Manriquez, V. Marín, P. Muñoz, S. A. Navarrete, E. Perez, E. Poulin, J. Sellanes, H. H. Sepúlveda, W. Stotz, F. Tala, A. Thomas, C. A. Vargas, J. Vásquez, and J. M. A. Vega. 2007. The Humboldt Current System of northern and central Chile. Oceanography and Marine Biology: An Annual Review **45**:195-344.

Till-Bottraud, I., L. Wu, and J. Harding. 1990. Rapid evolution of life history trait in populations of Poa annua. Journal of Evolutionary Biology **3**:205-224.

Tomicic, J. J. 1985. Efectos del fenomeno El Niño 1982/83 en las comunidades litorales de la peninsula de Mejillones. Investigacion Pesquera **32**:209-213.

Van Kleunen, M., and S. D. Johnson. 2007. Effects of Self-Compatibility on the Distribution Range of Invasive European Plants in North America. Conservation Biology **21**:1537-1544.

Vásquez, J. 2008. Production, use and fate of Chilean brown seaweeds: re-sources for a sustainable fishery. Journal of Applied Phycology **20**:457-467.

Wood, C. M., and C. G. McDonald. 1996. Global warming: implications for freshwater and marine fish. Cambridge University Press, New York.

Woodward, F. I. 1987. Climate and plant distribution. Cambridge University Press, New York.

Young, T. P. 1981. A general model od comparative fecundity for semelparous and iteroparous life histories. The American Naturalist **118**:27-36.

Table 1: Names and positions of the sampling locations along the Chilean coast, ordered from north to south. For each location, the corresponding cryptic species of *Lessonia nigrescens* is indicated (Northern and Southern species), as well as the population type (central and marginal). The sampling size was of 15 sporophyte individuals per site.

Location	Abbreviation	Species	Type	Latitude	Longitude	Sampling date
Iquique	IQ	Northern	Central	20°25'62"S	70°12'48"W	05-05-2008
Carrizal Bajo	CA	Northern	Central	28°04'27"S	71°08'36"W	05-05-2008
Chañaral de Aceituno	ACE	Southern	Marginal	29°04'03"S	71°29'26"W	03-06-2008
Choros Ventana	CH	Northern	Marginal	29°12'57"S	71°28'23"W	03-06-2008
Coquimbo-Cruz	COQ	Southern	Marginal	29°57'15"S	71°21'44"W	15-06-2008
Rio Limari	LI	Southern	Central	30°44'10"S	71°42'05"W	06-05-2008
Las Cruces	LCR	Southern	Central	33°30'09"S	71°38'01"W	06-05-2008
Valdivia	VAL	Southern	Central	39°46'75"S	73°23'49"W	04-06-2008

Table 2. Results of 2-way ANOVA (fixed factors) for each of the six considered vital rates. a. Comparison between both cryptic species (considering only central locations), and b. & c. Comparison between marginal and central locations within each species. d.f.: degree of freedom. Bold type indicates significant differences at $\alpha = 0.05$.

Vital rate		**Germination**		**Survival**		**Sex ratio**		**Veg. dev.**		**Female maturity**		**Female fertility**	
Groups of populations compared and sources of variation	**d.f.**	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
a. Comparison between Northern and Southern species (central locations only)													
Temperature	4	13.68	**<0.001**	0.58	0.674	11.71	**<0.001**	9.86	**<0.001**	38.13	**<0.001**	24.06	**<0.001**
Species	1	11.89	**0.001**	0.06	0.807	4.94	**0.028**	13.41	**0.001**	37.76	**<0.001**	45.16	**<0.001**
Temperature * Species	4	1.53	0.196	4.57	**0.002**	3.29	**0.013**	1.18	0.329	2.60	**0.039**	3.73	**0.007**
b. Northern species: comparison between marginal and central locations													
Temperature	4	6.29	**<0.001**	1.51	0.571	0.30	0.878	7.27	**<0.001**	57.43	**<0.001**	13.36	**<0.001**
Marginality	1	2.20	0.142	0.32	0.207	1.28	0.262	3.84	0.058	22.58	**<0.001**	31.47	**<0.001**
Temperature * Marginality	4	3.32	**0.015**	0.58	0.678	1.37	0.253	4.16	**0.007**	2.86	**0.029**	2.24	0.073
c. Southern species: comparison between marginal and central locations													
Temperature	4	3.64	**0.008**	4.95	**0.001**	8.59	**<0.001**	2.08	0.093	20.65	**<0.001**	13.76	**<0.001**
Marginality	1	6.58	**0.011**	5.55	**0.020**	20.92	**<0.001**	10.94	**0.002**	28.60	**<0.001**	9.98	**0.002**
Temperature * Marginality	4	1.05	0.386	0.10	0.984	8.41	**<0.001**	1.36	0.256	3.57	**0.008**	1.15	0.334

Table 3: Results of the Kruskal-Wallis tests comparing the population growth rates (lambda) between species (Northern *vs.* Southern species) and within species (central *vs.* marginal populations) for each of the five temperature treatments. In all cases, the degree of freedom for the *H*-statistic was 1. Bold type indicates significant differences at $\alpha = 0.05$.

Treatment (ºC)	Between species comparison		Within species comparisons			
			Northern species		Southern species	
	H	*p*	*H*	*p*	*H*	*p*
10ºC	1030.69	**<0.0001**	1965.67	**<0.0001**	1397.09	**<0.0001**
10-15ºC	559.20	**<0.0001**	1531.97	**<0.0001**	934.10	**<0.0001**
15ºC	2260.20	**<0.0001**	2.77	0.0960	1569.92	**<0.0001**
15-20ºC	3599.32	**<0.0001**	1827.07	**<0.0001**	0.00	0.9900
20ºC	3599.30	**<0.0001**	1999.30	**<0.0001**	395.32	**<0.0001**

FIGURE LEGENDS

Figure 1. Distribution of Northern and Southern cryptic species of *Lessonia nigrescens*: (a) along the Chilean coasts, (b) detail of the transition zone (28-31°S). The Southern species is represented in grey (names in italics) and the Northern species in black. For abbreviations see Table 1. Also, mean population growths (± SE) and elasticities of the different transition elements of the matrices for each treatment for each studied population.

Figure 2. Microscopic stages for the species of the *Lessonia nigrescens* complex. (1) spores, (2) germling spores, (3) gametophyte of 1-2 cells, (4) gametophyte of > 2 cells, (5) mature female, i.e. with oogonia, (6) fertilized females, bearing embryonic sporophyte, and (7) male gametophyte.

Figure 3. Mean vital rates (± SE) for each of the four defined groups of populations (Northern and Southern species, central and marginal populations) of *L. nigrescens* cryptic species, at each of the five temperature treatments. a. Germination rate (at day 2 of culture), b. Gametophyte survival (day 25), c. Sex ratio of gametophytes, i.e. the frequency of male gametophytes (day 15, please note that different *y*-axis label is used for this vital rate), d. Frequency of female gametophytes showing vegetative development, i.e. having more than 2 cells (day 24), e. Female maturity rate, i.e. frequency of females with oogonia (day 25), f. Female fertilization rate, i.e. frequency of females bearing a sporophyte (day 25).

Figure 4. Mean population growth (± SE) for each population type under the five tested temperature treatments.

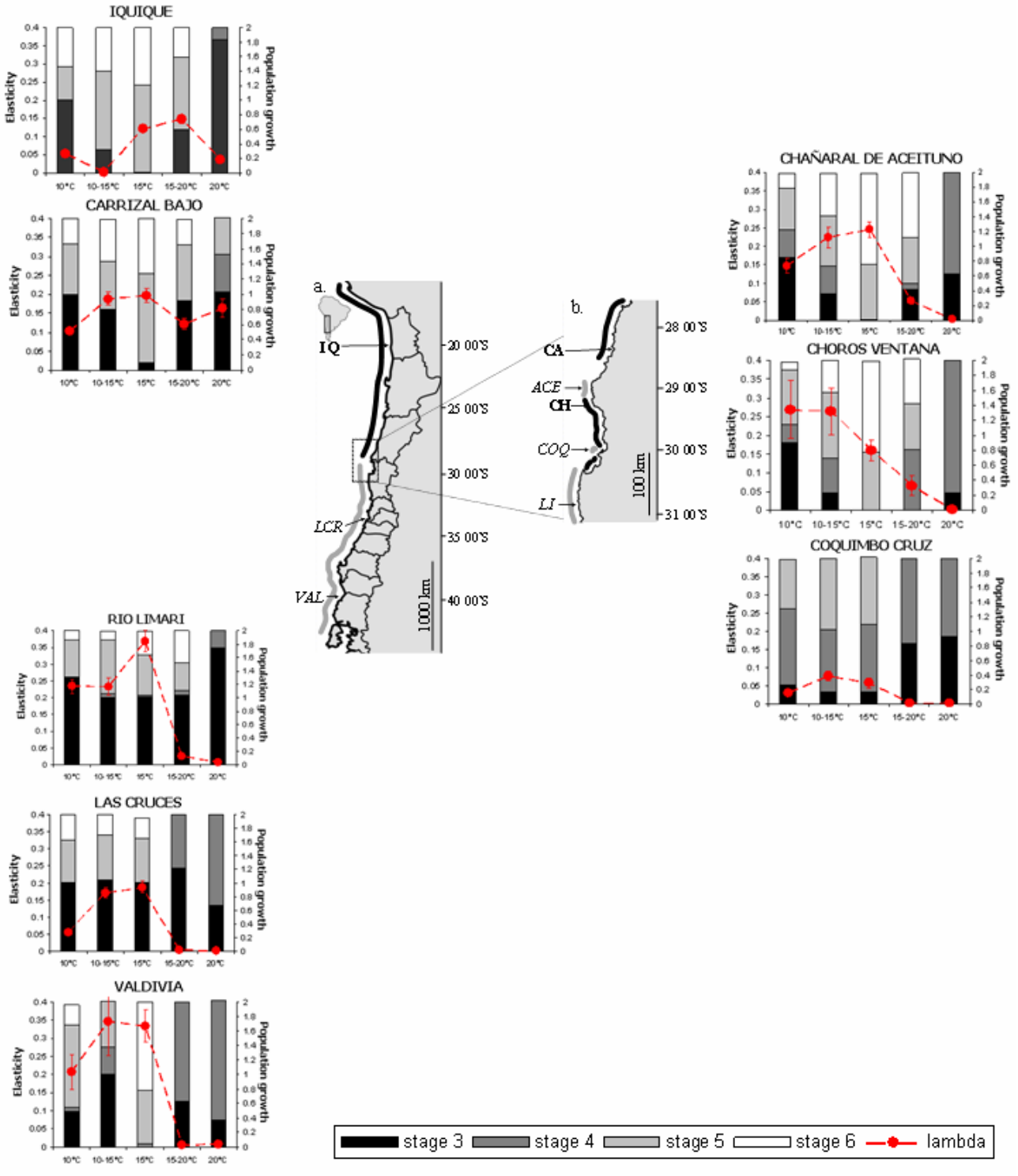


Figure 1. Oppliger et al.

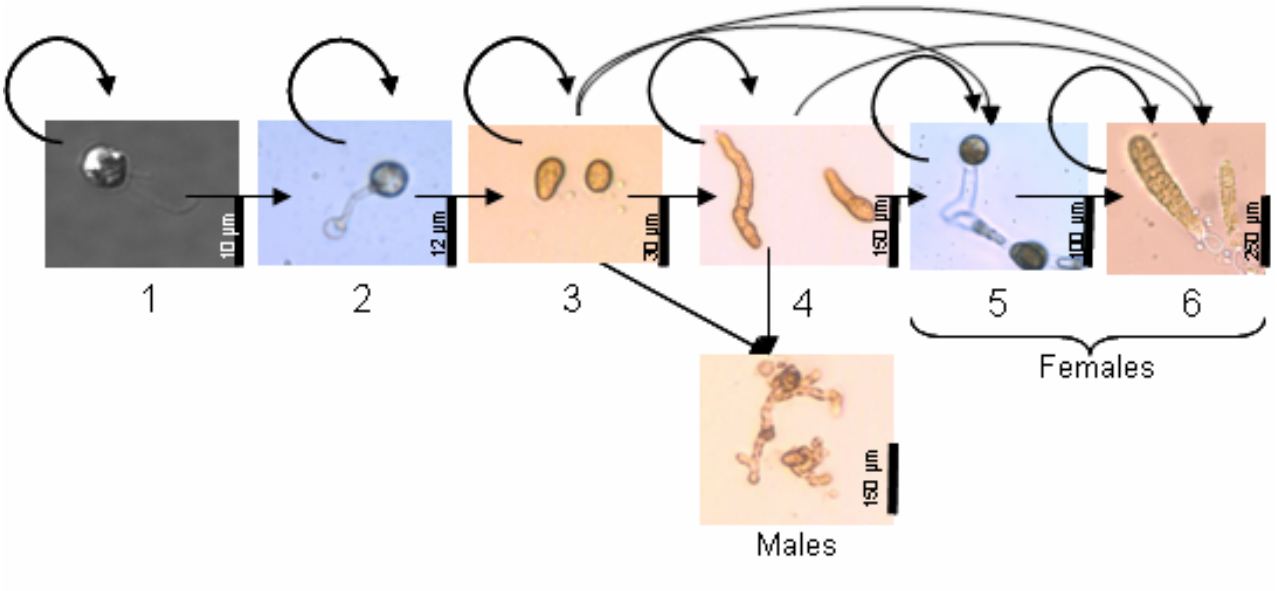


Figure 2. Oppliger et al.

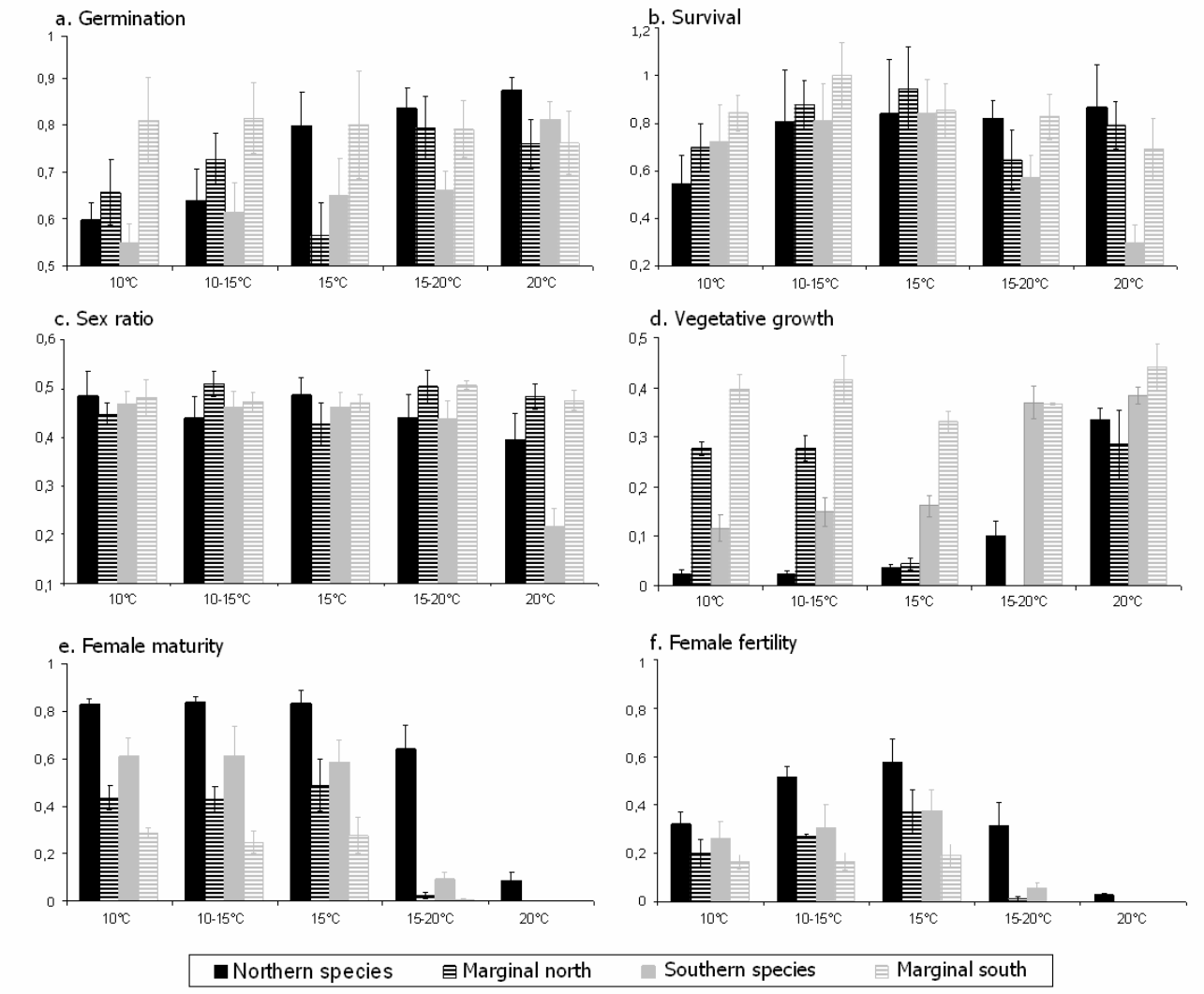


Figure 3. Oppliger et al.

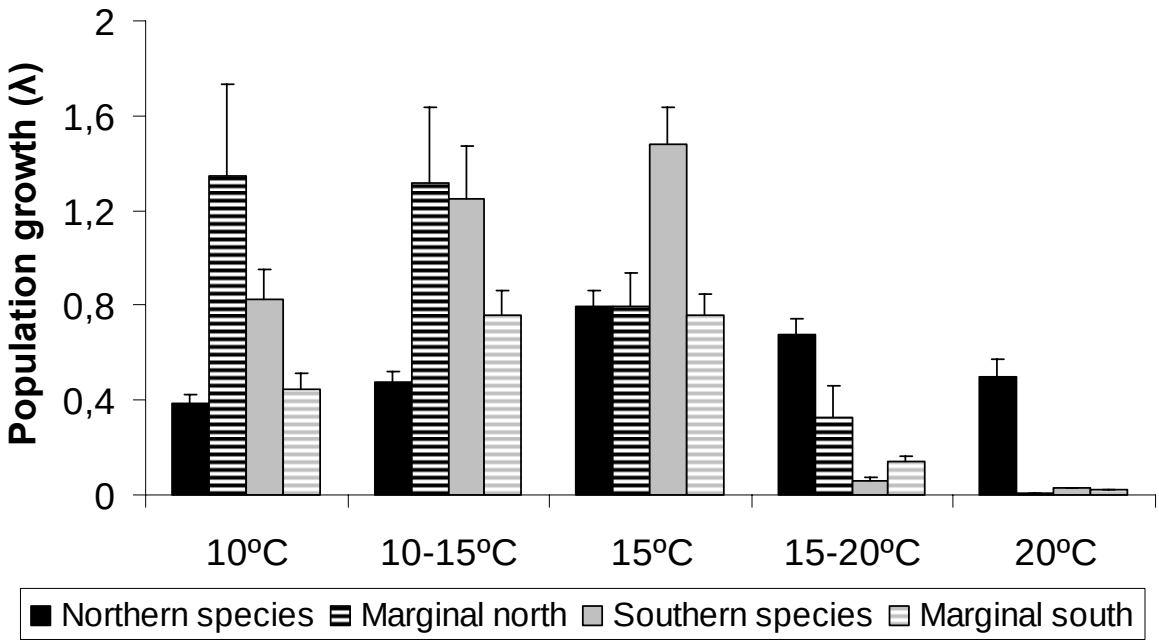


Figure 4. Oppliger et al.

