Pontificia Universidad Católica de Chile
Facultad de Ciencias Biológicas
Programa de Doctorado en Ciencias Biológicas
Mención Ecología

TESIS DOCTORAL:

“GROUP LIVING IN THE KELP *Lessonia spicata*“

Por

FERNANDA ARAUJO CASARES

Agosto de 2015

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Por

FERNANDA ARAUJO CASARES

Director de Tesis: Dr. Sylvain Faugeron

Comisión de Tesis: Dr. Luis Ebensperger
Dra. Fernanda Pérez
Dr.David Véliz

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RESÚMEN

La vida en grupo (sociabilidad) tiene consecuencias ecológicas que afectan las probabilidades de supervivencia y reproducción de los individuos. Ella crea oportunidad tanto para la cooperación cuanto para el conflicto. En última instancia, el balance entre los beneficios y costos en adecuación biológica determina el valor adaptativo de la vida en grupo en un dado ambiente. Cuando los individuos se agrupan con parientes cercanos, ellos pueden obtener beneficios indirectos a pesar de sufrir potenciales costos directos. Así, para la comprensión del valor adaptativo de la vida en grupo se requiere que se respondan cuestiones como: ¿Cuáles son los costos y beneficios relacionados a la sociabilidad? ¿Cuán frecuentemente parientes cercanos interactúan en poblaciones naturales? ¿Cuál es el grado de parentesco dentro de los grupos? En esta tesis, enfrenté el desafío de contestar estas preguntas utilizando el kelp *Lessonia spicata*, cuyos individuos (genotipos) se pueden fusionar originando plantas quiméricas, compuestas por dos o más líneas celulares provenientes de diferentes cigotos, como modelo de estudio para investigar las ventajas selectivas del quimerismo, un tipo peculiar pero común, de vida en grupo.

En el primer capítulo de esta tesis, evalué los efectos del quimerismo sobre la reproducción (un componente de adecuación biológica normalmente no evaluado en los estudios con quimeras) en los niveles del genotipo y de la planta. Cuantifiqué la frecuencia de quimerismo en poblaciones naturales de *L. spicata*, comparé la inversión reproductiva genotípica promedio entre plantas quiméricas y no quiméricas y estimé el éxito reproductivo genotípico y de la planta, en plantas quiméricas y no quiméricas. En el segundo capítulo, analicé la relación entre dispersión, estructuración genética a muy pequeña escala y formación de grupos compuestos por parientes cercanos. Utilizando analices de asignación parental y de autocorrelación espacial, evalué la forma del *kernel* de dispersión y la presencia de estructuración genética a muy pequeña escala (40-100m) en poblaciones de *L. spicata*. Posteriormente, investigué la influencia de la estructuración genética en la agrupación de parientes.

La frecuencia de plantas quiméricas fue alta (60-90%) en poblaciones naturales del kelp *L. spicata*. En general, no fueron encontrados beneficios o costos en éxito reproductivo genotípico relacionados a la vida en grupo. Sin embargo, en el nivel de la planta, el quimerismo aumentó tanto el éxito reproductivo, cuanto la probabilidad de reproducirse, principalmente debido al aumento del número de genotipos que se reproducen en plantas quiméricas. Este estudio sugiere que la vida en grupo resulta en beneficios en reproducción para *L. spicata*. Los *kernels* de dispersión mostraron que la mayoría de los eventos de reclutamiento ocurrió a cortas distancias de la fuente parental (14-40m), a pesar de la heterogeneidad observada entre los sitios de estudio. De la misma manera, uno de los sitios presentó estructuración genética significativa en la coorte de reclutas. Además, encontré concordancia entre la presencia de estructuración genética en reclutas y parentesco significativo dentro de grupos en plantas adultas, lo que sugiere que la estructuración genética a pequeña escala puede llevar a la agrupación de parientes cercanos en poblaciones de *L. spicata*. De esta forma, este estudio ha demostrado que la dispersión limitada puede causar

estructuración genética a pequeña escala en *L. spicata*. Entonces, la alta ocurrencia de quimeras en poblaciones naturales parece resultar de la fusión de genotipos altamente endogámicos, potencialmente involucrando también beneficios en adecuación biológica inclusiva.