CHAPTER 3

GEOGRAPHICAL PARTHENOGENESIS AT THE RANGE LIMIT OF *LAMINARIA DIGITATA*

(Article in preparation, to be submitted to PNAS)

L. Valeria Oppliger[1,2,3], Peter Von Dassow[1], Sarah Bouchemousse[2], Marine Robouchon[3], Myriam Valero[3], Juan A. Correa[1], Stéphane Mauger[3] & Christophe Destombe[2,3]

(1) Departamento de Ecología, Center for Advanced Studies in Ecology and Biodiversity, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Post-code 6513677, Santiago, Chile.
(2) UPMC Univ. Paris 06, UMR 7144, Equipe "BEDIM", Station Biologique de Roscoff, 29682 Roscoff, France.
(3) CNRS, UMR 7144, Station Biologique de Roscoff, Place Georges Teissier, 29682 Roscoff, France.

ABSTRACT

The coexistence of closely related sexual and asexual lineages raises the general question of the evolutionary potentials of asexuality (Haag and Ebert 2004, Hörandl 2006, Kawecki 2008). Closely related sexual and asexual organisms often exhibit distinct geographic distributions, with asexuality or parthenogenesis tending to dominate in marginal or stressful environments, a phenomenon referred to as geographical parthenogenesis. The capacity for parthenogenetic development of brown algae has been documented in culture, but it is unknown whether parthenogenesis occurs in nature or what role it might play. This study shows that the population of *Laminaria digitata* at its range limit displays irregular meiosis, a situation that is probably associated with the environmentally unstable conditions that affect the Southern Brittany coast. Furthermore, the marginal population at Quiberon displayed a decreased genetic diversity compared to central populations. Diploid spores developed normally as gametophytes. The full heteromorphic life cycle of kelps was expressed, but without expected change in ploidy. Syngamy, one of the costs of this life cycle, could potentially be eliminated. These results demonstrate the existence of geographical parthenogenesis in marine environments through meiosis modification. Hypotheses of cytological mechanism associated to this process are proposed.

INTRODUCTION

The ubiquity of sex among eukaryotes has long been a paradox due to the high costs associated with mating and recombination (Maynard-Smith 1978, Otto 2009). Sex incurs both significant genetic costs, as parents share only half their genes with their progeny, and energetic costs, as meiosis is slower than mitosis and more prone to failure, and sexual reproduction also requires investment of energy into encountering compatible mates. Theoretically asexual females transmit twice as many genes to each offspring compared to sexually reproducing females (Maynard-Smith 1978), yet sex is the predominant mode of reproduction in nearly all multicellular taxa. Several theories attempt to explain the evolutionary maintenance of sex through the benefits of genetic reshuffling which accelerates the production of advantageous new genotypes, facilitating adaptation, and limiting the accumulation of deleterious mutations (Bell 1980, Lynch 1984, Kondrashov and Kondrashov 2001). Sexual and asexual lineages may co-exist in the long term as a result of a dynamic equilibrium between the origin of new asexual lineages and their extinction (Nunney 1989).

Geographical parthenogenesis refers to the case where closely related sexual and asexual lineages exhibit distinct distributions (Vandel 1928). Asexual forms often tend to be prevalent in populations that occupy the margins of a species range, including high altitudes, deserts, or small islands. Geographical parthenogenesis has been observed in several organisms including *Daphnia* (Beaton and Hebert 1988), stick insects (Law and Crespi 2002), reptiles (Moritz et al. 1989, Kearney 2003) and plants (VanDijk 2003, Hörandl 2006). Marginal populations are generally characterised by increased genetic isolation, genetic differentiation, and variability in individual and population performance. Several mechanisms have been proposed to explain these patterns, including: 1. Sexuality may be more advantageous in habitats when selection results from biotic interactions, as co-evolutionary arms-race with parasites, predators and competitors favor continued generation of new gene combinations (Hamilton 1980, Lively et al. 1990). In contrast, asexuality might be favored in sparsely inhabited regions where abiotic factors dominate and the relative energetic costs of mating are higher (Hamilton et al. 1990). 2. Asexuals are superior colonists of new habitats because they do not have the two-fold cost of sex (Cuellar 1974). 3. Asexuality maintains locally adapted gene combinations (Peck et al. 1998). 4. Asexuals avoid the cost of inbreeding depression (Haag and Ebert 2004).

Brown algae (Phaeophyceae) are a closely-related group of multicellular organisms that form essential structural components of near-shore marine ecosystems.

These organisms exhibit a complex haplo-diplontic life cycle where both haploid and diploid phases exhibit vegetative growth. Diploid sporophytes produce spores by meiosis, which then undergo mitotic division to form multicellular haploid gametophytes. Fertilization eventually restores the diploid sporophyte state, which again grows vegetatively. Multiple mechanisms to avoid or modify sexuality exist within brown algae. Haploid cells from the gametophyte can undergo endomitosis to restore diploidy without fertilization in *Ectocarpus* sp., *Laminaria japonica*, *Undaria pinnatifida and Lessonia nigrescens* (Fang 1984, Oppliger et al. 2007, Bothwell et al. 2010). Apomeiosis, the replacement of meiosis with a mitotic division to produce diploid spores, has been documented in *Ectocarpus* sp. (Bothwell et al. 2010). Finally, almost all brown algae are capable of reproducing by fragmentation of either haploid or diploid multicellular phases, completely skipping the sexual cycle (Ar-Gall et al. 1996, Lewis 1996).

The kelp *Laminaria digitata* exhibits a broad distribution along the European coast, with the southern limit clearly defined by the population at Quiberon on the Atlantic coast of Southern Brittany. Considering Quiberon as a marginal population, the present study aims at understanding the reproductive systems of populations at both the center and the edge of distribution of *Laminaria digitata*. More precisely, we tested the occurrence of geographical parthenogenesis through combinational approaches including: population genetic analyses, spore flow cytometry, culture in vitro and microscopic observations.

MATERIAL AND METHODS

Site characterization

Sea surface temperature (SST) data obtained from satellite data during 9 years of survey in the Brittany region, show that: mean temperature values in Roscoff area ranges from 4.5 to 20.9°C whereas in Quiberon from 3.8 to 21.1°C. The Northern Brittany coast displayed a mean temperature value of 13.83 ± 3.38 °C, in contrast, the Southern Brittany coast showed a mean value of temperature of 14.45 ± 3.45 °C.

Sampling

Fertile thalli from 20 to 39 mature sporophytic individuals were sampled from 3

areas along the Brittany coast between Roscoff and Quiberon (Fig. 1) from July to September 2009. Two locations, Porspoder and Roscoff, were chosen for the continuous distribution of *Laminaria digitata* (hereafter referred to as central populations), and one location, Quiberon, was sampled at the southern range limit of the distribution. This latter site was considered to be marginal because it represents the southern-most population of *L. digitata*. This sampling was done to analyze spores by flow cytometry and to culture them. In addition, meristematic fragments of sporophytes were excised and stored in silica gel for genetic analyses (Table 1, 4). Two to three populations per site were analyzed for genetic diversity.

Spore release

Fertile thalli were sampled from Roscoff, Porspoder and Quiberon, and fertile fragments of equal size (3,8 cm^2) were cut off from each plant, cleaned in running tap water and exposed to air for 30 minutes. Ten fragments per plant were transferred to four 50mL plastic Falcons tubes containing 40µL of filtered seawater at 0,2 µm, set on ice and agitated. Fertile fragments were withdrawn from the tubes after 12h. Each spore suspension was divided in three aliquots. The first aliquot was used to estimate the number of released spores per ml under a binocular microscope with Neubauer chambers, the second one was used to obtain gametophyte cultures, and the third one was fixed with 2% formaldehyde for flow cytometry analyses.

Genetic analyses

To estimate genetic diversity in sporophytes of the studied populations, 7 microsatellite markers were used (Billot et al. 1998). DNA extractions were done with 5-10 mg of dried vegetative tissue from each plant using the NucleoSpin® kit (Macherey Nagel, Duren, Germany) and following the manufacturer's protocol. Microsatellites were amplified by touch down PCR, and PCR products were separated by an acrylamide gel in a LICOR® sequencer (Li-cor Bioscience, United Kingdom). DNA fragments were revealed by UV light and photos of these gels were used to determine allele size per locus for each individual.

Genetic diversity indexes included expected heterozygosity (He) and allelic richness (Na). Both indexes were estimated with the software GeneAlex 6.2 (Peakall & Smouse, 2006). To test the hypothesis that central populations were more genetically diverse than marginal populations, comparisons of observed heterozygosity and allelic diversity were done with the software FSTAT (Goudet 2001).

F_{IS} was estimated for each population. F_{IS} measures the within population genetic structure by examining the (genetic) correlation of the two alleles found within a single individual compared with the expected heterozygosity (under Hardy-Weinberg conditions) of the population. For this reason, F_{IS} can be calculated for diploid individuals only.

The number of diploid individual showing the same genotype (repeated multiplocus genotypes) was compared with the estimation of the expected number of individuals with the same multilocus genotype i) assuming Hardy-Weinberg equilibrium and ii) assuming sibs crosses according to GeneAlex 6.2 (Peakall & Smouse, 2006).

Culturing

Five drops of each spore suspension were inoculated in 55mmx14mm plastic Petri dishes with Provasoli enriched seawater and incubated in a culture chamber set at 15°C, 12:12 light:dark, 25-35 µmol photons $m^{-2}s^{-1}$. Culture medium was changed once a week and observations were done monthly for 10 months.

Sex ratio estimations

Sex ratio estimations were done on 9 progenies from Quiberon. Male and female gametophytes were identified according to their morphological characteristics using a Nikon Eclipse TE300 inverted microscope (Nikon Corp., Tokyo, Japan). Female gametophytes are characterized by large cells and filaments with few branches whereas male gametophytes are smaller and display highly branched filaments formed by small cells. These morphological differences make them unambiguously identifiable under the light microscope. The numbers of male and female gametophytes were determined by counting their occurrence in three visual fields per slide using the 10x objective. Sex ratio was expressed as the frequency of males per progeny (i.e. males/(males+females)).

Flow cytometry analyses

Ploidy levels of *L. digitata* spores were estimated by DNA content using flow cytometer analysis of fixed spores stained with Sybr Green I (Invitrogen, Carlsbad, CA, USA). In early trials isolated nuclei obtained from live spores (Marie et al. 2000, Marie et al. 2005). The principle of flow cytometry is described elsewhere (Ar-Gall et al. 1996, Marie et al. 2005) and the flow cytometry apparatus used in this work was the

FACSCanto II System (BD Biosciences, San Jose, CA, USA). Data was analyzed using the BD FACSDiva and FlowJo software (Treestar, Ashland, OR, USA).

Microscopy

Comparative ploidy levels of 10 male and female gametophytes from 2 progenies from Quiberon and 2 gametophytes from 2 progenies from Roscoff were estimated by measuring nuclear area using the fluorescent DNA Hoechst stain (Invitrogen, European Headquarters) and epifluorescence inverted microscope Zeiss Observer Z1 (Carl Zeiss Microimaging, Inc., USA), as described in Bothwell et al. (2010). Images of nuclei were analyzed with ImageJ software (National Institutes of Health, available at www.nih.gov).

Genotyping (microsatellite markers)

To identify the cytological mechanism that produces diploid spores in *L. digitata*, parental-descendants genotyping were done in 8 sporophytes and 12-24 gametophytes per parent with 2 microsatellite markers. DNA extractions were done with 5-10 mg of dried vegetative tissue from each parental plant and according to the protocol for the NucleoSpin®kit (Macherey Nagel, Duren, Germany). DNA extractions for gametophytes were done with Chelex 100® (Bio-Rad Laboratories, Hercules, CA), 5% and proteinase k 10mg/mL. Microsatellites were amplified by PCR touch down, and PCR products were separated under an acrylamide gel in a LICOR® sequencer (Li-cor Bioscience, United Kingdom). DNA fragments were revealed by UV light and photos of these gels were used to determine allele size per loci for each individual.

RESULTS

Spore release

The mean number of spores released per sporophyte sampled in Quiberon (179 +/-189 spores/µL) was significantly lower than the mean number produced per individual from Porspoder and Roscoff. (1860 +/- 1555 spores /µL, Kruskal-Wallis test: H=60.12, dl=1, p<0.0001) (Fig. 1).

Ploidy of spores

The spores produced by individuals from central populations were haploid whereas spores produced by individuals from Quiberon were predominantly diploid (Table 1, Figure 2). The microscopic observation of spores showed that diploid spores generally lacked flagella (data not shown).

Culture of gametophytes

The spores produced by the sporophytic individuals sampled in Quiberon, germinated and developed normally into gametophytes. These gametophytes did not differ in color, shape or texture from gametophytes obtained from germination of spores from Roscoff individuals. In culture, these gametophytes initiated new juvenile sporophytes that for the first steps of development did not differ from those obtained from gametophytes of Roscoff.

Sex-ratio

Sex ratios of the gametophytes obtained from spores of Quiberon were balanced (average of 0,461 +/- 0,185), however three of the nine studied progenies displayed female bias, as judged by comparison to the binomial law (Table 3).

Ploidy of gametophytes:

The nuclei of filamentous gametophytes were visible under epifluorescence microscopy (Figure 3). The gametophytes obtained from spores of Quiberon displayed a significantly larger nuclear area than gametophytes of Roscoff (Kruskal-Wallis test: H=104.44, dl=1, p<0.0001) (Table3, Fig.4). These results show that gametophytes produced by Quiberon individuals might be diploid or polypolyploid and they belong to diploid spores detected by flow cytometry.

Genetic analysis:

The results of the genetic analysis are given in table 4. These results showed that marginal populations located in the site of Quiberon showed lower genetic diversity (Na and He) than populations located in the central area (Porspoder and Roscoff). The lowest genetic diversity (He) was found in the marginal populations located in the area of Quiberon with an average of 0.50+/- 0.04 while central populations located in Roscoff and Porspoder showed higher values (respectively 0.61 +/- 0.01 and 0.67 +/- 0.02). Similarly, the allelic diversity (Na) was in average lower in marginal populations than in central ones (Mean allelic diversity in the site of Quiberon:

4.67 +/-0.66; in the site of Roscoff: 7.10 +/- 1.03; and in the site of Porspoder: 7.05+/-0.30) (Kruskal-Wallis test; H=5.4; dl=1; *p*=0.02).

The fixation index Fis, corresponding to the deficit of heterozygote in a population, is relatively low whatever the populations studied from Fis=0.00 in Ar Pourven and Sieck to Fis=0.078 in Molène. No clear difference was observed between marginal and central populations. Individuals with the same multilocus genotype were observed only in marginal populations. Two sporophytes with the same genotype (MLG) were found in Belle-Ile and two other in Pte de Conguel. This finding was made in populations where the probability to have repeated multilocus genotype, assuming sibs crosses was higher (Table 4).

Gametophytes genotyping:

The genotyping of the progeny of the heterozygous sporophyte sampled in Roscoff showed for both microsatellite loci (Ld371 and Ld531) a clear Mendelian segregation (Table 5). Similarly, the progenies of heterozygous sporophytic individuals from Quiberon displayed a Mendelian segregation excepted for 3 progenies (P1, P7 and P8) that showed heterozygous gametophytes. A total of 5 heterozygous gametophytes were detected using the two microsatellite loci over 124 gametophytes issued of the heterozygous sporophytes. This values corresponding of a minimum of 4% of heterozygous.

DISCUSSION

Spores produced by plants from Quiberon were mainly diploid. Three hypotheses can be proposed to explain this result: 1) Apomeiosis, the complete replacement of meiosis with mitotic divisions, would cause the production of gametophytes that were essentially genetically identical to the sporophyte parents, 2) Automixis, where two of the four nuclear products of meiosis fuse, resulting in diploid spores that were genetically different and, 3) Endomitosis, that would involve haploid spores restoring diploidy by chromosome doubling. There are several examples of apomeiosis in higher plants due to its potential application in agriculture. In the genus *Erigeron* (Asteraceae) unreduced egg formation (apomeiosis) is coupled with apospory or diplospory (Noyes 2005). In *Arabidopsis*, it was shown that apomeiosis is a deregulation of meiosis that results in a mitotic-like division and can arise by mutation

of a single gene that is directly involved in controlling the entry into the second meiotic division (d'Erfurth et al. 2009). In the diatom *Achnanthes* (Bacillariophyceae) uniparental auxospore formation occurs through apomeiosis followed by contraction of the contents of unpaired cells and then a mitotic division, with only one of the auxospores subsequently surviving (Sabbe et al. 2004). Automixis, has been reported mainly in animals such as stick insects (Schwander and Crespi 2009), sharks (Chapman et al. 2007), Drosophila (Gustafsson 1935). Endomitosis in fungi such as *Aspergillus nidulans* and yeast such as *Saccharomyces cerevisiae* undergo a "closed" mitosis, where chromosomes divide within an intact cell nucleus (Maton et al. 1997).

Overall sex ratio estimates in Quiberon were around 0.5. This can be explained by the fact that sex is thought to be genetically determined in Laminariales (Yasui 1992, Oppliger et al. 2010 in press). If we assume occurrence of a sex locus or sexual chromosomes, the genotype of an haploid gametophyte is either male (M) or female (F), whereas the diploid sporophyte is heterozygous for this locus (MF). If automixis or endomitosis occurs, gametophytes are expected to be diploid and their genotype either homozygous (MM or FF) or heterozygous (MF). The phenotype of the homozygous are expected to be respectively male and female and for the heterozygous, could depend on the allele dominance. In *Ectocarpus siliculosus*, male is dominant on female allele and the heterozygous are then phenotypically male (Cock pers.comm.). In our study, three out of nine progenies from Quiberon showed gametophyte female bias. These unbalance sex ratio are not compatible with the dominance of male allele and suggest that heterozygous and homozygous gametophytes could not have the same fitness according to their genotypes.