Esta tesis ha generado conocimiento importante sobre la vida en grupo en *L. spicata* que complementa estudios previos sobre quimerismo en algas marinas. Diversas evidencias parecen indicar que el quimerismo es una estrategia adaptativa en algas marinas: i) este es común en varias especies de algas verdes, rojas y cafés; ii) confiere ventajas selectivas en estadios tempranos del ciclo de vida; iii) su frecuencia parece estar relacionada a condiciones ambientales estresantes; iv) aumenta la densidad the reproductores; v) probablemente ocurre mayormente entre parientes cercanos, sugiriendo la posibilidad de beneficios indirectos dentro de grupos. Estudios futuros que investiguen los efectos del parentesco sobre diferentes componentes de adecuación biológica son necesarios para un completo entendimiento de los mecanismos responsables por la evolución y la mantención del quimerismo en *L. spicata* y en otras algas marinas.

ABSTRACT

Group living (sociality) has ecological consequences that affect the probabilities of survival and reproduction of individuals. It creates opportunity for both cooperation and conflict. Ultimately, the balance between fitness benefits and costs determines the adaptive value of group living in a given environment. When individuals group with relatives, they may gain indirect benefits despite potential direct fitness costs. Therefore, understanding the adaptive value of group living requires addressing questions like: What are the costs and benefits related to sociality? How likely are relatives to encounter each other in natural populations? What is the degree of relatedness within groups? In this thesis I took the challenge of answering these questions for the kelp *Lessonia spicata*, whose individuals may fuse originating chimeric plants, made of two or more germ lines originated by different zygotes, to investigate the selective advantages of chimerism, a peculiar, yet widespread, type of group living.

In the first chapter of this thesis I evaluated the effects of chimerism in reproductive success (a fitness component usually neglected in studies on chimerism) at both the genotype and plant levels. I quantified the frequency of chimerism in natural populations of *L. spicata*, compared genotypic reproductive investment between chimeric and non-chimeric plants and estimated reproductive success at the genotype and plant levels in chimeric and non-chimeric plants. In the second chapter of this thesis I analyzed the interplay among dispersal, fine-scale genetic structure and kin group formation within the same populations of *L. spicata*. Using parentage and spatial autocorrelation analysis, I analyzed the shape of the dispersal kernel and the presence of genetic structure at fine scales (40-100m) within *L. spicata´*s populations. I further investigated the influence of genetic structure in kin group formation.

Chimeric plants were abundant (60-90%) in natural populations of the kelp *L. spicata*. Overall, no benefits (and no costs) in group living were found in terms of genotypic reproductive success. Yet, at the plant level, chimerism enhanced both reproductive success and the probability of reproducing, mainly due to higher number of genotypes that reproduce in chimeric plants. This study suggests that group living results in reproductive benefits for *L. spicata*. Dispersal kernels revealed that recruitment events mainly occurred at short distances from parental source (14-40 m), despite heterogeneity between study sites. Accordingly, recruit kin structure was observed in one of the study sites. I found concordance between the presence of kin structure in recruits and significant adult within-group relatedness, suggesting that fine-scale genetic structure leads to kin group formation within populations of *L. spicata*. This study demonstrated that limited dispersal may drive fine-scale genetic structure in *L. spicata*. Hence, the high occurrence of chimeras in natural populations seems to result from the fusion of highly inbred individuals, potentially also providing benefits in terms of inclusive fitness.

This study has generated important knowledge on group living in *L. spicata* that complements previous studies on chimerism in seaweeds. Several lines of evidence seem to indicate that chimerism is an adaptive strategy in seaweeds: i) it is common in several species

of green, red and brown algae; ii) it confers selective advantages at early life-cycle stages iii) its frequency seems to be related to stressful conditions; iv) it enhances the density of potential reproducers; v) it probably occurs mostly among kin, suggesting the possibility of indirect fitness benefits within groups. Future studies unraveling the fitness effects of kinship in different fitness components are needed for a complete understanding of the mechanisms underlying the evolution and maintenance of chimerism in *L. spicata* and in other seaweeds.

GENERAL INTRODUCTION

Levels of population spatial structure: basic concepts

The distribution of individuals in space has long attracted the attention of ecologists and evolutionary biologists, since it can influence many important processes including genetic structure (Slatkin 1987), social interactions (Hamilton 1964), population dynamics (Kareiva et al 1990), species coexistence (Amarasekare 2003), and speciation and extinction probabilities (Hanski 1998). While the geographic range of a species is mainly determined by historical events and intrinsic biological requirements and attributes, at a smaller scale, the spatial distribution of a species depends on factors including resource distribution, life-history characteristics, dispersal ability, and both intra- and interspecific interactions. Given the usual patchy configuration of suitable habitats, species are rarely found continuously distributed in space. As a consequence, instead of a single population, species are usually composed by subdivided populations characterized by a set of local populations (or subpopulations) more or less connected to each other by dispersal (i.e. metapopulation). Delimitation of local populations is a difficult task and depends on the species of interest and the aim of the study in question. Here I define a local population as a collection of individuals that potentially mate at random (i.e. a panmitic unity), following the evolutionary definition of Waples and Gaggiotti (2006).

Within local populations, a further level of spatial substructure may be present if individuals aggregate with some conspecifics. Gregariousness is a conspicuous feature in nature. From microorganisms to humans, the tendency to live close to conspecifics is present.

Several types of groupings exist ranging from those that are just a collection of individuals gathered in close proximity (an aggregation) - as observed for example in some intertidal organisms like barnacles and mitylids - to very organized groups, in which complex interactions between members occur, like the division of labor between them (e.g eusocial insects). In-between these two extremes lie many other kinds of groups that may vary according to some characteristics including for example: the degree of relatedness within groups (e.g. kin groups, colonies), the existence of dominance hierarchies (not all individuals reproduce within groups), the presence of alloparental care (individuals help other group members to raise offspring), and group stability in space and time. Here I define a group as a collection of two or more conspecific individuals that are connected to one another by social interactions (adapted from Foursyth 2006). A behavior can be considered social whenever it influences the fitness of other individuals in addition to the actor, either negatively or positively (Wilson and Wilson 2007). I consider that the merepresence of one individual may affect the fitness of the other. Conversely, sociality means group living (Alexander 1974). Note that the definition of group adopted here does not imply reproductive unity, which means that reproduction may or may not occur among group members. Hence, in most cases groups are nested within local populations. According to this broad definition, a group can be a simple aggregation of individuals, a chimera (group of genetically different individuals fused to each other forming a single macroscopic entity), social bacteria, a school (fishes), a breeding group (birds, mammals), a pack (mammals), an insect colony, a human family, etc.

Group living: ecological consequences and evolutionary explanation

Living in groups may bring several benefits that include increased survival and reproduction through protection from stressful environmental conditions, increased protection from predators, and enhanced probability of obtaining food and mates (Krause and Ruxton 2002). However, individuals within groups may also suffer fitness costs like increased competition (for resources and mates) and enhanced disease transmission. So, group living intensifies two opposing forces: cooperation and conflict (Frank 2007). This is why biologists have long been interested in understanding how sociality has evolved in so many types of organisms.

In theory, group living is adaptive if fitness benefits related to sociality outweigh the costs (Alexander 1974). Within groups, individuals may gain fitness benefits either directly through their own reproductive success or indirectly through the reproduction of closely related individuals (that share a great portion of their genes), as predicted by kin selection (Hamilton 1964). Therefore, understanding fitness effects of group living also requires answering questions including: How likely are relatives to encounter each other in natural populations? What is the degree of relatedness within groups?

Although high intra-group relatedness can offer an opportunity for kin selection to act on social traits, it can also lead to a high level of competition between group members – kin competition (Queller 1992, West et al 2002), given that related individuals tend to exhibit similar ecological requirements. Moreover, assumed altruistic traits may confer direct fitness benefits (Griffin and West 2002), so care must be taken when relating high group relatedness to the occurrence of kin selection. It is important to measure fitness effects of group living and

also to assess the relationship between fitness and relatedness, before claiming the action of one or other type of selection.

Despite the selective advantages of sociality found for some organisms, the overall adaptive value of group living still remains controversial, since there are several empirical studies that document no benefits and others that even report costs related to group living (see Silk 2007, Ebensperger et al 2012 for examples and metanalysis in mammals). Indeed the relative fitness costs and benefits of group living may depend on various factors including: environmental conditions, group attributes like size (Shen et al 2014) and relatedness (Griffin et al 2004), the fitness component analyzed (Bilde et al 2007) and the ontogenetic stage of the organism (Despland and Le Huu 2007).

Traditionally, group living has been mainly studied in mammals, birds and insects. However, during the last 15 years, other kinds of organisms, including microorganisms, have begun to be widely pictured through a social perspective (Crespi 2001, West et al 2006, Aanen et al 2008, Velicer and Vos 2008, Strassman and Queller 2011- see also Buss 1982).

Group living, dispersal, relatedness, and SGS

Sociality can influence spatial genetic structure (SGS) – the non-random distribution of genotypes (Sugg et al 1996). If groups are formed by relatives, a substructure of the genetic diversity may occur within local populations, where intra-group relatedness is predicted to be higher than intergroup relatedness (Sugg et al 1996). High relatedness among group members can arise due to limited dispersal or assortative interactions based on genetic similarity – kin

recognition (Hamilton 1964). Limited dispersal can be a particularly powerful means of generating within-group relatedness, because it will foster interactions among relatives even if individuals group indiscriminately with neighbors (Queller 1994). This may have evolutionary consequences, since a high level of relatedness within groups represents opportunity for the action of kin selection on social traits. Therefore, evaluating how dispersal and sociality interact to generate local patterns of SGS is important to gain insights into the mechanisms that underlie the evolution and maintenance of group living and social traits.