Nuclear area of male and female gametophytes developed from spores of Quiberon suggests that gametophytes are at least diploid. These diploid gametophytes are able to survive and to develop normally *in vitro.* Polyploidy (or diploidisation) is often associated with asexuality (Vandel 1928, Suomalainen et al. 1987) and may give ecological advantages in harsh environments, as is well documented for polyploidy plants (Stebbins 1950), or animals (Kearney 2003). An example is the study done on the ostracod *Eucypris virens* that reported that triploid, but not diploid asexual clones were able to colonized higher latitudes expanding the range of distribution. It was suggested that the wider distribution range of triploids was due to elevated ploidy rather than asexuality (Adolfsson et al. 2009). This idea was previously discussed examining if it was polyploidy itself or the generation of elevated ploidies by hybridization that promoted asexuality and further range expansion (Kearney 2005,

2006, Lundmark 2006). However in our study no evidence of diploid gametophytes resulting from either of triploid or tetraploid sporophytes exists in natural populations of Quiberon. Indeed, for each microsatellite locus analyzed, genotypes of the sporophytes are either homozygous or heterozygous (showing respectively only 1 or 2 bands). This result suggests that gametes from diploid gametophytes are not able to fuse to give a new sporophyte. The only possibility is that diploid gametophytes produce a new sporophyte by parthenogenesis.

Among the variety of mechanisms for parthenogenesis or cloning demonstrated to be available to Laminiarian algae (Fang 1983, Funano 1983, Fang 1984, Ar-Gall et al. 1996, Lewis 1996, Asensi et al. 2001, Oppliger et al. 2007), the dominant process observed in the marginal population of *L. digitata* is the one that maintains diploidy and lowers the costs of sex. The mechanism of parthenogenesis found in the population at Quiberon maintains the complex heteromorphic life cycle. The only life cycle cost that has been reduced is the cost of syngamy (fertilization), however the genetic cost of sex has been reduced. One of the strongest genetic consequences of being diploid is that nearly all deleterious mutations within the genome are masked, which has been hypothesized to hasten the rate of adaptation to new environments by permitting a larger pool of mutant alleles. This pool provides a source of genetic variability that may prove to be beneficial as the environment changes. Likewise, the acquisition of new functions may be faster in diploids because the "extra" gene copy can preserve mutant alleles that serve a new purpose, while in haploids gene duplication must predate the evolution of novel functions (Mable and Otto 1998).

The genotyping of the progenies of the sporophytes from Quiberon showed that heterozygous gametophytes were detected in the progenies in low frequency. The frequency is lower than expected frequency assuming automixis and corresponding to half homozygous and half heterozygous. This observation suggests that survival of gametophytes originated from diploid spores is lower than gametophyte growing from haploid ones. Finally, our results on population genetics indicated that the reproduction of *L.digitata* was mainly panmictic whatever the population studied (marginal or central). Paradoxally, the fact that repeated multilocus genotypes were found only in marginal populations and that corresponded to the expected number of repeated genotype assuming sibs crossing, suggests that automixis might occur in this species. The low genetic diversity observed in marginal populations compared to central ones could be the consequence and/or the cause of this particular reproductive system.

Marginality is usually associated with higher abioitic stresses, and Quiberon is subjected to higher and more fluctuating regimes of temperature than central population. As all kelps, *L.digitata* is sensitive to elevated temperature, and the observed change in its reproductive system seems to be an adaptation to isolation and to temperature instablility of its habitat. However, there is no evidence for relaxation of biotic selection; for example, species richness of epiphytes is actually higher at the Quiberon than other sites (data not shown). Environmental conditions in Quiberon sustain the basis of geographical parthenogenesis where abiotic factors are more important at marginal environments than the biotic ones, and thus probably have an influence on the reproductive system.

ACKNOWLEDGEMENTS

We thanks to S. Coelho for technical help and critical discussions, D. Marie for technical help with flow cytometry, D. Roze for critical evolutionary discussions, M. Oriot for help in taking samples. V. Oppliger was supported by a CONICYT-FRENCH EMBASSY PhD grant and F. Tellier by a doctoral grant from the Ministry of Education and Research (MENRT). This study is part of the research programs FONDAP 1501-0001 Program 7, ANR ECOKELP (ANR 06 BDIV 012), "ARCUS" of the French Ministry of Foreign Affairs, and the Laboratoire International Associé "Dispersal and Adaptation of Marine Species (LIA DIAMS).

REFERENCES

Adolfsson, S., Y. Michalakis, D. Paczesniak, S. N. Bode, R. K. Butlin, D. K. Lamatsch, M. J. F. Martins, O. Schmit, J. Vandekerkhove, and J. Jokela. 2009. Evaluation of the elevated ploidy and asexual reproduction as an alternative explanations for geographic parthenogenesis in *Eucypris virens* ostracods. Evolution **64**:986-997.

Ar-Gall, E., A. Asensi, D. Marie, and B. Kloareg. 1996. Parthenogenesis and apospory in the Laminariales: a flow cytometry analysis. European Journal of Phycology **31**:369-380.

Asensi, A., E. A. Gall, D. Marie, C. Billot, P. Dion, and B. Kloareg. 2001. Clonal propagation of *Laminaria digitata* (Phaeophyceae) sporophytes through a diploid cell-filament suspension. Journal of Phycology **37**:411-417.

Beaton, M. J., and P. D. N. Hebert. 1988. Geographical Parthenogenesis and Polyploidy in Daphnia pulex. The American Naturalist **132**:837-845.

Bell, G. 1980. The costs of reproduction and their consequences. The American Naturalist **116**:45-76.

Billot, C., S. Rousval, A. Estoup, J. T. Epplen, P. Saumitou-Laprade, M. Valero, and B. Kloareg. 1998. Isolation and characterization of microsatellite markers in the nuclear genome of the brown alga *Laminaria digitata* (Phaeophyceae) Molecular Ecology **7**:1771-1788.

Bothwell, J. H., D. Marie, A. F. Peters, J. M. Cock, and S. M. Coelho. 2010. Role of endoreduplication and apomeiosis during parthenogenetic reproduction in the model brown alga Ectocarpus. New Phytologist **188**:111-121.

Chapman, D. D., M. S. Shivji, E. Louis, J. Sommer, H. Fletcher, and P. A. Prodöl. 2007. Virgin birth in a hammerhead shark. Biology letters **3**:425-427.

Cuellar, O. 1974. On the origin of parthenogenesis in vertebrates: the cytogenetic factors. The American Naturalist **108**:625-648.

d'Erfurth, I., S. Jolivet, N. Froger, O. Catrice, M. Novatchkova, and R. Mercier. 2009. Turning Meiosis into Mitosis. Plos Biology **7**:1-10.

Fang, T. C. 1983. A summary of the genetic studies of *Laminaria japonica* in China. Pages 123-136 *in* C. K., editor. Tseng. Science Press, Beijing

Fang, T. C. 1984. Some genetic features revealed from culturing the haploid cells of kelps. Hydrobiologia **116/117**:317-318.

Funano, T. 1983. The ecology of *Laminaria religiosa* Miyabe. I. The life history and alternation of nuclear phases of *Laminaria religiosa*, and the physiological ecology of the gametophytes and the embryonal sporophytes. Hokusui-Shiho **25**:61-109.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices Version 2.9.3.

Gustafsson, A. 1935. Studies on the mechanism of parthenogenesis. Hereditas **21**:1-112.

Haag, C. R., and D. Ebert. 2004. A new hypothesis to explain geographic parthenogenesis. Annales Zoologici Fennici **41**:539-544.

Hamilton, W. D. 1980. Sex versus non-sex versus parasite. Oikos **35**:282-290.

Hamilton, W. D., R. Axelrod, and R. Tanese. 1990. Sexual reproduction as an adaptation to resist parasites (a review). Proceedings of the National Academy of Sciences of the United States of America **87**:3566-3573.

Hörandl, E. 2006. The complex causality of geographical parthenogenesis. New Phytologist **171**:525-538.

Kawecki, T. J. 2008. Adaptation to Marginal Habitats. Annual Review of Ecology and Systematics **39**:321-342.

Kearney, M. 2005. Hybridization, glaciation and geographical parthenogenesis. Trends in Ecology and Evolution **20**:495-502.

Kearney, M. 2006. Response to Lundmark: Polyploidization, hybridization and geographical parthenogenesis. Trends in Ecology and Evolution **21**:10.

Kearney, M. R. 2003. Why is sex so unpopular in the Australian desert? Trends in Ecology & Evolution **18**:605-607.

Kondrashov, F. A., and A. S. Kondrashov. 2001. Multidimensional epistasis and the disadvantage of sex. PNAS **98**:12089-12092.

Law, J. H., and B. J. Crespi. 2002. The evolution of geographic parthenogenesis in Timema walking-sticks. Molecular Ecology **11**:1471-1489.

Lewis, R. J. 1996. Chromosomes of the brown algae. Phycologia **35**:19-40.

Lively, C. M., C. Craddock, and R. C. Vrijenhoek. 1990. Red Queen hypothesis supported by parasitism in sexual and clonal fish. Nature **344**:864-866.

Lundmark, M. 2006. Polyploidization, hybridization and geographical parthenogenesis. Trends in Ecology and Evolution **21**:9.

Lynch, M. 1984. Destabilizing hybridization, general purpose genotypes and geographic parthenogenesis. The Quarterly Review of Biology **59**:257-290.

Mable, B. K., and S. P. Otto. 1998. The evolution of life cycles with haploid and diploid phases. BioEssays **20**:453-462.

Marie, D., N. Simon, L. Guillou, F. Partensky, and D. Vaulot. 2000. Chapter 11.12. DNA/RNA analysis of phytoplankton by flow cytometry. Pages 1–18 *in* J. Robinson, Z. Darzynkiewicz, P. Dean, A. Orfao, P. Rabinovitch, C. Stewart, H. Tanke, and L. Wheeless, editors. Current protocols in cytometry. John Wiley & Sons, Inc., New York.

Marie, D., N. Simon, and D. Vaulot. 2005. Phytoplancton cell counting by flow cytometry. Pages 1-15 *in* R. A. Andersen, editor. Algal Culturing Techniques. Elseviewer Academic Press, London.

Maton, A., J. Hopkins, S. Johnson, D. LaHart, W. Quon, D. Wright, and D. Jill. 1997. Cells: Building Blocks of Life. Prentice Hall, New Jersey.

Maynard-Smith, J. 1978. The evolution of sex. Cambridge University Press, Cambridge.

Moritz, C., S. Donnellan, M. Adams, and P. R. Baverstock. 1989. The Origin and Evolution of Parthenogenesis in Heteronotia binoei (Gekkonidae): Extensive Genotypic Diversity Among Parthenogens. Evolution **43**:994-1003.

Noyes, R. D. 2005. Inheritance of apomeiosis (diplospory) in fleabanes (*Erigeron*, Asteraceae). Heredity **94**:193-198.

Nunney, L. 1989. The Maintenance of Sex by Group Selection. Evolution **43**:245-257.

Oppliger, L. V., J. A. Correa, S. Faugeron, F. Tellier, J. Beltran, M. Valero, and C. Destombe. 2010 in press. Sex ratio variation in the *Lessonia nigrescens* complex (Laminariales, Phaeophyceae): effect of latitude, temperature and marginality. Journal of Phycology.

Oppliger, L. V., A. Peters, and J. A. Correa. 2007. Parthenogenesis in the brown algae *Lessonia nigrescens* (Phaeophyceae) from Central Chile. Journal of Phycology **43**:1295-1301.

Otto, S. P. 2009. The Evolutionary Enigma of Sex. The American Naturalist **174**:S1-S14.

Peck, J. R., J. M. Yearsley, and D. Waxman. 1998. Explaining the geographic distributions of sexual and asexual populations. Nature **391**:889-892.

Sabbe, K., V. A. Chepurnov, W. Vyverman, and D. G. Mann. 2004. Apomixis in *Achnanthes* (Bacillariophyceae); development of a model system for diato reproductive biology. European Journal of Phycology **39**:327-341.

Schwander, T., and B. J. Crespi. 2009. Multiple direct transitions from sexual reproduction to apomictic parthenogenesis in Timema stick insects. Evolution **63**:84-103.

Stebbins, G. 1950. Variation and Evolution in plants. Columbia University Press, Ney York.

Suomalainen, E., A. Saura, and J. Lokki. 1987. Cytology and evolution in parthenogenesis. CRC Press, Boca Raton.

Vandel, A. 1928. La parthénogenese geographique. Contribution a l'étude biologique et cytologique de la parthénogenese naturelle. Bulletin Biologique de la France et de la Belgique **62**:164-182.

VanDijk, P. J. 2003. Ecological and evolutionary opportunities of apomixis: insights from Taraxacum and Chondrilla. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **358**:1113-1121.

Yasui, H. 1992. Chromosome numbers and a sex chromosome of *Laminaria yendoana* Miyabe (Phaeophyta). Nippon Suisan Gakkaishi **58**:1385.

FIGURES LEGENDS

Figure 1. Spore release (number of spores per µL) by central and marginal populations of *L.digitata*.

Figure 2. Genetic diversity and ploidy of spores from *Laminaria digitata* in the studied sites.

Figure 3. Gametophytes dyed with DNA stain. A) Female gametophyte from Quiberon. B) Female gametophyte from Roscoff.

Figure 4. Nuclear area of gametophytes from central and marginal (Quiberon) populations.

Table 1. Flow cytometry results in the studied populations. **n**: nb. of progenies displaying haploid profiles of DNA content. **2n**: nb. of progenies displaying diploid profiles of DNA content**. n & 2n:** nb. of progenies displaying haploid and diploid profiles of DNA content**.**

Population	n	2n	n & 2n
Roscoff	24/31	0/31	7/31
Porspoder	20/20	0/20	0/20
Quiberon	6/39	10/39	23/39

Table 2: Sex ratio in nine progenies of Quiberon. Bold numbers represent deviated sex ratios according to the binomial law.

Progenie	Sex ratio
1	0,56410256
2	**0,328125**
3	**0,13333333**
4	0,8
5	0,51304348
6	0,37931034
7	0,55932203
8	**0,39694656**
9	0,475

Table 3. Nuclear area of gametophytes from Quiberon and Roscoff

	Roscoff		**Quiberon**									
	Male	Female	Male	Male	Female	Female	Male	Male	Male	Male	Male	Male
N° nuclei	Area	Area	Area	Area	Area	Area	Area	Area	Area	Area	Area	Area
1	365	292	476	560	891	772	592	758	996	505	1100	488
2	374	396	487		791	810	735	1454	803	622	698	448
3	302	259	614			709	626		991	575	647	478
4	344	349	434			559	447		695	706	621	552
5	257	228	486				440		1030	652	705	577
6	397	209	460				542			543	975	677
7	310	307	405				512			820	870	402
8	243	244	516				543			949		810
9	377	280	296				571					541
10	282	293					585					674
11	427	273										
12	244	275										
13	303	312										
14	242	302										
15	444	433										
16	327	426										
17	295											
18	268											
19	279											
20	212											
Mean	**314,6**	**304,88**	**463,8**	**560**	**841**	**712,5**	**559**	**1106**	**903**	**672**	**802**	**565**
Sd	65,524	65,903	85,86		70,71	110,49	86,2	492	146	150	183	124

Table 4. Information of study sites and sampled individuals. N: number of sporophytes sampled for genetic analysis, in bracket number of samples for flow cytometry analysis, He: genetic diversity, Na: allelic diversity, Fis: Fixation index, MG: multilocus genotype, Exp. N° of repeated MLG in panmixia: Expected Number of Individuals with the same Multilocus Genotype Based on the Probability of Identity assuming panmixia. Exp. N° of repeated MLG sibs crosses: Expected Number of Individuals with the same Multilocus Genotype Based on the Probability of Identity assuming sibling crossing.

Location	Population	Population type	N	He (SE)	Na (SE)	Fis (SE)	N° unique MLG	N° Repeat MLG	Exp N° Repeat MLG in panmixia $.10^{-3}$	Exp N° Repeat MLG sibs crosses
Roscoff 48°43'N-3°58'W	Ar Pourven	Central	62(31)	0.601 (0.077)	6.429 (1.702)	0.000 (0.031)	62	0	0.24	0.43
	Sieck	Central	68	0.623 (0.073)	8.286 (2.222)	0.000 (0.023)	68	0	0.08	0.37
	Duons	Central	45	0.604 (0.076)	6.571 (1.378)	0.057 (0.041)	45	0	0.21	0.31
	Mean +/- sd			0.61 +/- 0.01	7.10 +/- 1.03	0.02 +/- 0.03				
Porspoder 48°30'N-4°46'W	Porspoder	Central	28(20)	0.666 (0.045)	7.143 (1.317)	0.042 (0.024)	28	0	0.02	0.11
	Le Conquet	Central	30	0.654 (0.060)	6.714 (1.796)	0.026 (0.052)	30	0	0.03	0.13
	Molène	Central	30	0.696 (0.041)	7.286 (1.190)	0.078 (0.059)	30	0	0.01	0.09
	Mean +/- sd			0.67 +/- 0.02	7.05 +/- 0.30	0.05 +/- 0.03				
Quiberon 47°28'N-3°05'W	Pte Conguel	Marginal	41 (39)	0.488 (0.071)	4.286 (0.944)	0.059 (0.047)	39	1	5.30	0.85
	Belle-île	Marginal	47	0.469 (0.066)	4.286 (0.066)	0.017 (0.045)	45	1	12.00	1.20
	Quiberon int	Marginal	49	0.549 (0.057)	5.429 (0.896)	0.039 (0.029)	49	0	2.1	0.61
	Mean +/- sd			0.50 +/- 0.04	4.67 +/- 0.66	0.04 +/- 0.02				

Table 5. Genotyping results for 8 progenies of Quiberon with two locus microsatellite.

		PROGENIES								
Locus	**Phenotype**	**1**	**3**	**5**	**6**	**7**	**8**	**9**	**10**	**R**
Ld 371	**117**	0	0	0	7	0	4	0	0	0
	120	0	0	0	0	2	0	0	0	0
	123	7	2	0	0	7	0	5	3	4
	130	0	4	0	0	0	0	0	0	0
	132	0	0	6	0	0	3	7	0	0
	135	6	0	0	0	0	0	0	0	0
	138	0	0	6	7	0	0	0	0	0
	142	0	0	0	0	0	0	0	1	0
	148	0	0	0	0	0	0	0	0	4
	123-135	1	0	0	0	0	0	0	0	0
Ld 531	**220**	0	0	0	0	5	0	0	0	0
	233	0	0	0	0	0	0	0	3	0
	236	0	0	7	17	0	0	16	4	2
	238	0	0	0	0	0	4	0	0	0
	239	7	0	0	0	0	0	0	0	0
	240	0	0	0	0	6	0	0	0	7
	244	0	0	0	0	0	0	0	0	0
	245	5	7	5	0	0	0	0	0	0
	239-245	1	0	0	0	0	0	0	0	0
	240-220	0	0	0	0	2	0	0	0	0
	244-238	0	0	0	0	0	1	0	0	0

The heterozygote individual from progeny 1 in locus Ld371 is also heterozygote for locus Ld531.