As Starrfeld and Kokko (2012) highlighted, dispersal can be seen as a cause and as a consequence. "Dispersal can produce ecological patterns, but these patterns can again influence the selective pressures on dispersive traits" (Starrfeld and Kokko 2012). In this sense, limited dispersal and the resulting kin structure (a negative relationship between pairwise relatedness and geographic distance) can create proper ecological conditions to the evolution of social behavior that would in turn tend to select for the maintenance of low dispersal. On the other hand, limited dispersal can also enhance inbreeding rates and therefore the possibility of inbreeding depression (i.e. the decreased fitness of inbred individuals), which could then trigger the evolution of dispersal at longer distances or specific mating behaviors to avoid reproducing with relatives (Gandon 1999 but see Szulkin et al 2013). Alternatively, purging of deleterious alleles following inbreeding (Crnokrak and Barrett 2002) could allow the maintenance of low dispersal and kin group formation. Hence, there could be a feedback structure between the evolution of limited dispersal and sociality, mediated by the fitness effects of living in kin groups and the relative strength of other selective pressures that act on dispersal.

Several studies have evaluated the relationship between social structure and dispersal in mammals and birds by assessing local SGS, mainly in species that form cooperative breeding groups, in which some group members forego reproduction but contribute to the rearing of others' offspring. These studies have demonstrated that the pattern of local SGS mainly depends on the frequency and distance of offspring and adult dispersal events, and on mating behaviors (e.g. extra-group mating). For example, genetic structure is lower within populations when natal dispersal occurs (i.e. juveniles disperse), since relatedness within groups is similar to among-group relatedness (Sugg et al 1996). In cases in which sex-biased dispersal occurs (only males or females disperse and the other sex remains philopatric), SGS is stronger in the most philopatric sex (e.g. Temple et al 2006, Woxvold et al 2006). Moreover, if dispersal occurs at short distance, neighboring groups tend to exhibit higher relatedness than distant located groups (e.g. Beck et al 2008). Lastly, when extra-group mating is observed, low within-group relatedness and consequently decreased SGS are found (e.g. Brower et al 2011). Some studies have suggested natal dispersal (e.g. Lukas and Clutton-Brook 2011) and specific mating behaviors (Brower et al 2011) evolved as mechanisms of inbreeding avoidance (but see Szulkin et al 2013).

The interplay between sociality, limited dispersal, and local genetic structure has been less explored in sessile species, despite its potential importance. This is probably related to the underestimation of sociality in these organisms. In sessile species, adults cannot move and propagules (spores, pollen, seeds, larvae, etc.) constitute the dispersive phase. So, life-history characteristics related to propagule dispersal and settlement patterns will play a key role in shaping the social environment of individuals. For example, species with restricted propagule dispersal are likely to exhibit fine-scale genetic structure within populations (Vekemans and

Hardy 2004). Because ecological interactions occur primarily among neighbors in populations of sessile organisms, kin structure in turn will result in ecological interactions among relatives (Heywood 1991) and possibly in kin group formation. Under these conditions, average within-group relatedness is expected to be high compared to average within-population relatedness and neighboring groups are likely to be more related to themselves than to more distant groups in local populations. In species in which propagule dispersal ability is higher, kin group formation will depend on factors other than local kin structure (only produced by limited dispersal), including kin propagule aggregation followed by kin settlement and/or kin recognition. It has been proposed that these specific traits have evolved in species in which grouping with non-kin can lead to high fitness costs, as documented in some chimeric marine invertebrates (Grosberg and Quinn 1986 - see below). This illustrates that fitness effects of kin group formation can mediate the intensity of the feedback between dispersal and sociality. In these species, higher within-group relatedness compared to among-group relatedness is expected.