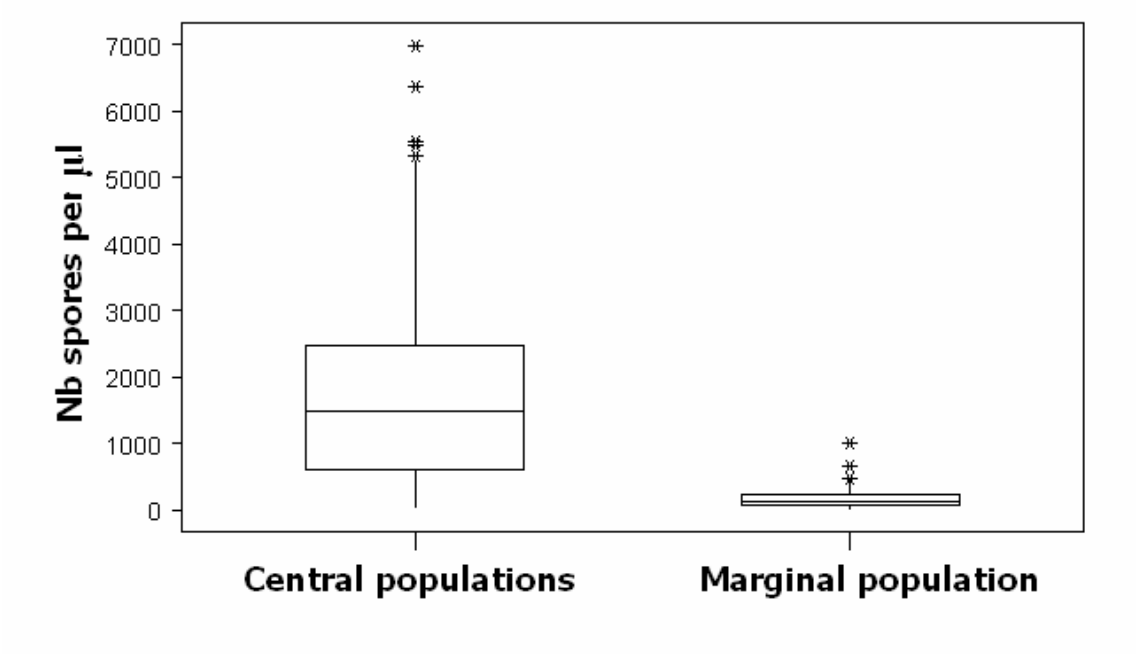


Figure 1. Oppliger et al.

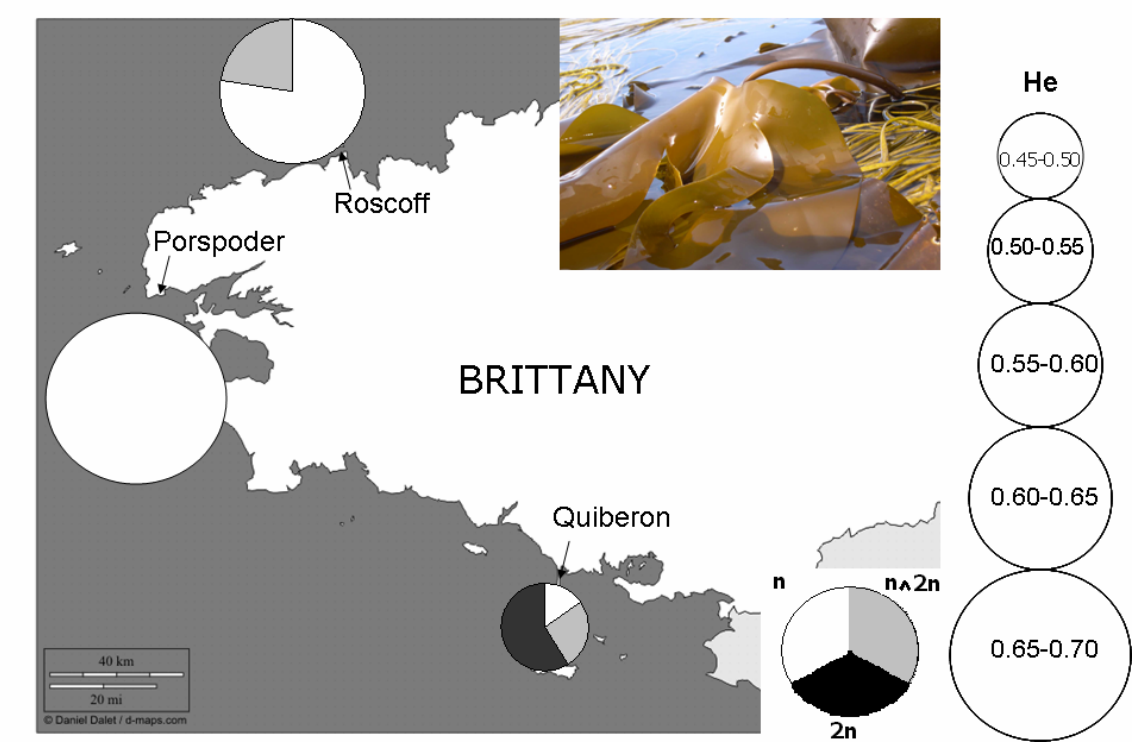


Figure 2. Oppliger et al.

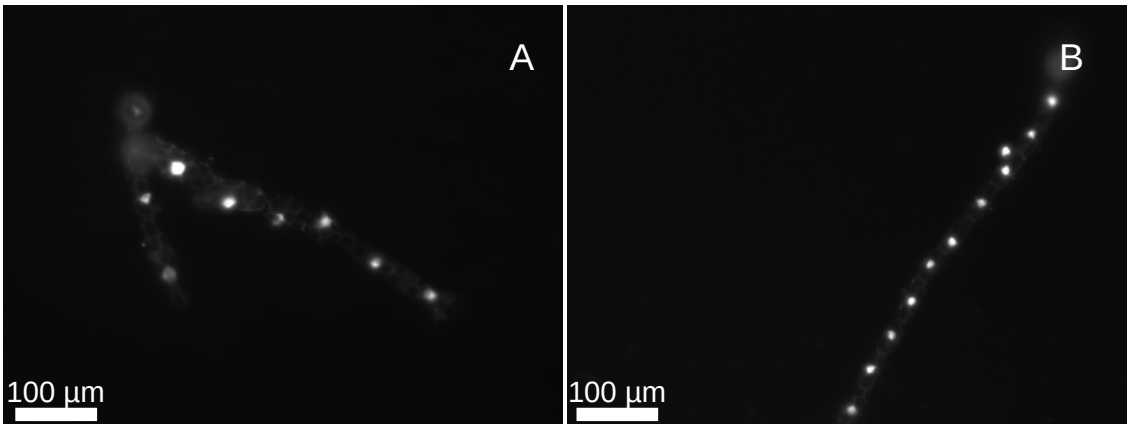


Figure 3. Oppliger et al.

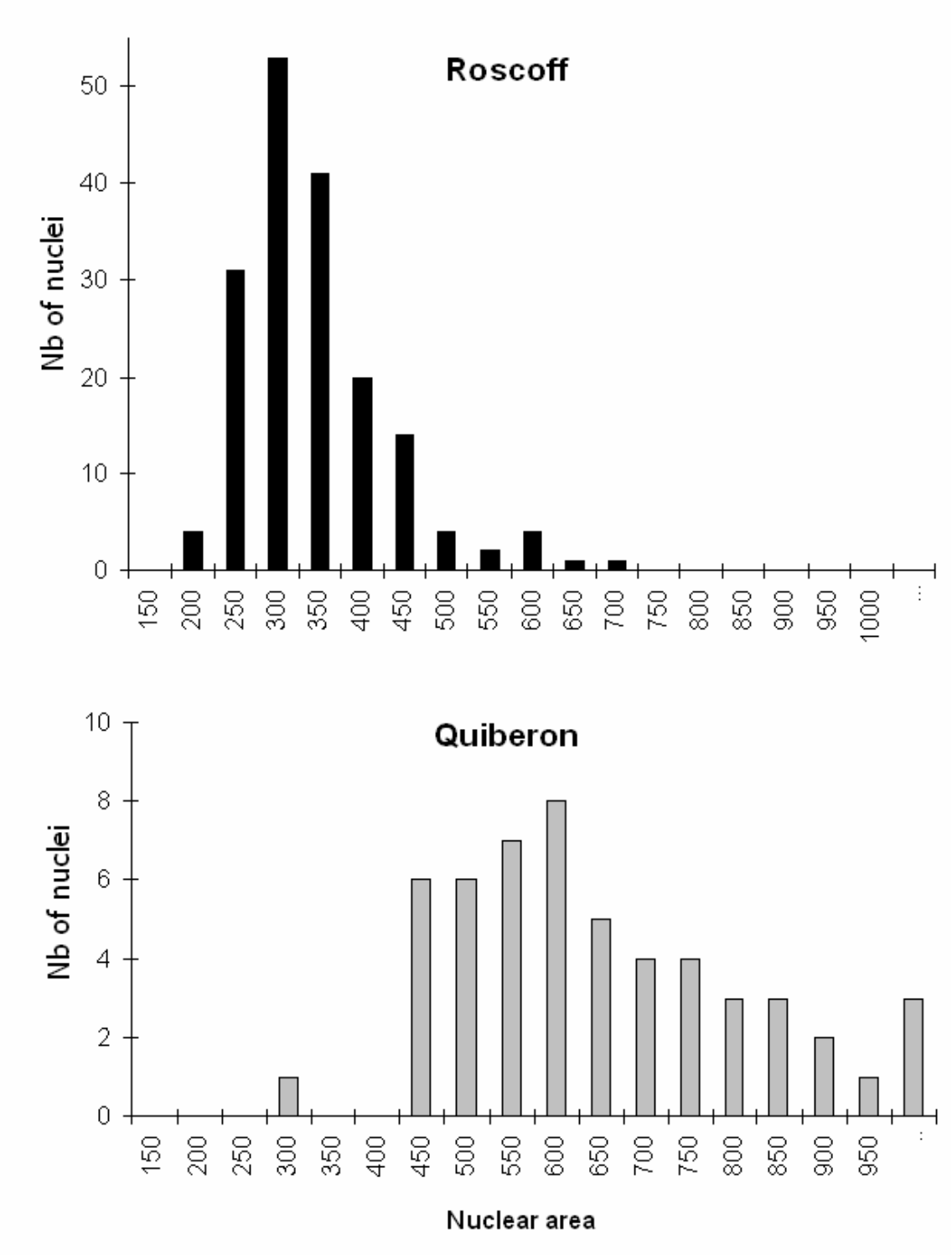


Figure 4. Oppliger et al.

GENERAL DISCUSSION

AND PERSPECTIVES

The Laminarian species investigated in this thesis proved to be interesting models for exploring the evolution and ecology of heteromorphic haplo-diplontic life cycles. The models used in this thesis were the two cryptic species of the *Lessonia nigrescens* complex occurring along the Chilean coast (Tellier et al. 2009) and *Laminaria digitata* in the Brittany coast. The *L. nigrescens* species complex provides a good example of two cryptic species that are within the same morphospecies and use similar ecological niche, but have evolved separately.

All Laminarian species display heteromorphic separated phases of diploid sporophytes and haploid gametophytes (Sauvageau 1918). This characteristic allowed us to perform detailed studies of reproductive aspects of the microscopic phases of the three species in culture conditions. Our results confirm that the sexual reproduction of kelps depends broadly on temperature and is involved in determining geographical ranges of species.

Sex determination

Sex-ratio, the expression of a given sexual phenotype reflects key information about the mechanism of sex determination operating in a species. Our results on sex-ratio of the two cryptic species of *L. nigrescens* suggest that sex determinism is predominantly genetic, although we also found that temperature affects the microscopic stages of the life cycle, leading in some conditions to unbalanced sex-ratios. Our results on sex-ratio are in agreement with the cytological observation suggesting the existence of a sexual chromosome in Laminarian species (Evans 1963, Evans 1965, Yasui 1992). Studies on other algae have reported sex determination through a sexual locus. Some examples are the pennate diatom *Seminavis robusta* (Vanstechelman et al. 2009), the green microalgae *Chlamydomonas reinhardtii* (Ferris and Goodenough 1994, Ferris et al. 2002) and the colonial *Volvox* sp. (Ferris et al. 2010). Recently, a non-recombinant zone of about 800 kpb was considered as a putative sex locus in *Ectocarpus siliculosus* (Coelho, pers.comm., Heesch et al. 2010 in press, Dittami et al. Submitted).

The potential "sex locus" found in *Ectocarpus siliculosus* should be conserved in related species and, given the phylogenetic relatedness between Ectocarpales and Laminariales (Draisma et al. 2001), it would be interesting to test its occurrence in male gametophytes of Laminarian species. For this purpose, I created a bank of separated genders of gametophytes of *L. digitata*, from different locations, which is intended to be

used in genetic studies of sex determination of this species. The idea is to have high polymorphism in autosomic regions of gametophytes so that the common region in males should correspond to the sex region for *E. siliculosus* and should not be amplified in female gametophytes. If this sex marker does not amplify in male gametophytes of *L.digitata*, a detailed study with Amplified Fragment Length Polymorphism (AFLP) must be done to identify loci that exclusive to male or female gametophytes. The identification of a genetic loci for sex determination could permit better understanding of the mating system of the cryptic species of *L.nigrescens*, for instance, how unbalanced sex ratios appear at the range limits.

Range limits

Marginal populations were studied with three different approaches. Sex-ratio variations and local adaptation of the microscopic stages were detected at the range limit of both cryptic species of the *Lessonia nigrescens* complex along the Chilean coast. A clear case of geographical parthenogenesis was found at the range limit of *Laminaria digitata* in the Brittany coast. These findings are discussed below.

The geographically distinct distribution of sexuals and asexuals is called geographical parthenogenesis (Vandel 1928). In *Lessonia nigrescens*, we expected to find sex ratios biased only towards females, in particular in marginal populations, because a previous study suggested that the cytological mechanisms associated with parthenogenesis was endomitosis (Oppliger et al. 2007) (see annex 1). This idea was also supported by the observations in two others species : *Undaria pinnatifida* and *Laminaria japonica* (Fang 1983, 1984). If parthenogenesis happened in natural populations. we expected that such a mechanism would produce 100% of female progenies

The results obtained in marginal populations of both cryptic species in the *Lessonia nigrescens* complex showed unbalanced sex-ratios towards both female and male gametophytes. The fact that, contrary to the expected, no progeny was 100% female gametophytes lead us to examine other possibilities. The biased sex ratios found in marginal populations of *L. nigrescens* could be due to differential mortality between sexes as shown for some Laminariales where females displayed more tolerance to stress (Bartsch 1993), but this hypothesis could only explain female-biased progenies. Studies of dioecious terrestrial plants have found a recurrent pattern of the presence of male-biased sex ratios in marginal populations experiencing higher levels of environmental stress. One proposed explanation is

that female reproduction requires more expenditure of resources (Delph 1999). Biased sex ratios can also be produced by segregation distortion, where one gene or allele segregates in excess of the Mendelian ratio as it is present in more than half of the gametes produced by meiosis. This phenomenon is common in animals (Hartl et al. 1967, Silver 1985) and fungi (Nauta and Hoekstra 1993, Thomas et al. 2003), but few reports are available for plants.

We can suppose that marginal populations are not at equilibrium. Evolutionary processes that determine equilibrated sex ratios of 1:1 might be in disequilibrium, resulting in biased sex ratios. In the *Lessonia nigrescens* complex, it would be interesting to test the potential sexual locus found in Ectocarpus at the range limits. If gene distorters are the responsible for the biased sex ratio found at the transition zone at 30°S (marginal populations) we would probably found disequilibrium between genotype and phenotype (Kawecki 2008).

Another possibility is that parthenosporophytic individuals in *L. nigrescens* are not able to reach maturity. To verify this hypothesis a hierarchical sampling was done in 9 locations, including marginal and central populations, for the Southern species of *L. nigrescens* for genetic analyses with microsatellite markers (Faugeron et al. 2009). This sampling comprised an area of around 1500 km (see annex 2). The strategy was to sample juveniles and adults in the same quadrats following the hierarchical design (annex 2, Figure 3) and to compare juvenile genotypes to adult genotypes located in the same quadrats. If the hypothesis of parthenogenesis by endomitosis occurs in *L. nigrescens* in nature, we would expect to find homozygotes to all loci at the juvenile stage and not at the adult stage. Also, this pattern was expected to occur more in marginal populations than in central populations. The genetic indexes of diversity (He and Na) were expected to be lower at the margins than at the center, and the indexes of deficit in heterozygotes (Fis) and genetic differentiation (Fst) to show excess of homozygotes and stronger genetic differentiation at the margins than at the center of distribution. This genetic study started with DNA extractions, PCRs and setting the protocols for sequencing, however, it could not be completed for this thesis because of the lack of time.

The population genetic study on juveniles and adults from the Southern species of *L.nigrescens* must be finished. The broad hierarchical sampling on marginal and central populations will give key insights in the genetic correspondence between new recruits settling in the shore, and older sporophyte plants. This will provide crucial information of the viability of parthenosporophytes, if they do ocurr by endomitosis, but also about the mating system, dispersion and genetic structure of kelps forests in the Chilean coast.

Geographical parthenogenesis was detected in *Laminaria digitata*. The low genetic diversity displayed by the population of Quiberon when compared with the central populations of Porspoder and Roscoff, added to higher temperature variations in Southern Brittany, corresponded well to the classical description of a marginal population (Eckert 2001, Sexton et al. 2009). Spores from Quiberon, analysed by flow cytometry, were mainly diploid when compared to those from central populations. These results suggested the occurrence of abnormal meiosis, which has been documented in *Ectocarpus siliculosus* grown in laboratory (Bothwell et al. 2010). Well developed gametophytes from spores from Quiberon and Roscoff, which later developed into sporophytes, were used to compare nuclear area. Comparisons between gametophytes initiated from Quiberon and Roscoff spores were consistent with a possible higher ploidy level in Quiberon gametophytes. Further, microstaellite analysis showed that some gametophytes from Quiberon were heterozygotes.