SGS and group living in the sea

The fact that most marine organisms possess complex life-cycles in which sessile or sedentary adults release propagules (gametes, zygotes, spores or larvae) in the water column has led to the paradigm that most marine populations were open, widely connected through a well-mixed propagule pool and genetically homogeneous (Bohonak 1999). However, over the last 15 years several studies have demonstrated the occurrence of genetic structure at various spatial scales, contrary to the view of the absence of dispersal barriers in the sea (e.g. Swearer

et al 1999, Jones 2005). Moreover, recent studies have revealed the occurrence of small-scale genetic structure within many marine populations of fishes and invertebrates, even in species with long distance dispersal potential (due to high pelagic larval duration) as the spiny lobster *Panulirus interruptus* (Iacchei et al 2013). This casts doubt on the predicted inverse relationship between larval duration in the plankton and population genetic differentiation (Kinlan and Gaines 2003) and highlights that effective dispersal distance is likely to be much lower than potential dispersal distance in some cases. The main mechanisms proposed to limit dispersal distances in marine organisms are (Selkoe et al 2010, Broquet et al 2013): i) oceanographically and biologically driven patterns of collective dispersal of related individuals (e.g. Veliz et al 2006, Bernardi et al 2012); ii) larval retention near parental source (e.g. Jones 2005, Planes et al 2009, Christie et al 2010); iii) high stochastic individual variance in reproductive success (Hedgecock 1994, Hedgecock et al 2007). These findings indicate that small-scale genetic structure seems to be more widespread than once expected in marine populations.

Furthermore, genetic structure at very fine scales (few meters) has been documented in species with poor dispersal ability, like some invertebrates (e.g. Calderon et al 2007 Ledoux 2010) and seaweeds (Faugeron et al 2001, Kusumo et al 2006, Krueger-Hadfield 2013). In addition, there is evidence of inbreeding (Raimondi et al 2006, Maier et al 2009, Barner et al 2011, Johansson et al 2013) in many marine species.

As a result, kin interactions and the formation of kin groups (for those species that live in groups) with important (positive and negative) fitness consequences are likely to be frequent in the marine environment (Kamel et al 2010). Hence, marine organisms represent

interesting models to study with a social perspective (Kamel and Grosberg 2013). One type of group living commonly found in the marine realm is chimerism – the formation of genetically heterogeneous entities due to the fusion of different individuals (i.e. originated from different zygotes) into a single macroscopic entity (Pineda-Krch and Lehtila 2004). Chimeras are a peculiar type of group in the sense that, differently from groups of mammals, birds, or insects, group members (called here the genotypes) are sticked to each other and often integrate part or all of their tissues into a single macroscopic entity. Chimeras occur frequently in natural populations of some sessile marine sponges (Maldonado 1988), cnidarians (Amar et al 2008, Puill-Stephan et al 2009, Mercier et al 2011), bryozoans (Buss 1982), ascidians (Bishop and Sommerfeldt 1999, Rinkevich 2005) and macroalgae (Santelices et al 1999, Wernberg 2005, González and Santelices 2008, González et al 2013, Segovia et al 2014).

Physiological integration among members of the chimera may occur in some cases (Pineda-Krch and Lehtila 2004) and individual identification within chimeras is not always visually possible in the field. The intimate connection between group members within chimeras, possibly involving the exchange of cells and molecules, may lead to specific benefits and costs at both the genotype and the group levels. The main cost associated to fusion between conspecifics is intraspecific competition. If there is physiological integration between fused genotypes, it can be present in the form of competition between cell lineages of fused genotypes (i.e. cellular parasitism), that occurs when, after fusion, cells from one genotype invade areas of the other genotype, integrating part of its soma or germ line (Buss 1982, Rinkevich 2005). Another potential cost is disease transmission, mainly studied in microorganisms (Velicer and Vos 2000). Benefits of chimerism may result from increased protection from predation and stressful environmental conditions and enhanced chance of

finding mates. The formation of chimeras may also lead to size-related benefits, considering that the fusion of spores, larvae, juveniles or adults generates an instantaneous increase of the size of the entity (Buss 1982). This can bring higher survival probability (e.g. Santelices et al 1999, Wernberg 2005), competitive ability and decreased time to reach reproductive maturity (e.g. Raymundo and Maypa 2004). Another potential benefit attributed to chimeras is an increased likelihood of responding to changes in the environment (due to higher genetic diversity within groups) if compared to solitary individuals (Buss 1982). Given the potential severe costs involved with chimera formation, including cellular parasitism and death of some partners (following rejection), the independent evolution and maintenance of chimerism in so many types of phyla strongly suggest this strategy might be adaptive.

Most studies that evaluated the fitness costs and benefits related to group living in chimeras measured group fitness and not the fitness of group members (or genotypes - genotype fitness) (Pineda-Krch & Lehtilla 2004, but see Amar et al 2008). This can reflect the difficulty in identifying morphologically each genotype after fusion (Raymundo and Maypa 2004). Furthermore, the effects of fusion between conspecifics have mostly been measured as consequences in survival and growth. A general understanding of the overall fitness consequences of chimerism requires to quantify the effects of living integrated into a single entity on the reproductive success of both the genotypes and the chimera.

In species in which fusion between conspecifics occurs, the probability of fusion is determined by the proximity between individuals (Amar et al 2008). Therefore, factors that lead to an increase in the proximity between individuals including aggregated settlement of propagules, low dispersal ability and life-history characteristics can be important (and non-

mutually exclusive) factors in determining chimerism frequency in a given environment. Besides from proximity, the level of relatedness between conspecifics will dictate whether two individuals in contact will successfully fuse, in species that have allorecognition systems (self/non-self recognition systems that mediate the outcome of fusion between conspecifics - Hart and Grosberg 1999) (e.g. the ascidian *Botryllus schloserii* – Rinkevich 2005). Interestingly, it has been shown that in most chimeric species there is a high probability of proximity between relatives due to the aggregated settlement of kin larvae (e.g. hidrozoan *Hydractinia simbiolongicarpus* Grosberg et al 1996 the ascidians (*Botryllus schloserii* – Grosberg and Quinn 1986 and *Molgula complanata* – Schmidt et al 1982, and the corals *Stylopora pistillata* - Amar et al 2008, *Acropora millepora* - Puill-Stephan et al 2009). Fusion with relatives among species without allorecognition systems and with limited dispersal is expected to be frequent simply because they tend to be found close to each (i.e., as a result of kin structure within the population). Therefore, characterizing dispersal patterns at small scales and fine-scale genetic structure of chimeric species as well as relating them to specific biological characteristics is important to revealing the processes involved in chimera formation. This knowledge in conjunction to fitness effect analyses may provide a more throughout understanding of how chimerism is maintained in natural populations.

In this thesis I evaluated the fitness consequences of fusion between conspecifics, as well as the fine-scale genetic structure within populations and its influence on kin group formation in the kelp *Lessonia spicata* as a model species.

Study species and objectives of this thesis

Lessonia spicata (Suhr) Santelices (Pheophyta, Laminariales) is a dominant species in the low intertidal in the Southeast Pacific. It is an ecologically and economically important species, since it constitutes habitat for several kinds of organisms (Ojeda and Santelices 1984) and is used as a raw material for alginate extraction (Vasquez 2008). *Lessonia spicata* has an heteromorphic life cycle, in which the macroscopic form is the diploid sporophyte and the haploid male and female gametophytes are microscopic (Hoffman and Santelices 1982). Sporophytes are composed by a holdfast that adheres the alga into the substrate, from where stipes emerge. Stipes, in turn ramify into fronds that bear reproductive organs. Traditionally, each plant was considered as an independent individual (one genotype). However, adjacent holdfasts of *L. berteroana* (a sister species of *L. spicata*, González et al 2012) were recorded to fuse under natural conditions (Vásquez et al 2008). Recent molecular studies have demonstrated that plants of *L. spicata* and *L. berteroana* can be composed of different genotypes (González et al 2013, Segovia et al 2014). In this case, a single holdfast may include emerging stipes from up to 5 different genotypes, whereas each stipe exhibits only one genotype (see Fig. 1). In other words, chimeras are composed by different genetic entities that are fused through the holdfast while retaining their identity at the stipe level, and potentially therefore, its reproductive ability. So, chimeric plants of *L. spicata* can be considered as groups of different genotype members.