Given the high potentiality of kelps to display diverse types of asexual reproduction (Fang et al. 1978, Fang and Dai 1980, Fang 1983, 1984, Ar-Gall et al. 1996, Lewis 1996, Oppliger et al. 2007, Bothwell et al. 2010), we consider that at Quiberon is probably operating a combination of sexual and asexual strategies. Flow cytometry analyses done on Quiberon plants show that, although diploid spores were often dominant, most individuals often produced at least some haploid spores. These results suggest that some spores might be the result of apomeiosis and others of complete meiosis. Also the genotyping of progenies made us suspect the occurrence of apomixis, but not excluding the possibility of apomeiosis.

In *Laminaria digitata,* the preliminary study done on some gametophytes from Quiberon displayed heterozygotes and homozygotes for two microsatellite locus. These finding made us suspect of apomixis. A complete study with the 7 microsatellites available for *L.digitata* must be done, to verify this hypothesis.

Developed gametophytes from Quiberon preserved diploidy but evaded the haploid phase. The complex heteromorphic life cycle of kelps has been maintained, but syngamy, one of the costs of this life cycle due to the need for encounter of compatible partners, has been reduced by the addition of asexual reproduction. One of the strongest genetic consequences of being diploid is that nearly all deleterious mutations within the genome are masked, which has been hypothesized to hasten the rate of adaptation to new environments by permitting a larger pool of mutant alleles. This pool provides a source of genetic variability that may prove to be beneficial as the environment changes. Likewise, the acquisition of new functions may be faster in diploids because the "extra" gene copy can

preserve mutant alleles that serve a new purpose, while in haploids gene duplication must predate the evolution of novel functions (Mable and Otto 1998, Otto and Gerstein 2008).

A reciprocal transplant experiment was performed between a range edge population (Quiberon) and a central population (Roscoff) of *L.digitata* between July 2009 and June 2010 (annex 3). We estimated the responses of the transplanted individuals in terms of fitness. If range edge was solely explained by genetic limitations, we expected a higher fitness for those individuals belonging to the same site and a lower fitness for transplanted individuals. In contrast, if range edge was solely explained by environmental selective factors, we expected no differences among transplants within each transplantation site.

Fitness estimates consisted in individual growth, survival and reproduction (annex 3). This latter one included spore production and quality. We also measured meiosis success in these spores by flow cytometry, to estimate ploidy level indirectly by DNA content. Analyses of fitness estimates are not included in this thesis, however, spore production and quality, and flow cytometry results of spores are summarized in annex 3, Table 1.

Spore quality and ploidy was different among individuals of different origin in each site. Spores produced from Quiberon individuals transplanted to Roscoff were non-motile (lacking flagella) and were purely diploid or a mix of haploid and diploid spores. In contrast, spores produced by individuals originally from Roscoff were motile and haploid. These results suggest that range edge responses might be explained mainly by genetic limitations instead of environmental selective factors. However, these results must be verified by increasing replicates and culturing spores, in order to observe developmental patterns and genotyping of parent-descendants to study the cytological mechanism associated.

The idea of apomeiosis in spores of *L. digitata* leads us to think of a possible mechanism for parthenogenesis at the range margins of *L. nigrescens*. Fertile plants were sampled in two central populations (Concepcion n=6 and Las Cruces n=30) and one marginal population (Ermitaño n=16) of the Southern species (data not shown). Spore analyses were performed with flow cytometry to make ploidy estimates. Consistently, central populations displayed haploid profiles, while spores from Ermitaño displayed diffused peaks at three ploidy levels of DNA content. Although still unclear, these results might suggest a case of apomeiosis followed by apoptosis. The frequent existence of one or more peaks with lower DNA fluorescence might indicate the controlled destruction of nuclei, as it occurs during apoptosis (a form of programmed cell death) (Fraker et al. 1995). However these data must be verified with another method, for example, using a vital colorant that discriminates between dead/alive spores by microscopy (e.g., with Sytox Green staining of

live cells). With this simple approach it would be possible to detect apomeiosis in marginal populations of the two cryptic species of the *L. nigrescens* complex.

The occurrence of apomeiosis followed by apoptosis must be verified in marginal and central populations. As enounced above, a vital colorant can be use to distinguish between dead or alive spore. Repetitions on the flow cytometer could be done, increasing the number of samples and the number of marginal populations.

A more detailed study could be done to identify the cytological mechanism associated with parthenogenesis by using microsatellite markers (Faugeron et al. 2009). Parents and progenies from marginal and central populations can be genotyped to distinguish between apomeiosis and other asexual possibilities (i.e. endomitosis or automixis). Also the number of progenies can be increase in the analyses and the number of marginal populations as well. This way a more complete vision of the reproductive system in the *L.nigrescens* complex could be obtain.

The study of geographical parthenogenesis in *Laminaria digitata* is the first one reported on Laminariales. However, studies done on the red algae *Mastocarpus papillatus* (Fierst et al. 2010) and in the fucalean *Fucus serratus* (Serrão et al. 1999) have previously suggested the existence of this phenomenon in nature based on culture approaches alone. There is a lack of marine studies done on this field and the few available are based on only one approach (either cultures or genetic) (i.e. the case of the Baltic Sea (Johannesson and André 2006)). It is important to characterize geographical parthenogenesis with several approaches (i.e. genetic, culturing, microscopy) to have a broader view of the mechanisms operating and to enlighten the ecological and evolutionary consequences in a species. We could imagine that for exploited species, it would be interesting to identify how marginal populations work to create different conservation programs according to reproductive capacities of the different populations paying special attention to small, fragmented and isolated marginal populations. This study done in *L.digitata* identified a new asexual mechanism in nature that must be consider in the evolutionary potentials and consequences of asexuality in marine communities. The phenomenon of geographical parthenogenesis opens more questions of about the paradoxical maintenance of sex, given the variety of different asexual mechanisms already reported not only in marine, but also in terrestrial environments.

This thesis explored ecological and evolutionary aspects of reproduction species with haplo-diplontic life cycle with different approaches. We obtained insights of about sex

determination, the distribution of species and marginal population responses. It was very interesting to study marine species, but more interesting was to realize that are common ecological patterns with terrestrial environments and similar evolutionary consequences. This work raised several questions of reproductive systems in population biology, that will probably have unexpected responses. Sex still remains a mistery.

Literature cited

Ar-Gall, E., A. Asensi, D. Marie, and B. Kloareg. 1996. Parthenogenesis and apospory in the Laminariales: a flow cytometry analysis. European Journal of Phycology **31**:369-380.

Bartsch, I. 1993. Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. Marine Ecology Progress Series **100**:253-264.

Bothwell, J. H., D. Marie, A. F. Peters, J. M. Cock, and S. M. Coelho. 2010. Role of endoreduplication and apomeiosis during parthenogenetic reproduction in the model brown alga Ectocarpus. New Phytologist **188**:111-121.

Delph, L. F. 1999. Sexual dimorphism in life history. Springer-Verlag, Berlin.

Dittami, S. M., C. Proux, S. Rousval, S. M. Coelho, A. F. Peters, J. M. Cock, J. Coppée, C. Boyen, and T. Tonon. Submitted. Comparative genome hybridization of different strains and ecotypes of *Ectocarpus* reveal high intra-generic variability. New Phytologist.

Draisma, S.G.A., Prud'Homme van Reine, W.F., Stam, W.T. and Olsen, J.L. 2001. A reassesment of phylogenetic relationships within the Phaeophyceae based on RUBISCO large subunit and ribosomal DNA sequences. Journal of Phycology **37**:586-603.

Eckert, C. G. 2001. The loss of sex in clonal plants. Evolutionary Ecology **15**:501-520.

Evans, L. 1963. A large chromosome in the laminarian nucleus. Nature **198**:215.

Evans, L. 1965. Cytological studies in the Laminariales. Annals of Botany **116**:541-562.

Fang, F. X., and J. X. Dai. 1980. The use of haploid phases in the genetic study of *Laminaria japonica*. Acta Genetica Sinica **7**:19-25.

Fang, T. C. 1983. A summary of the genetic studies of *Laminaria japonica* in China. Pages 123-136 *in* C. K., editor. Tseng. Science Press, Beijing

Fang, T. C. 1984. Some genetic features revealed from culturing the haploid cells of kelps. Hydrobiologia **116/117**:317-318.

Fang, Z. X., C. H. Tai, Y. L. Oü, C. C. Tsuei, and T. C. Chen. 1978. Some genetic observations on the monoploid breeding of *Laminaria japonica*. Scientia Sinica **21**:401-408.

Faugeron, S., D. Veliz, G. Peralta, J. Tapia, F. Tellier, C. Billot, and E. Martinez. 2009. Development and characterization of nine polymorphic microsatellite markers in the Chilean kelp *Lessonia nigrescens*. Molecular Ecology Resources **9**:937-939.

Ferris, P., B. J. S. C. Olson, P. L. De Hoff, S. Douglass, D. Casero, S. Prochnik, S. Geng, R. Rai, J. Grimwood, J. Schmutz, I. Nishii, T. Hamaji, H. Nozaki, M. Pellegrini, and J. G. Umen. 2010. Evolution of an Expanded Sex-Determining Locus in *Volvox*. Science **328**:351-354.

Ferris, P. J., E. V. Armbrust, and U. W. Goodenough. 2002. Genetic structure of the mating-type locus of *Chlamydomonas reinhardtii*. Genetics **160**:181-200.

Ferris, P. J., and U. W. Goodenough. 1994. The mating-type locus of *Chlamydomonas reinhardtii* contains highly rearranged DNA-sequences. Cell **76**:1135-1145.

Fierst, J. L., J. E. Kübler, and S. R. Dudgeon. 2010. Spatial distribution and reproductive phenology of sexual and asexual *Mastocarpus papillatus* (Rhodophyta). Phycologia **49**:274-282.

Fraker, P. J., L. E. King, D. Lill-Elghanian, W. G. Telford, and M. S. a. B. A. O. Lawrence. 1995. Chapter 4 Quantification of Apoptotic Events in Pure and Heterogeneous Populations of Cells Using the Flow Cytometer. Pages 57-76 *in* Methods in Cell Biology. Academic Press.

Hartl, D. L., Y. Hiraizumi, and J. F. Crow. 1967. Evidence for sperm dysfunction as the mechanism of segregation distortion in *Drosophila melanogaster*. Proc Natl Acad Sci USA **58**:2240-2245.

Heesch, S., G. A. Cho, A. F. Peters, G. LeCorguillé, C. Falentin, G. Boutet, S. Coëdel, C. Jubin, G. Samson, E. Corre, S. M. Coelho, and J. M. Cock. 2010 in press. A sequence-tagged genetic ma for the brown algae Ectocarpus siliculosus provides large-scale assembly of the genome sequence. New Phytologist.

Johannesson, K., and C. AndrÉ. 2006. Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. Molecular Ecology **15**:2013-2029.

Kawecki, T. J. 2008. Adaptation to Marginal Habitats. Annual Review of Ecology and Systematics **39**:321-342.

Lewis, R. J. 1996. Chromosomes of the brown algae. Phycologia **35**:19-40.

Mable, B. K., and S. P. Otto. 1998. The evolution of life cycles with haploid and diploid phases. BioEssays **20**:453-462.

Nauta, M. J., and R. F. Hoekstra. 1993. Evolutionary dynamics of spore killers. Genetics **135**:923-930.

Oppliger, L. V., A. Peters, and J. A. Correa. 2007. Parthenogenesis in the brown algae *Lessonia nigrescens* (Phaeophyceae) from Central Chile. Journal of Phycology **43**:1295-1301.

Otto, S. P., and A. C. Gerstein. 2008. The evolution of haploidy and diploidy. Current Biology **18**:1121-1124.

Sauvageau, C. 1918. Recherches sur les Laminaires des côtes de France. Mémoires de l'Académie des Sciences de Paris **56**:1-240.

Serrão, E. A., S. H. Brawley, J. Hedman, L. Kautsky, and G. Samuelsson. 1999. Reproductive success of *Fucus vesiculosus* (Phaeophyceae) in the Baltic sea. Journal of Phycology **35**:254-269.

Sexton, J. P., P. J. McIntyre, A. L. Angert, and K. J. Rice. 2009. Evolution and Ecology of Species Range Limits. Annual Review of Ecology, Evolution and Systematics **40**:415-436.

Silver, L. M. 1985. Mouse t-haplotype Annual Review of Genetics **19**:179-208.

Tellier, F., A. P. Meynard, J. A. Correa, S. Faugeron, and M. Valero. 2009. Phylogeographic analyses of the 30°S south-east Pacific biogeographic transition zone establish the occurence of a sharp genetic discontinuity in the kelp *Lessonia nigrescens*: vicariance or parapatry? Molecular Phylogenetics and Evolution **53**:679-693.

Thomas, A., J. Shykoff, O. Jonot, and T. Giraud. 2003. Sex-ratio bias in populations of the phytopathogenic fungus *Microbotryum violaceum* from several host species. International Journal of Plant Sciences **166**:649-657.

Vandel, A. 1928. La parthénogenese geographique. Contribution a l'étude biologique et cytologique de la parthénogenese naturelle. Bulletin Biologique de la France et de la Belgique **62**:164-182.

Vanstechelman, I., M. Vuylsteke, P. Vanormelingen, W. Vyverman, and K. Sabbe. 2009. Identification of the mating type locus in a pennate diatom. *in* 10th VLIZ Young Scientists' Day, Oostende.

Yasui, H. 1992. Chromosome numbers and a sex chromosome of *Laminaria yendoana* Miyabe (Phaeophyta). Nippon Suisan Gakkaishi **58**:1385.

ANNEXES

ANNEX 1:

PARTHENOGENESIS IN THE BROWN ALGA *LESSONIA NIGRESCENS* (LAMINARIALES, PHAEOPHYCEAE) FROM CENTRAL CHILE

J. Phycol. **43,** 1295–1301 (2007)

DOI: 10.1111/j.1529-8817.2007.00408.x

PARTHENOGENESIS IN THE BROWN ALGA *LESSONIA NIGRESCENS* (LAMINARIALES, PHAEOPHYCEAE) FROM CENTRAL CHILE[1*]

L. Valeria Oppliger, Juan A. Correa[2]

Departamento de Ecología, Center for Advanced Studies in Ecology & Biodiversity and LIA-DIAMS, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile

and Akira F. Peters

UMR 7139 CNRS-UPMC, Station Biologique, Place Georges Teissier, BP 74, 29682 Roscoff Cedex, France

Parthenogenesis, the development of female gametes without fertilization, is relatively common in brown algae, although limited quantitative information on the phenomenon is available. Its occurrence is reported for the first time in *Lessonia nigrescens* Bory, a member of the Laminariales and a key ecological component of the benthic algal communities along the Chilean coast. Isolated female gametophytes developed into parthenosporophytes throughout the year, with a maximum in spring to early summer. Isolated male gametophytes, on the other hand, never developed fronds. Parthenosporophytes obtained in the laboratory developed normally when cultivated under greenhouse conditions, and the resulting individuals were indistinguishable in size, shape, texture, and color from heterozygous sporophytes. Quantification of DNA of various tissues demonstrated that early during their development, parthenosporophytes duplicated their DNA content, displaying levels similar to heterozygous sporophytes and almost twice the level found in gametophytes. One out of 45 individuals from a field population yielded only female gametophytes, strongly suggesting that parthenogenesis does occur in wild stands of *L. nigrescens*.

Key index words: **DNA quantification; flow cytometry; *Lessonia nigrescens;* parthenogenesis; Phaeophyceae**

Abbreviation: **SFC, sterile filtered enriched seawater medium C**

Parthenogenesis is a form of reproduction in which a gamete develops into a new individual without fertilization. This apomictic process has been interpreted as being advantageous because it avoids some of the costs of sexual reproduction, such as finding the gamete of the opposite sex for fertilization (Judson and Normark 1996). Parthenogenesis has been observed in a wide diversity of organisms, including animals, plants, and algae (Maynard-Smith 1986, Judson and Normark 1996, Vielle Calzada et al. 1996), but the frequency of the phenomenon varies among species (Judson and Normark 1996). The phenomenon has been recently reviewed in the context of the adaptive potential that parthenogenesis provides to a wide range of living organisms (Lushai et al. 2003).

Parthenogenesis occurs in both micro- and macroalgae and in the latter group has been reported in Chlorophyta, Rhodophyta, and Phaeophyceae. Within the brown algae, parthenogenetic development of both female and male gametes (the latter also being referred to as androgenesis) is the rule in isogamous species. In anisogamous taxa, usually only the female gametes are capable of parthenogenesis, although exceptions may exist. In oogamous brown algae, such as *Dictyota* (Dictyotales), parthenogenetic development of unfertilized eggs appears to be related to the presence of centrioles in oocytes: unfertilized eggs missing a centriole do not develop (Motomura 1994, Nagasato et al. 1998), whereas eggs of members of the order Laminariales, which possess a vestigial flagellum and contain a centriole (Motomura and Nagasato 2004), may show parthenogenetic development. However, parthenogenetic sporophytes of *Laminaria saccharina* (L.) J. V. Lamour., *Lam. longicruris* Bach. Pyl., *Lam. ochotensis* Miyabe, *Lam. digitata* (Huds.) J. V. Lamour., *Eisenia arborea* Aresch., *Nereocystis luetkeana* (K. Mert.) Postels et Rupr., *Macrocystis pyrifera, M. integrifolia* (L.) C. Agardh, *Alaria marginata* Postels et Rupr., *Lessoniopsis littoralis* (Farl. et Setch. ex Tilden) Reinke, and *Costaria costata* (C. Agardh) D. A. Saunders—all originated in cultures containing only female gametophytes—have been described to develop into abnormally shaped and fragile plants, with abnormal levels of ploidy and high mortality rates (Kemp and Cole 1961, Nakahara 1984, Yabu and Notoya 1985, Yabu and Sanbonsuga 1985, 1987, 1990, Yabu and Taniguchi 1990, Lewis et al. 1993, Druehl et al. 2005). Furthermore, these parthenogenetic plants never grew more than a few

[1]Received 21 August 2006. Accepted 14 May 2007.
[2]Author for correspondence: e-mail jcorrea@bio.puc.cl.
*This is a paper of the International Associated Laboratory 'Dispersal and Adaptation in Marine Species' (Station Biologique de Roscoff and Center for Advanced Studies in Ecology and Biodiversity).

millimeters (Bolton et al. 1983, Le Gall et al. 1996). An exception to the apparently negative effect of parthenogenesis on development seems to be *Lam. japonica* Aresch., which was reported to produce normal, fertile, haploid parthenosporophytes from unisexual female gametophytes cultured in the laboratory (Fang et al. 1978, Nakahara 1984, Lewis et al. 1993). Fang also noted that spontaneous chromosome doubling occasionally occurred in haploid cell cultures producing homozygous diploid cells, from which fertile plants could be obtained (Chen and Lin 1976, Fang 1984). Moreover, female gametophytes of *Lam. japonica* derived from mature parthenosporophytes were again capable of parthenogenesis (Fang and Dai 1980). A similar phenomenon was reported for *Undaria pinnatifida* (Harv.) Suringar, in which gametophytes derived from mature parthenosporophytes experienced parthenogenesis (Fang et al. 1983).

Lessonia nigrescens belongs to the Laminariales, and it is a species that dominates in cover and biomass the lower-intertidal and shallow-subtidal areas, which are wave exposed and rocky, along most of the temperate Pacific coasts of South America, between 18 and 56° S (Kim 1971, Santelices et al. 1980, Hoffmann and Santelices 1997). This species is economically important because it is used as raw material for alginate extraction (Cancino and Santelices 1984). From an ecological point of view, *L. nigrescens* is used as food, shelter, and area for larval settlement by invertebrates and fish (Santelices et al. 1980, Cancino and Santelices 1981, Ojeda and Santelices 1984, Vásquez and Santelices 1984). *Lessonia nigrescens* has been described to have the typical *Laminaria*-type life history with dioecious, morphologically different microscopic gametophytes, and where the female-borne oocytes, after fertilization by the male-produced spermatozoids, develop into macroscopic sporophytes (Olivari 1974, Searles 1978, Avila et al. 1985). As both male and female gametophytes of *L. nigrescens* are commonly present and easily recognized in laboratory cultures due to their sexual dimorphism even at very early stages of development, the occurrence of sexual reproduction has never been questioned. Following the same rationale, the occurrence of alternative ways of producing the macroscopic sporophytes has never been tested in this species. However, our preliminary studies on the early stages of development in gametophytes and sporophytes of *L. nigrescens* revealed that male gametophytes were rarely mature, and, in spite of that, juvenile sporophytes always developed in cultures.

Thus, the objectives of the present work were to (i) assess the occurrence of parthenogenesis in *L. nigrescens*, (ii) characterize and quantify the phenomenon, (iii) assess the eventual development of parthenosporophytes, (iv) quantify DNA content of parthenosporophytes, and (v) test the occurrence of parthenogenesis in a natural population.

MATERIALS AND METHODS

Sampling and cultures. Fertile sporophytic fronds from at least five individuals of *L. nigrescens* were collected monthly from March to December 2003 in Las Cruces, central Chile (33°27′ S; 71°38′ W).

Mature sori were cleaned with running tap water, rinsed several times with sterile seawater, and incubated in 250 mL Erlenmeyer flasks with sterile seawater for 2–4 h at 16°C to obtain a spore suspension. Three drops of this suspension were inoculated into petri dishes with 4 mL of sterile filtered enriched seawater medium C (SFC; Correa et al. 1988). Spores were cultured at 10°C and a 12:12 light:dark (L:D) photoperiod, with cool-white fluorescent tubes (tlt 20W/54 RS Day Light; Philips, Sao Paulo, Brazil) yielding a photon fluence rate of 35–45 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Culture medium was changed once a week, and observations were made every 3 d.

After 12–15 d in culture, gametophytes with two to six cells and typical female morphology were selected and isolated from the starting culture using a micropipette manipulated on a Nikon Eclipse TE300 inverted microscope (Nikon Corp., Tokyo, Japan). Monthly, a total of 100 female gametophytes isolated as indicated were cultivated individually in plastic multiplates under the same conditions as the starting culture. After 50 d in culture, the isolated female gametophytes were monitored to record the presence of parthenogenetic sporophytes.

Development of the parthenosporophytic progeny. Parthenosporophytes obtained during April–May were sent to indoor culture facilities available at Universidad Austral in Puerto Montt, southern Chile. These plants were fastened to plastic frames and maintained in 100 L tanks with semicontinuous water exchange and aeration. The maximum length of each individual was recorded weekly. Heterozygous sporophytes (obtained from cultures containing mature gametophytes of both sexes) were used as controls.

DNA content. The ploidy level of parthenosporophytes and of heterozygous sporophytes was assessed by measurements of the DNA content using flow cytometry of isolated nuclei, according to a methodology described elsewhere (Le Gall et al. 1993, 1996, Asensi et al. 2001). Briefly, nuclei obtained by chopping tissue were stained with SYBR Green I (Molecular Probes Inc., Eugene, OR, USA), and DNA quantification was performed in a FACSort flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with an adjustable laser (coherent innova 90) emitting at 353–357 nm with the appropriate emission filters. Stained nuclei of *Ectocarpus siliculosus* (Dillwyn) Lyngb. (Phaeophyceae) gametes and of *Chondrus crispus* Stackh. (Rhodophyta) gametophytes were added to the samples as internal standards.

The experimental ratios of fluorescence—that is, the ratio of the intensity of fluorescence emitted by the sample population of nuclei to that of reference nuclei—were taken into account. Using this procedure, the relative DNA content was estimated for 100–1200 nuclei per sample. The ploidy level of sporophytes was estimated on the basis of their nuclear DNA content relative to that of haploid cells (Le Gall et al. 1996).

Parthenogenesis in natural populations. A total of 46 mature sori, each from a different plant of *L. nigrescens,* were collected in April 2004 from the same population sampled to obtain the reproductive material used in the first part of the study. They were prepared as explained above prior to incubation. Once cleaned, each sorus was inoculated into a 15 mL plastic tube with sterile seawater and incubated for 2–4 h to obtain a spore suspension. Three drops from each suspension were inoculated into individual petri dishes to create individual cultures for each parental plant. These cultures were maintained under the same conditions as described above. After 25 d in culture,

the ratio between male and female gametophytes was recorded for each plant.

RESULTS

Shortly after release, spores of *L. nigrescens* withdrew their flagella, became rounded, and initiated the process of settlement (Fig. 1A). Large-celled early female gametophytes (Fig. 1B) developed rapidly and within 7–12 d were clearly different from male gametophytes, which appeared at this time as smaller and thinner unbranched filaments. Female gametophytes became reproductive, both in mixed and clonal cultures, by developing at least one apical cell of their radiating vegetative filaments into an oogonium (Fig. 1C). Oocytes (Fig. 1C) were clearly distinguishable in female-only cultures after 7 d of isolation. These egg cells underwent mitosis and gave rise to erect fronds (Fig. 1D), representing the beginning of the development of parthenogenetic sporophytes. The presence of small bladelike formations attached to the female gametophytes was recorded as early as 4 d after the development of the oocyte was completed. The parthenogenetic fronds remained attached to the gametophyte (Fig. 1E), and it was possible to observe multiple parthenogenetic fronds attached to a single gametophyte (Fig. 1F). Isolated male gametophytes, maintained for up to 50 d under the same culture conditions as their female counterparts, only developed into spherical masses of thin filaments that never developed fronds. Furthermore, we found no microscopic evidence of maturation (i.e., mature male gametangia) in these isolated male gametophytes.

Parthenogenesis in cultures of *L. nigrescens* occurred during most of the year, with a maximum in spring to early summer (October–December; Fig. 2) when almost all female gametophytes produced parthenosporophytic fronds. During April and May, the same protocol used to isolate female gametophytes of *L. nigrescens* was used to isolate 100 male gametophytes each month. Androgenesis, however, was not observed in these cultures.

Parthenosporophytic and heterozygous sporophytes from April, May, and June (raised in the greenhouse) grew at similar rates, reached similar sizes, and had the same shape, texture, and pigmentation after 3 months of indoor cultivation (Fig. 3, A–C).

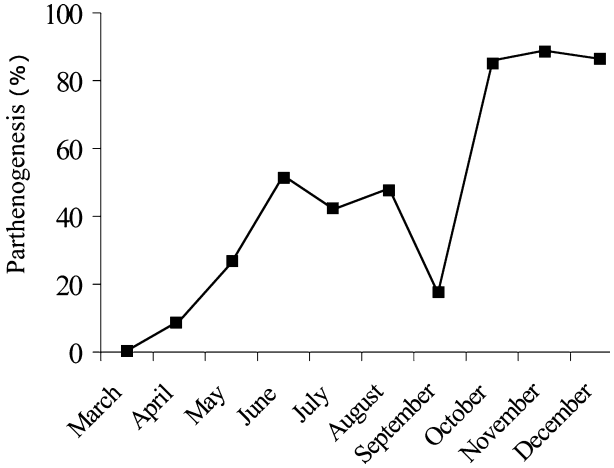


FIG. 2. Frequency of parthenogenesis in female gametophyte cultures of *Lessonia nigrescens* isolated from March to December 2003.

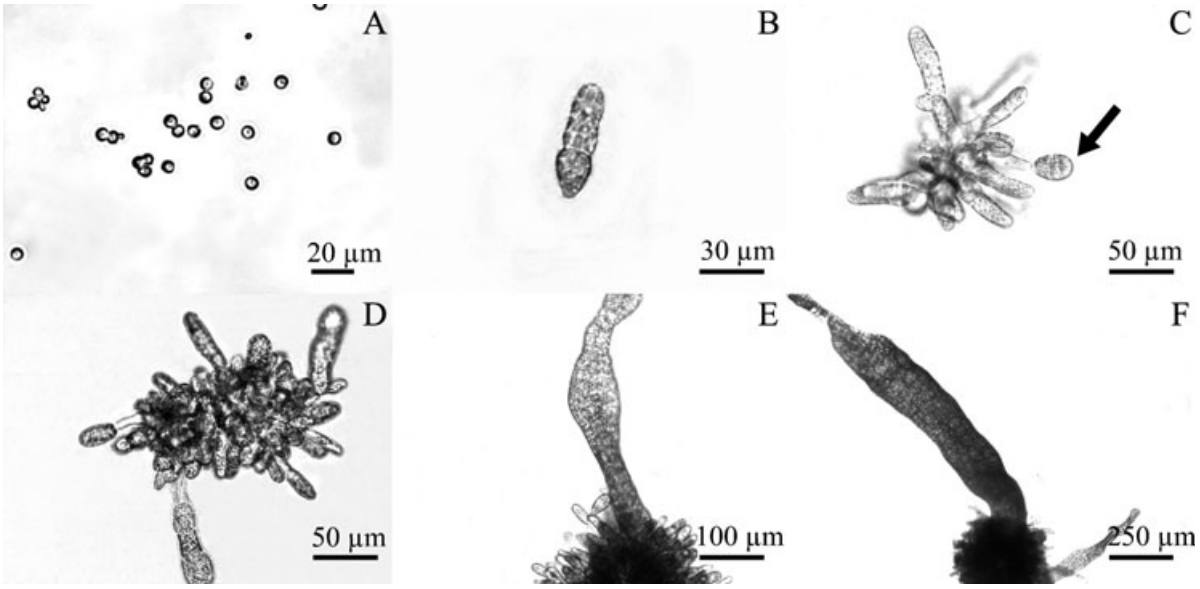


FIG. 1. Parthenogenetic development of sporophytes in *Lessonia nigrescens*. (A) One-day-old meiospores. (B) Four-celled female gametophyte at day 12. (C) Mature female gametophyte with egg-bearing oogonium (arrow) at day 21. (D) Young parthenosporophyte attached to a female gametophyte at day 31. (E) Parthenosporophyte still attached to a female gametophyte in a 43-day-old culture. (F) Two parthenosporophytes attached to a single female gametophyte at day 50.

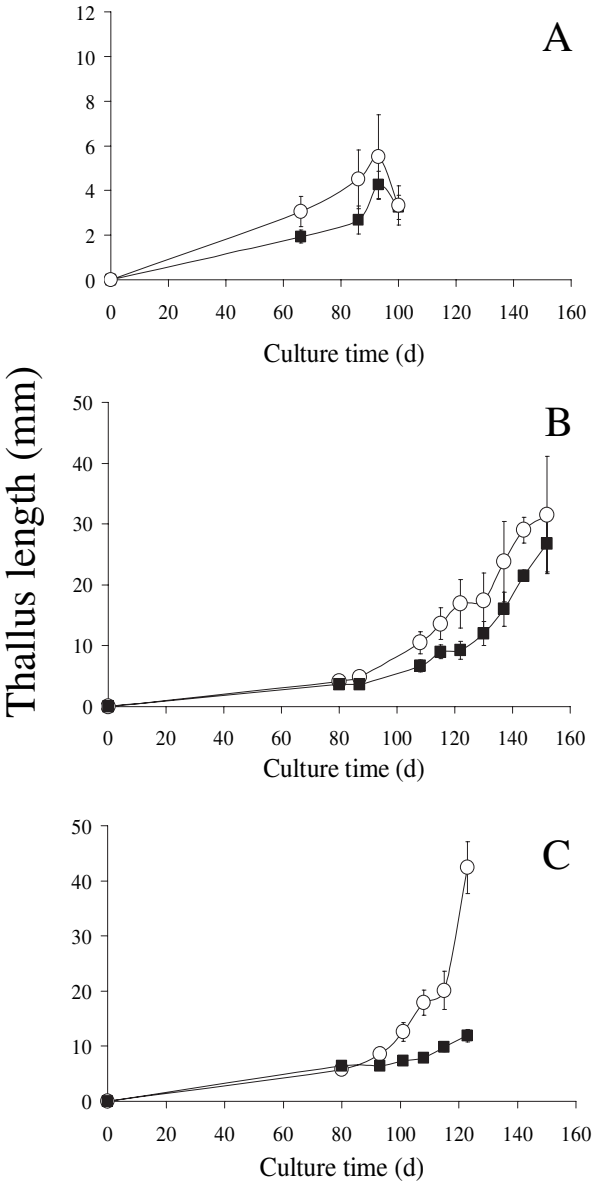


FIG. 3. Growth of tank-cultivated *Lessonia nigrescens* sporophytes from spores isolated in April (A), May (B), and June (C). ○, parthenosporophytes ($n = 5$); ■, control thalli (heterozygous sporophytes, $n = 5$). Error bars show standard error.

DNA content. The ratio between mean values of nuclear fluorescence emission of parthenosporophytic tissue from five different thalli and gametophyte filaments ($n = 5$) ranged from 1.75 to 1.86 (Table 1). The ratio between mean values of nuclear fluorescence emission of heterozygous sporophytic tissue ($n = 5$) and gametophytes ($n = 5$) was 1.8–1.91 (Table 2).

Parthenogenesis in natural populations. The ratio between male and female gametophytes obtained from 45 wild individuals was 41:59, a ratio not significantly different from 1:1 (Kruskal–Wallis test, $H = 1.08$; df = 1, $P = 0.299$), indicating that the sampled population of *L. nigrescens* consists almost exclusively of heterozygote individuals. However, one individual produced only female offspring.

TABLE 1. Mean values of fluorescence emitted from nuclei from five different parthenosporophytic individuals of *Lessonia nigrescens*.

Measurement	Individuals	No. nuclei	Emission	2*n*:*n*
1	PS-1	445	181.8	1.81
2	PS-2	567	199.7	1.75
3	PS-3	437	229.0	1.83
4	PS-4	888	231.7	1.85
5	PS-5	183	217.4	1.86

2*n*:*n* = parthenosporophytic:gamethophyte emission ratio.

TABLE 2. Mean values of fluorescence emitted from nuclei from five different heterozygous sporophyes of *Lessonia nigrescens*.

Measurement	Individuals	No. nuclei	Emission	2*n*:*n*
1	NS-1	747	183.6	1.84
2	NS-2	337	205.4	1.91
3	NS-3	187	169.4	1.8
4	NS-4	522	169.7	1.84
5	NS-5	274	167.5	1.82

2*n*:*n* = heterozygous sporophyte:gamethophyte emission ratio.

DISCUSSION

Our results demonstrate that the phenomenon of parthenogenesis takes place in *L. nigrescens*, both in nature and in culture. One out of 45 individuals sampled in the wild population studied yielded female gametophytes only. The latter condition has been used in *Lam. japonica* as a diagnostic feature of parthenogenesis (Fang 1983), and as Lewis et al. (1993) commented, it is consistent with earlier observations that sex determination in brown algae is genotypic (Schreiber 1930, 1935, Evans 1965, Müller 1967, Yabu and Sanbonsuga 1981). In culture, a surprisingly large proportion of clonal female gametophytes (up to 90%) produced sporophytes in the absence of male gametes. We rule out contamination by male gametophytes, mainly due to the differential development rate and time to reach maturity that was observed for female and male gametophytes. These differences allowed us to identify and isolate female gametophytes early in the process, well before any indication of maturity was detected in the co-occurring male gametophytes. Furthermore, microscopic observation found no signs of cryptic monoecism (i.e., male gametangia on female gametophytes).

Parthenosporophytes of *L. nigrescens* developed normally both in the laboratory and when cultivated in tanks, resembling previous findings in *Lam. japonica* (Fang 1983, 1984, Lewis et al. 1993), and *U. pinnatifida* (Yan 1984, Kawashima and Tokuda 1993), in which parthenosporophytes produced in

the laboratory were raised in culture. In *Lam. japonica*, such parthenosporophytes were even cultivated for up to nine successive generations in the sea (Lewis et al. 1993). The apparent normality in the development of parthenosporophytes of *L. nigrescens* and *Lam. japonica* contrasts with several reports on other Laminariales, suggesting that parthenogenesis is more of a rarity and that most of the resulting parthenosporophytes are abortive or abnormal (Sundene 1958, Kemp and Cole 1961, Svendsen and Kain 1971, Nakahara and Nakamura 1973, Chapman 1974, Lüning 1975, Fang et al. 1978, Sanbonsuga and Neushul 1978, Bolton et al. 1983, Fang 1983, Bharathan and Shinmura 1986, tom Dieck 1992, Lewis and Neushul 1994, Druehl et al. 2005). In a broader context, abnormal development of sporophytes in brown algae has been associated with their parthenogenetic origin (Clayton 1988), but there are also several reports of normal development as haploid individuals, as in *Arthrocladia villosa* (Huds.) Duby (Müller and Meel 1982), *Desmarestia viridis* (O. F. Müll.) J. V. Lamour. (Nakahara 1984), *Halosiphon tomentosus* (Lyngb.) Jaasund (Maier 1984), and *Haplospora globosa* Kjellm. (Kuhlenkamp and Müller 1985).

In the case of *L. nigrescens*, there are at least three elements that suggest that parthenogenesis is not a rarity. First, the phenomenon involved female gametophytes isolated through most of the year. Second, parthenogenesis was expressed in a large proportion of the female gametophytes, with levels close to 90% in spring gametophytes. Lastly, the phenomenon also occurs in the wild, as concluded from the presence of a sporophyte containing only the female sex factor.