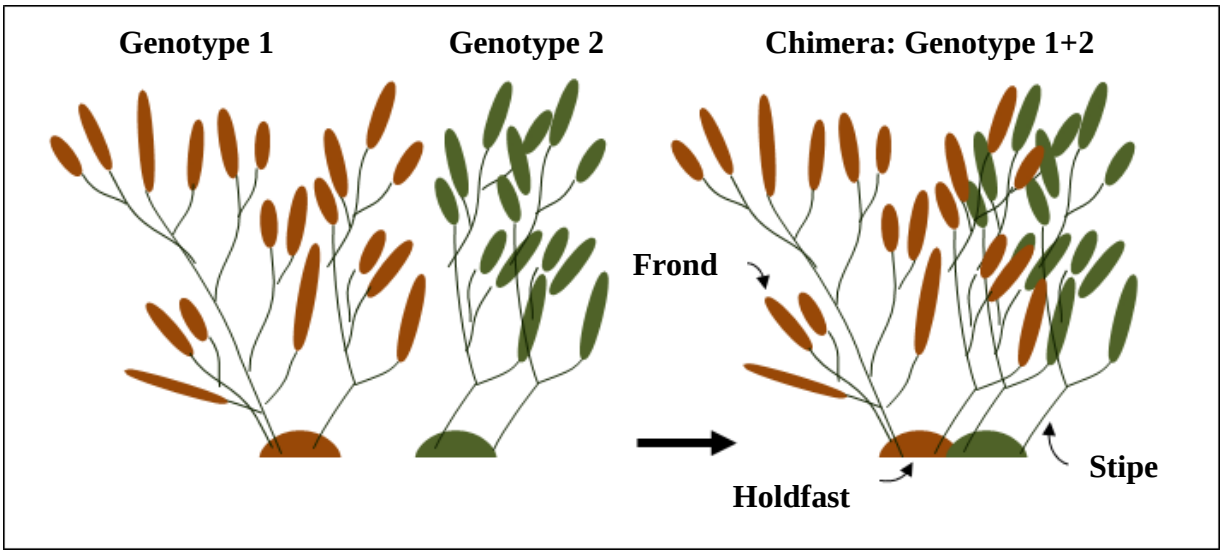

Figure 1. Schematic representation of the process of fusion between macroscopic holdfasts originating chimeric plants in *Lessonia* sp.

In natural populations of *L. spicata*, both non-chimeric and chimeric plants may co-occur, allowing the comparison of fitness between genotypes and making this species a good biological model to evaluate the fitness consequences of chimerism. Recently, Segovia et al (2014) analyzed the frequency of chimeric plants of *L. berteroana* in high and low density areas. They found that under low density conditions 100 % of the plants analyzed were chimeric, whereas under high density areas 62.5 % of the holdfasts were chimeric. This finding suggests that fusion is not a consequence of high population density that would increase the probability of encounter, but rather a consequence of higher survival of fused genotypes at low density conditions, potentially representing relatively more stressful conditions. Intriguinly, significant kinship was also detected within chimeric plants of *L. berteroana* (Segovia et al 2014), implying a substructure of the genetic diversity at short spatial scales.

There are several lines of evidence that suggest that *L. spicata* is a poor disperser. First, life-history characteristics of *L. spicata* are associated with restricted dispersal ability, including life-cycle (short spore duration in the plankton and viability, necessity of close proximity of male and female gametophytes for successful reproduction) (Avila et al 1985, Parada 2001, Boland et al 1983), morphological (the absence of floating structures to travel long distances) and habitat (intertidal) characteristics (Valero et al 2011). Second, high genetic differentiation has been reported among neighbor populations (Tellier et al 2009) and accentuated small-scale (20m) genetic structure has been detected for *L. berteroana* (Faugeron et al 2005). Third, *L. berteroana* exhibited a limited recolonization rate after an ENSO event that caused massive mortality in Northern Chile (Martinez et al 2003). The limited dispersal ability and the possibility of self-reproduction in *L. spicata* (as in all kelps) raise the following questions: At which spatial scale does genetic structure occur within populations of this species? Does it influence the formation of kin groups?

All the previous characteristics make the kelp *L. spicata* a suitable organism for the study of the fitness effects of group living and the interplay between limited dispersal, SGS and kin group formation. Two general hypothesis were tested in this thesis: i) Chimerism is selectively advantageous and ii) Limited dispersal leads to kin group formation in *L. spicata* via fine-scale genetic structure. Two sites with similar characteristics (high wave exposure, strong upwelling - following Tapia et al. 2014 - and no kelp harvesting) were studied to evaluate the generality of the patterns observed.

In the first chapter of this thesis I evaluated the fitness effects of chimerism in *L. spicata*, through the conceptual framework of social evolution theory. Specifically, I

quantified the effects of chimerism on reproductive success (usually neglected in studies on chimerism) at both the genotype and group levels. I also analyzed the effect of group size and within-group relatedness on genotypic reproductive success and reproductive investment.

In the second chapter of this thesis I evaluated the fine-scale genetic structure within populations of *L. spicata*. Using parentage and spatial autocorrelation analysis, I analyzed the shape of the dispersal kernel and the presence of kin structure at small scales (40-100m) within *L. spicata´*s populations. I further investigated the influence of SGS in kin group formation.