Quantification of DNA contents indicated that both parthenosporophytes and heterozygous sporophytes of *L. nigrescens* were diploid. The results obtained from flow cytometry were in agreement with microfluorometric measurements, in which DAPI-stained nuclei of parthenogenetic sporophytes showed 1.7 times the fluorescence of those in gametophyte filaments (data not shown in detail). These results suggest that parthenosporophytes of *L. nigrescens* acquired diploid DNA content during the development of the frond. In this context, it seems possible that an early diploidization of parthenosporophytes in *L. nigrescens* is responsible for their vigor. The evidence available in the literature is controversial on this issue. On the basis of chromosome counts and the fact that parthenosporophytes reproduced normally in farms, Fang (1983, 1984) and Fang et al. (1978) concluded that sporophytes of *Lam. japonica* developed from female gametophytes were diploid and that spores from these parthenosporophytes were the result of meiosis during maturation of the sporophytes (Fang and Dai 1980). Likewise, Lewis et al. (1993) recognized that a large proportion of their parthenogenetic kelp sporophytes were diploid, but they also showed that young and mature parthenogenetic sporophytes of *Lam. japonica* could be haploid and that sporogenesis in those individuals did not involve a reduction in the chromosome numbers. The same study demonstrated that in spite of the fact that parthenosporophytes could display up to hexaploidy, the three strains that became reproductive were haploid (Lewis et al. 1993).

There is no consistent developmental pattern following parthenogenesis in isogamous brown algae. In *E. siliculosus*, Müller (1967) observed that nonfused gametes developed into parthenosporophytes, but chromosome counts showed that parthenosporophytes remained either haploid or underwent vegetative diploidization. Nonfused gametes of other isogamous brown algae, whose chromosomes were counted, may develop into haploid sporophytes (Henry and Müller 1983), haploid gametophytes (Müller 1984), either haploid gametophytes or sporophytes (Nakahara 1984, Clayton 1986), or either haploid gametophytes or haploid or diploid sporophytes (Peters 1988). Thus, the mechanisms regulating the development into sporophyte or gametophyte generations in brown algae remain to be elucidated.

Even though our study was not focused on the fate of the male gametophytes, the only experiment performed with this purpose did not reveal any signs of frond development. This response differs from that of *Lam. japonica*, where androgenesis (Laviolette and Grassé 1971), or the formation of the so-called male sporophytes, has been reported. It was recognized, however, that these individuals were abnormal in morphology, grew very slowly, and rarely reached a normal size when farmed (Fang 1983). Furthermore, even in cases where *Lam. japonica* male sporophytes reached normal size, they never became reproductive. Androgenesis regularly occurs in isogamous Phaeophyceae such as *E. siliculosus* (Müller 1967). In the anisogamous brown alga *Colpomenia peregrina* Sauv., both female and to a much lesser extent male nonfused gametes produced partenosporophytes (Yamagishi and Kogame 1998). In fact, this greater success of female parthenosporophytes in culture was considered responsible for the female dominance observed in wild stands of *C. peregrina* in Japan. Androgenesis in oogamous species appears to be rare. In dioecious *Desmarestia firma* (C. Agardh) Skottsb. from Chile, Ramírez et al. (1986) observed that sporophytes developed from settled male gametes. Chromosome counts showed that the ploidy of such androgenetic sporophytes was either haploid or diploid.

For technical reasons, we were not able to transplant parthenosporophytes of *L. nigrescens* to their natural habitat (i.e., the wave-swept lower intertidal zone of exposed rocky shores), and we do not know whether they could reproduce like the cultivated strains of *Lam. japonica* (Lewis et al. 1993).

However, indirect evidence that parthenosporophytes of *L. nigrescens* may reach maturity is provided by the finding of a parthenosporophyte from the wild stand at Las Cruces. Certainly, more experimental work is needed, particularly to address the ecological consequences of this reproductive strategy. In this context, it is worthwhile to note that some studies have reported the dominance of female individuals in wild populations of brown algae, including *C. peregrina* (Yamagishi and Kogame 1998), *Cutleria multifida* (Turner) Grev. (Fletcher 1987, Womersley 1987), and *Cutleria cylindrica* Okamura (Kitayama et al. 1992). No information on this aspect is available for Laminariales, let alone for *L. nigrescens*. Unless large-scale germination experiments with sporophyte-derived spores are undertaken, there is little hope of unmasking hidden parthenosporophytes in wild stands of *L. nigrescens*. However, molecular markers designed to assess the level of homozygosity or to directly identify sex loci could be useful tools to quantify the frequency of parthenogenetic sporophytes in natural kelp populations.

We deeply thank D. Marie, S. Faugeron, R. Westermeier, D. Patiño, V. Flores, E. Martínez, and J. Beltrán for their help during various stages of the research. We also thank Drs. E. Henry and M. Graham for advice and suggestions that improved the first version of the paper. We thank the support by Fundación Andes. This study is part of the research program FONDAP 1501 0001 funded by CONICYT, to the Center for Advanced Studies in Ecology & Biodiversity (CASEB) Program 7.

Asensi, A., Ar Gall, E., Marie, D., Billot, C., Dion, P. & Kloareg, B. 2001. Clonal propagation of *Laminaria digitata* (Phaeophyceae) sporophytes through a diploid cell-filament suspension. *J. Phycol.* 37:411–7.

Avila, M., Hoffmann, A. J. & Santelices, B. 1985. Interacciones de temperatura, densidad, flujo fotónico y fotoperíodo sobre el desarrollo de etapas microscópicas de *Lessonia nigrescens* (Phaeophyta, Laminariales). *Rev. Chil. Hist. Nat.* 58:71–82.

Bharathan, G. & Shinmura, I. 1986. A note on parthenogenesis in four species of Laminariales. *Jpn. J. Phycol.* 34:13–8.

Bolton, J. J., Germann, I. & Lüning, K. 1983. Hybridization between Atlantic and Pacific representatives of the Simplices sections of *Laminaria* (Phaeophyta). *Phycologia* 22:133–40.

Cancino, J. & Santelices, B. 1981. The ecological importance of kelp-like holdfast as a habitat of invertebrates in central Chile. II. Factors affecting community organization. *Proc. Int. Seaweed Symp.* 10:241–6.

Cancino, J. & Santelices, B. 1984. The ecological importance of holdfasts of *Lessonia nigrescens* Bory (Phaeophyta) in Central Chile. *Rev. Chil. Hist. Nat.* 57:23–33.

Chapman, A. R. O. 1974. The genetic basis of morphological differentiation in some *Laminaria* populations. *Mar. Biol. (Berl.)* 24:85–91.

Chen, C. C. & Lin, M. H. 1976. Introduction of rice plantlets from anther culture. *Bot. Bull. Acad. Sin.* 17:18–24.

Clayton, M. N. 1986. Culture studies on the life history of *Scytothamnus australis* and *Scytothamnus fasciculatus* (Phaeophyta) with electron microscope observations on sporogenesis and gametogenesis. *Br. Phycol. J.* 21:371–86.

Clayton, M. N. 1988. Evolution and life histories of brown algae. *Bot. Mar.* 31:379–87.

Correa, J. A., Nielsen, R. & Grund, D. W. 1988. Endophytic algae of *Chondrus crispus* (Rhodophyta). II. *Acrochaete heteroclada* sp. nov., *A. operculata* sp. nov., and *Phaeophila dendroides* (Chlorophyta). *J. Phycol.* 24:528–39.

Druehl, L. D., Collins, J. D., Lane, C. E. & Saunders, G. W. 2005. An evaluation of methods used to assess intergeneric hybridization in kelp using Pacific Laminariales (Phaeophyceae). *J. Phycol.* 41:250–62.

Evans, L. V. 1965. Cytological studies in the Laminariales. *Ann. Bot.* 24:541–62.

Fang, T. C. 1983. A summary of the genetic studies of *Laminaria japonica* in China. *In* Tseng, C. K. [Ed.] *Proceedings of the Joint China-U.S. Phycology Symposium.* Science Press, Beijing, pp. 123–36.

Fang, T. C. 1984. Some genetic features revealed from culturing the haploid cell of kelps. *Hydrobiologia* 116/117:317–8.

Fang, Z. X. & Dai, J. X. 1980. The use of haploid phases in the genetic study of *Laminaria japonica. Acta Genet. Sin.* 7:19–25.

Fang, T. C., Tai, C. H., Oü, Y. L., Tsuei, C. C. & Chen, T. C. 1978. Some genetic observations on the monoploid breeding of *Laminaria japonica. Sci. Sin.* 21:401–8.

Fang, Z., Yan, Z. & Wang, Z. 1983. Some preliminary observations on tissue cultures in *Laminaria japonica* and *Undaria pinnatifida. Kexue Tongbao* 28:247–9.

Fletcher, R. L. 1987. *Seaweeds of the British Isles. Vol. 3. Fucophyceae (Phaeophyceae) Part I.* British Museum (Natural History), London, 359 pp.

Henry, E. C. & Müller, D. G. 1983. Studies on the life history of *Syringoderma phinneyi* n. spec. (Phaeophyceae). *Phycologia* 22:387–94.

Hoffmann, A. & Santelices, B. 1997. *Marine Flora of Central Chile.* Ediciones Universidad Católica de Chile, Santiago, 434 pp.

Judson, O. P. & Normark, B. B. 1996. Ancient asexual scandals. *Trends Ecol. Evol.* 11:41–6.

Kawashima, Y. & Tokuda, H. 1993. Regeneration from callus of *Undaria pinnatifida* (Harvey) Suringar (Laminariales, Phaeophyta). *Hydrobiologia* 260/261:385–9.

Kemp, L. & Cole, K. 1961. Chromosomal alternation of generations in *Nereocystis luetkeana* (Mertens) Postels and Ruprecht. *Can. J. Bot.* 39:1711–24.

Kim, D. H. 1971. A guide to the literature and distribution of the benthic algae in Chile. Part I. Chlorophyceae-Phaeophyceae. *Gayana Bot.* 1:3–82.

Kitayama, T., Kawai, H. & Yoshida, T. 1992. Dominance of female gametophytes in field populations of *Cutleria cylindrica* (Cutleriales, Phaeophyceae) in the Tsugaru Strait, Japan. *Phycologia* 31:449–61.

Kuhlenkamp, R. & Müller, D. G. 1985. Culture studies on the life history of *Haplospora globosa* and *Tilopteris mertensii* (Tilopteridales, Phaeophyceae). *Br. Phycol. J.* 20:301–12.

Laviolette, P. & Grassé, P. P. 1971. *Allgemeine Biologie. Vol. 2. Fortpflanzung und Sexualität.* Gustav Fisher, Stuttgart, Germany, 256 pp.

Le Gall, E., Asensi, A., Marie, D. & Kloareg, B. 1996. Parthenogenesis and apospory in the Laminariales: a flow cytometry analysis. *Eur. J. Phycol.* 31:369–80.

Le Gall, Y., Brown, S., Marie, D., Mejjad, M. & Kloareg, B. 1993. Quantification of nuclear DNA and G–C content in marine macroalgae by flow cytometry of isolated nuclei. *Protoplasma* 173:123–32.

Lewis, R. J., Jiang, B. Y., Neushul, M. & Fei, X. G. 1993. Haploid parthenogenetic sporophytes of *Laminaria japonica* (Phaeophyceae). *J. Phycol.* 29:363–9.

Lewis, R. J. & Neushul, M. 1994. Northern and southern hemisphere hybrids of *Macrocystis* (Phaeophyceae). *J. Phycol.* 30:346–53.

Lüning, K. 1975. Kreuzungsexperimentean *Laminaria saccharina* von Helgoland und von der Isle of Man. *Helgol. Wiss. Meeresunters.* 27:108–14.

Lushai, G., Loxdale, H. D. & Allen, J. A. 2003. The dynamic clonal genome and its adaptive potential. *Biol. J. Linn. Soc.* 79:193–208.

Maier, I. 1984. Culture studies of *Chorda tomentosa* (Phaeophyta, Laminariales). *Br. Phycol. J.* 19:95–106.

Maynard-Smith, J. 1986. Contemplating life without sex. *Nature* 324:300–1.

Motomura, T. 1994. Electron and immunofluorescence microscopy on the fertilization of *Fucus distichus* (Fucales, Phaeophyceae). *Protoplasma* 178:97–110.

Motomura, T. & Nagasato, C. 2004. The first spindle formation in brown algal zygotes. *Hydrobiologia* 512:171–6.

Müller, D. G. 1967. Generationswechsel, Kernphasenwechsel und Sexualität der Braunalge *Ectocarpus siliculosus* im Kulturversuch. *Planta* 75:39–54.

Müller, D. G. 1984. Culture studies on the life history of *Adenocystis utricularis* (Phaeophyceae, Dyctiosiphonales). *Phycologia* 23:87–94.

Müller, D. G. & Meel, H. 1982. Culture studies on the life history of *Arthrocladia villosa* f. *australis* (Desmarestiales, Phaeophyceae). *Br. Phycol. J.* 17:419–25.

Nagasato, C., Motomura, T. & Ichimura, T. 1998. Selective disappearance of maternal centrioles after fertilization in the anisogamous brown alga *Cutleria cylindrica* (Cutleriales, Phaeophyceae): paternal inheritance of centrioles is universal in brown algae. *Phycol. Res.* 46:191–8.

Nakahara, H. 1984. Alternation of generations of some brown algae in unialgal and axenic cultures. *Sci. Pap. Inst. Algol. Res. Fac. Sci. Hokkaido Univ.* 7:77–292.

Nakahara, H. & Nakamura, Y. 1973. Parthenogenesis, apogamy and apospory in *Alaria crassifolia* (Laminariales). *Mar. Biol. (Berl.)* 18:327–32.

Ojeda, F. P. & Santelices, B. 1984. Ecological dominance of *Lessonia nigrescens* (Phaeophyta) in Central Chile. *Mar. Ecol. Prog. Ser.* 19:83–91.

Olivari, R. 1974. Nota sobre algunos aspectos del desarrollo inicial de gametofitos y ciclo reproductivo de *Lessonia nigrescens. Inv. Mar.* 5:85–96.

Peters, A. F. 1988. Culture studies of a sexual life history in *Myriotrichia clavaeformis* (Phaeophyceae, Dictyosiphonales). *Br. Phycol. J.* 23:299–306.

Ramírez, M. E., Müller, D. G. & Peters, A. F. 1986. Life history and taxonomy of two populations of ligulate *Desmarestia* (Phaeophyceae) from Chile. *Can. J. Bot.* 64:2948–54.

Sanbonsuga, Y. & Neushul, M. 1978. Hybridization of *Macrocystis* (Phaeophyta) with other float-bearing kelps. *J. Phycol.* 14:214–24.

Santelices, B., Castilla, J. C., Cancino, J. & Schmiede, P. 1980. Comparative ecology of *Lessonia nigrescens* and *Durvillaea Antarctica* (Phaeophyta) in Central Chile. *Mar. Biol.* 59:119–32.

Schreiber, E. 1930. Untersuchungen über Parthenogenesis, Geschlechtsbestimmung und Bastardisierungsvermögen bei Laminarien. *Planta* 12:331–53.

Schreiber, E. 1935. Über Kultur und Geschlechtsbestimmung von *Dictyota dichotoma. Planta* 24:266–75.

Searles, R. B. 1978. The genus *Lessonia* Bory (Phaeophyta, Laminariales) in Southern Chile and Argentina. *Br. Phycol. J.* 13:361–81.

Sundene, O. 1958. Interfertility between forms of *Laminaria digitata. Nytt. Mag. Bot.* 6:121–8.

Svendsen, P. & Kain, J. M. 1971. The taxonomic status, distribution and morphology of *Laminaria cucullata* sensu Jorde and Klavestad. *Sarsia* 46:1–22.

tom Dieck, I. 1992. North Pacific and North Atlantic digitate *Laminaria* species (Phaeophyta): hybridization experiments and temperature responses. *Phycologia* 31:147–63.

Vásquez, J. A. & Santelices, B. 1984. Comunidades de macroinvertebrados en discos adhesivos de *Lessonia nigrescens* Bory (Phaeophyta) en Chile Central. *Rev. Chil. Hist. Nat.* 57:131–54.

Vielle Calzada, J., Crane, C. F. & Stelly, D. M. 1996. Apomixis: the asexual revolution. *Science* 274:1322–3.

Womersley, H. B. S. 1987. *The Marine Benthic Flora of Southern Australia. Part II.* South Australian Government Printing Division, Adelaide, 484 pp.

Yabu, H. & Notoya, M. 1985. Nuclear divisions in the young sporophytes of *Ecklonia stolonifera. Bull. Fac. Fish. Hokkaido Univ.* 36:83–6.

Yabu, H. & Sanbonsuga, Y. 1981. A sex chromosome in *Cymathere japonica* Miyabe et Nagai. *Jpn. J. Phycol.* 29:79–80.

Yabu, H. & Sanbonsuga, Y. 1985. Mitosis in the gametophytes and young sporophytes of *Macrocystis angustifolia. Jpn. J. Phycol.* 33:1–4.

Yabu, H. & Sanbonsuga, Y. 1987. Chromosome count in *Macrocystis integrifolia* Bory. *Bull. Fac. Fish. Hokkaido Univ.* 38:339–42.

Yabu, H. & Sanbonsuga, Y. 1990. Mitosis in the female gametophytes and young sporophytes of *Laminaria diabolica* f. *longipes* Miyabe et Tokida. *Bull. Fac. Fish. Hokkaido Univ.* 41:8–12.

Yabu, H. & Taniguchi, K. 1990. Mitosis in the young sporophytes of *Ecklonia kurome* Okamura. *Bull. Fac. Fish. Hokkaido Univ.* 41:57–60.

Yamagishi, Y. & Kogame, K. 1998. Female dominant population of *Colpomenia peregrina* (Scytosiphonales, Phaeophyceae). *Bot. Mar.* 41:217–22.

Yan, Z. M. 1984. Studies on tissue culture of *Laminaria japonica* and *Undaria pinnatifida. Hydrobiologia* 116/117:314–6.

ANNEX 2:

HIERARCHICAL SAMPLING OF THE SOUTHERN SPECIES OF *LESSONIA NIGRESCENS* FOR POPULATION GENETIC ANALYSES

Table 1: Summary of sites included the geographical location and the sampling dates.

Locality	Site	Population type	Code	Date	Latitude (South)	Longitude (West)
Valdivia	Punta Mision	Central	PM	03/01/2009	39°40'15	73°21'13
Valdivia	Pilolcura	Central	PILOL	04/01/2009	39°47'47	73°24'04
Las Cruces	Ecim	Central	LCR	06//01/09	33°30'06	71°37'48
El Quisco	Caleta	Central	Q	08/01/2009	33°24'32	71°41'59
Concepcion	Cocholgue	Central	CON	10/01/2009	36°33'44	72°59'04
Algarrobo	El Canelillo	Central	AL	13/01/2009	33°21'53	71°41'13
Rio Limari	Desembocadura	Marginal	RL	08/02/2009	30°44'23	71°42,08
Coquimbo	Fuerte Coquimbo	Marginal	COQ	09/02/2009	29°56'01	71°20'10
Chanaral de Aceituno	Ch. Aceituno	Marginal	ACE	10/02/2009	29°03'59	71°29'19

Table 2: Number of sporophytic individuals sampled in hierarchical transects, in different sites along the Chilean coast

Site		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Total
PM	**Adults**	23	16	26	29	25	25	28	26			**198**
	Recruits	14	19	25	27	24	26	29	20			**184**
PILOL	**Adults**	27	27	25	25	25	27	26	29			**211**
	Recruits	9	22	14	29	25	25	27	13			**164**
LCR	**Adults**	25	26	26	25	25	26	25	25	25	25	**253**
	Recruits	22	25	26	28	27	10	25	25	10	21	**219**
Q	**Adults**	25	25	25	25	25	25	25	25	25	25	**250**
	Recruits	14	9	23	25	25	25	25	25	19	25	**215**
CON	**Adults**	25	25	25	25	25	25	25	25	25	25	**250**
	Recruits	25	25	24	24	23	25	25	23	25	25	**244**
AL	**Adults**	25	25	25	25	25	26	25	25	25	25	**251**
	Recruits	25	25	2	12	12	25	25	25	24	23	**198**
RL	**Adults**	25	25	25	25	25	22	25	25	25	25	**247**
	Recruits	5	10	25	22	13	13	18	21	23	0	**150**
COQ	**Adults**	25	25	25	25	25	14	25	25	25	25	**239**
	Recruits	25	25	16	25	25	25	15	25	25	25	**231**
ACE	**Adults**	23	25	24	19	25	25	25	25			**191**
	Recruits	25	25	25	24	25	24	25	25			**198**
												3893

Table 3: Table of distances between sites (kms)

	ACE	COQ	RL	AL	Q	LCR	CON	PILOL	PM
ACE	0	105	195	495	500	512	862	1212	1228,3
COQ	105	0	90	390	395	407	757	1107	1123,3
RL	195	90	0	300	305	317	667	1017	1033,3
AL	495	390	300	0	5	17	367	717	733,3
Q	500	395	305	5	0	12	362	712	728,3
LCR	512	407	317	17	12	0	350	700	716,3
CON	862	757	667	367	362	350	0	350	366,3
PILOL	1212	1107	1017	717	712	700	350	0	16,3
PM	1228,3	1123,3	1033,3	733,3	728,3	716,3	366,3	16,3	0

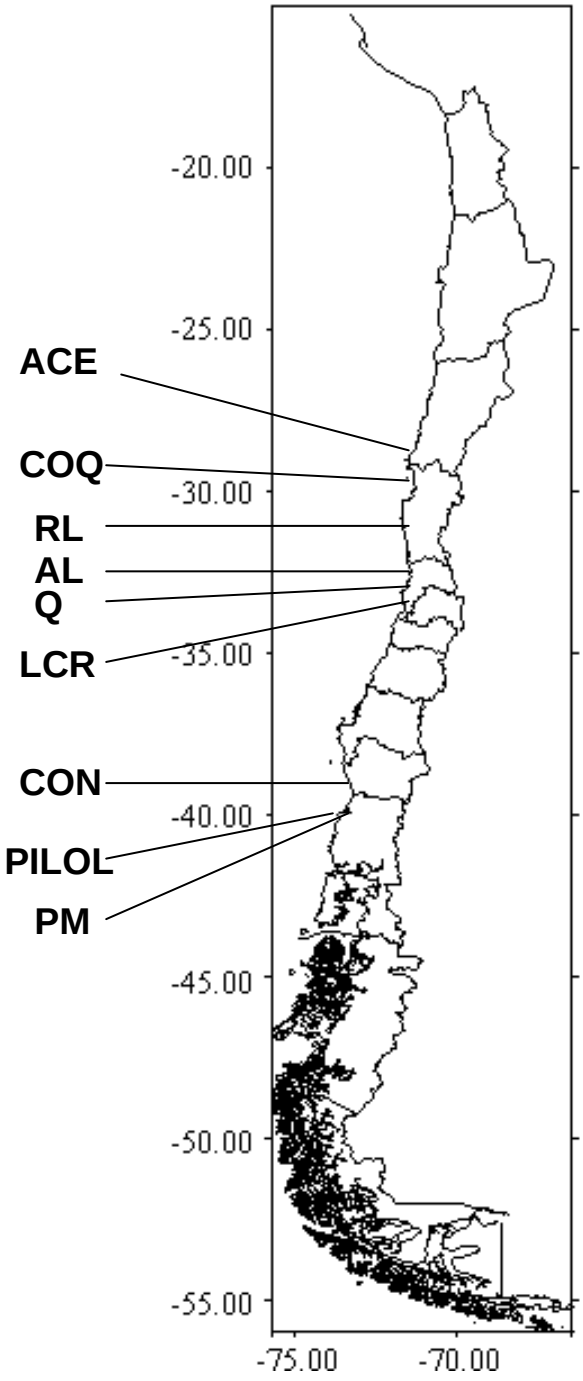
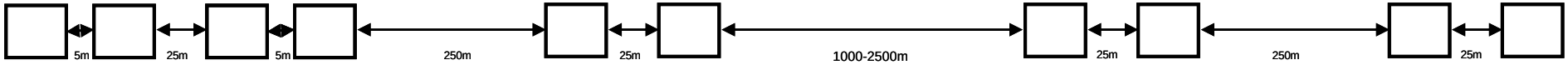
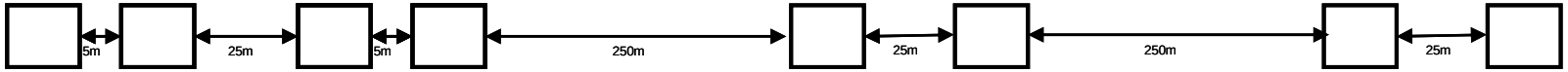


Figure 1: Map of the sites sampled for genetic analyses.

(A) For COQ, RL, LCR, Q, AL, CON



(B) For ACE



(C) For PM and PILOL

Figure 2: Hierarchical transects used for sampling. (A) Original transect used for Coquimbo, Rio Limari, Las Cruces, El Quisco, Algarrobo and Concepcion. (B) Transect used for Chanaral de Aceituno and, (C) transect used for Punta Mision and Pilolcura.

ANNEX 3:

THE RECIPROCAL TRANSPLANTATION EXPERIMENT IN *LAMINARIA DIGITATA*

Table 1: Spore information of fertile plants from the transplant experiment.

Label	Origin	Destiny	Nb spores per µL	Spore quality	Ploidy
627	Quiberon	Quiberon	0	non motile	non determined
647	Quiberon	Quiberon	7500	non motile	2n
609	Quiberon	Quiberon	11250	non motile	2n
560	Roscoff	Quiberon	1250	motile	n
558	Roscoff	Roscoff	12500	motile	n
549	Roscoff	Roscoff	0	motile	non determined
550	Roscoff	Roscoff	2500	motile	n
656	Quiberon	Roscoff	2500	non motile	n and 2n
654	Quiberon	Roscoff	56250	non motile	n and 2n
637	Quiberon	Roscoff	1250	non motile	n and 2n

Figure 1 : Location of studied sites and experimental design.

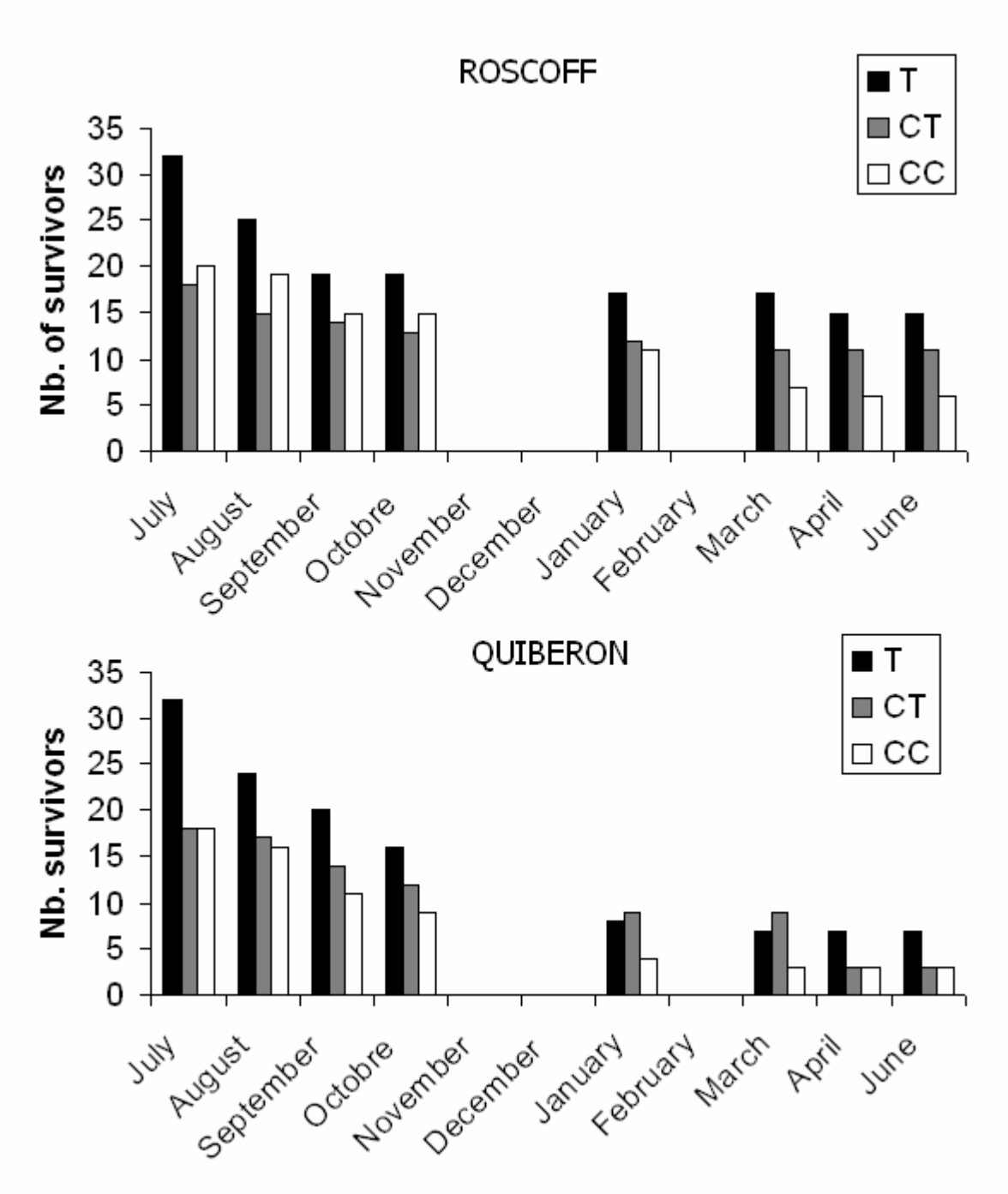


Figure 2: Survivorship of individuals in the transplant experiment of *L.digitata.*

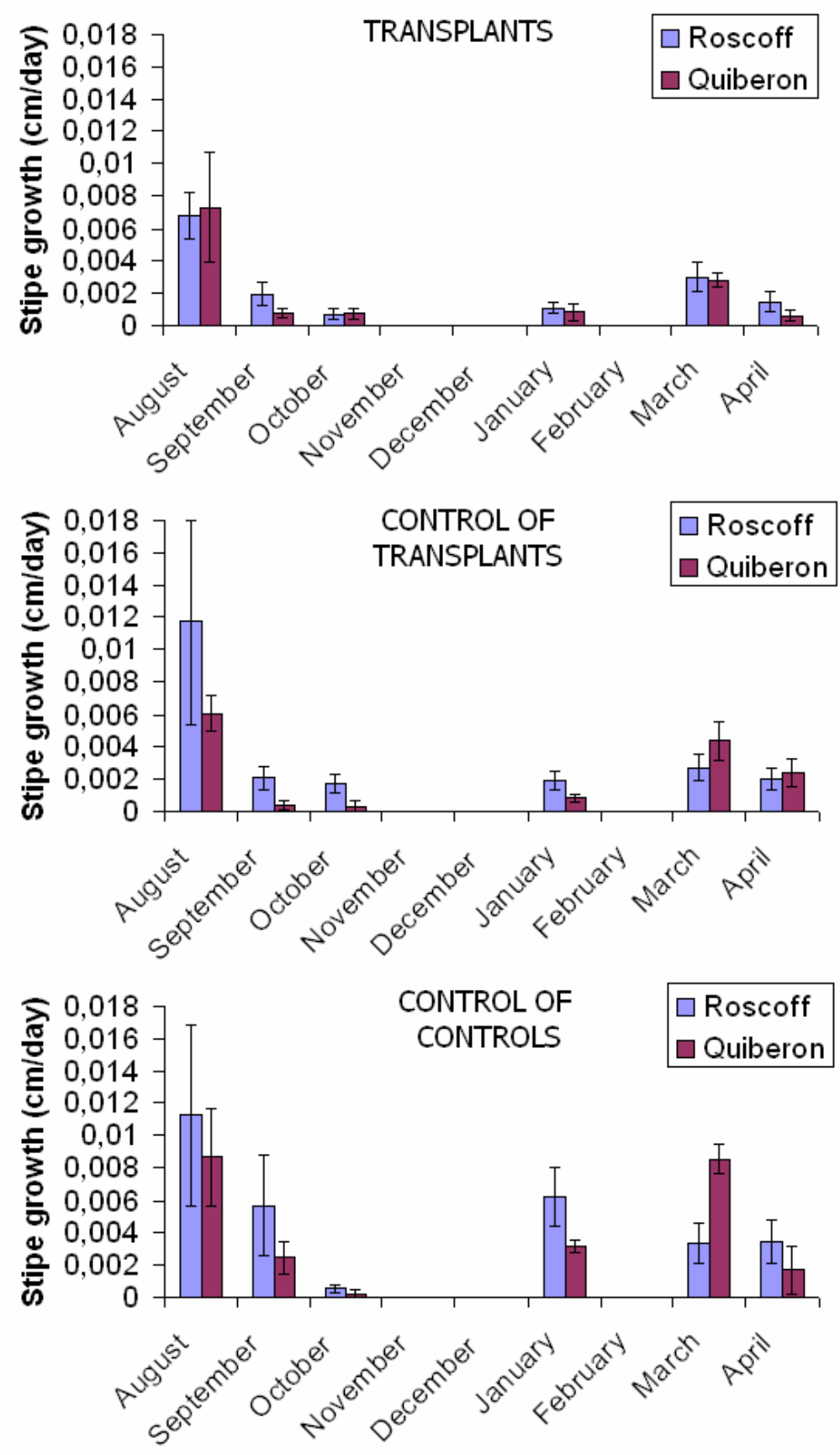


Figure 3 : Plant growth at the 9th month of experiment. Symbols represent mean values and SE.

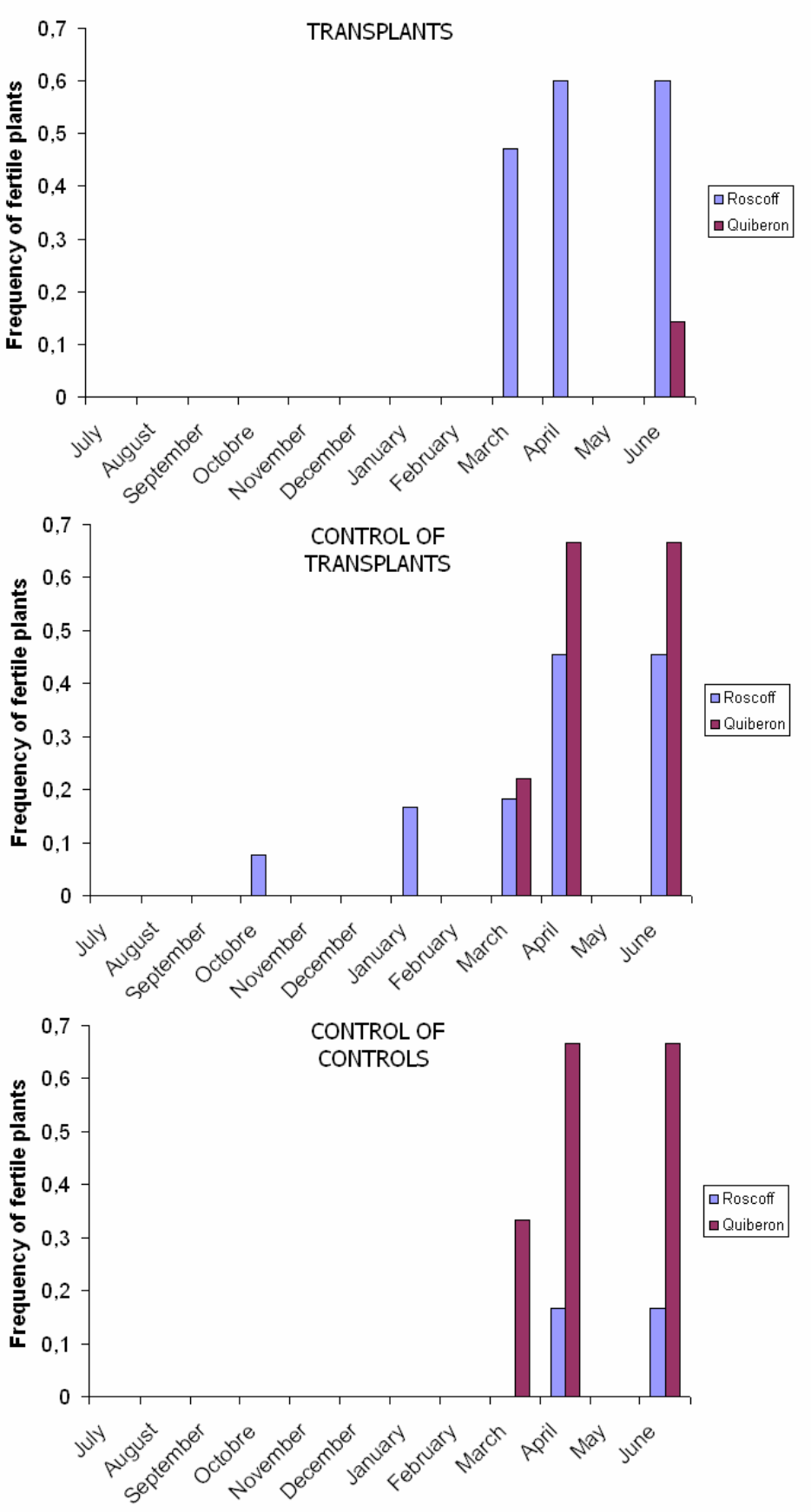


Figure 4: Reproduction of plants in the transplant experiment.