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CHAPTER 1

FITNESS EFFECTS OF CHIMERISM IN THE KELP *Lessonia spicata*

Abstract

Chimerism is a type of group living in which genetically heterogeneous entities are originated through fusion between conspecifics. Here we tested whether chimerism provides direct benefits to the kelp *Lessonia spicata*, by analyzing its consequences on reproductive investment and success, both at the genotype and plant levels. In addition, we quantified the frequency of chimerism in two natural populations, tested if group members are close kin, and evaluated the effects of relatedness and number of genotypes per plant on reproduction. Chimeric plants were frequent (>60 %) in both populations of *L. spicata*. In most cases, average intragroup relatedness was not significantly different from interplant relatedness. Reproductive investment was not significantly affected by the type of plant (chimeric versus non-chimeric), or by the number of genotypes per plant or average plant relatedness. Genotypic reproductive investment did not correlate with genotypic reproductive success. Chimerism did not result in net benefits or costs in terms of genotypic reproductive success. Chimerism had no effect on the probability of reproducing nor on the number of offspring produced by each genotype. Neither the number of genotypes per plant or relatedness significantly affected genotypic reproductive success. Yet, at the plant level, chimerism increased reproductive success and the probability of reproducing. Apparently, chimerism affects *L. spicata* reproductive success by allowing the coexistence of a higher number of potential reproducers and mates compared with unigenotypic plants.

Introduction

Group living (sociality) is a widespread phenomenon that has long been studied by behavioral ecologists. When groups are formed, by definition, individuals interact with each other more intensely than with other members of the population. Therefore, sociality involves costs including competition for resources and mates, and disease transmission, as well as benefits like cooperation. For sociality to be adaptive, fitness benefits of living in a group must exceed its costs (Allee 1931; Alexander 1974). In an adaptive scenario, social evolution is expected to result from increased direct individual fitness (Bernasconi and Strassman 1999) or, if groups are composed by genetically related individuals, from inclusive fitness benefits due to augmented fitness of relatives, as predicted by kin selection theory (Hamilton 1964).

A peculiar type of group living is found in the chimeras - genetically heterogeneous entities formed either by the fusion of individuals, or the exchange of cells originated from different reproductive events (i.e. different zygotes; Pineda-Krch and Lehtila 2004). Chimeras challenge the notion of individual and represent interesting entities to study through a social perspective (Buss 1982, Aanen et al 2008). In comparison to the traditionally studied mammals, birds and insects, chimeras constitute a less flexible type of group, since components are attached to each other, and in most cases they are sessile (e.g. marine invertebrates, algae and plants). This restriction implies that there is no choice of leaving the group after successful fusion takes place.

Chimerism is a common phenomenon in several kinds of organisms including amoebas (Foster et al 2002), fungi (Aanen et al 2008), bacteria (Velicer and Vos 2009), sponges (Maldonado 1998), briozoans (Buss 1982), hydrozoans (Hart and Grosberg 1999), corals (Raymundo and Maypa 2004), ascidians (Bishop and Sommerfeldt 1999; Rinkevich 2005),

vertebrates (Rinkevich 2001; Ross et al 2007), marine algae (Santelices et al 1999; Wernberg 2005; González and Santelices 2008; Segovia 2014) and angiosperms (Thomson et al 1991; McIntire and Fajardo 2011). Relatedness within chimeras is thought to be high due to developed kin recognition systems that only allow successful fusion to occur between related conspecifics (e.g. most marine invertebrates) and/or to limited dispersal that enhances the chance of fusion among kin (Grosberg and Quinn 1986).

The adaptive value of chimerism remains controversial since there are empirical evidences of benefits, costs and no effects. Demonstrated costs include intraspecific competition (Stoner and Weissman 1996; Pancer et al 1995; Stoner et al 1999; Rinkevich 2002), as well as disease transmission, described mainly in microorganisms (Velicer and Vos 2009). Reported benefits are synergistic complementation (Buss 1982), protection from adverse environmental conditions (Wernberg 2005; McIntire and Fajardo 2011), and increased probability of finding mates (Pietsch 2005; Høeg and Lutzen 1995 – see Rinkevich 2011). Moreover, considering that chimerism generates an instantaneous increase in size of the entity as a whole (Foster et al 2002; Amar et al 2008), size-related benefits like greater probability of survival (Raymundo and Maypa 2004; Santelices and Aedo 2006), earlier onset of reproduction and higher intra- and interspecific competitive ability (Buss 1981) have been attributed to chimeras. Another important benefit is related to the genetic diversity (sometimes called the "chimeric vigor" – Buss 1982). The increase in the genetic diversity in chimeric entities in comparison to solitary individuals might allow a better ability of phenotypic responses to changes in the environment (Buss 1982; Rinkevich and Yankelevich 2004). There are also studies that did not find differences between solitary individuals and chimeras (Maldonado 1998; Rinkevich and Shapira 1999).

The difficulty in identifying members of chimeras has often led to the comparison of chimeras as a whole to solitary individuals in studies on the fitness effects of chimerism. Consequently, possible differences in the response of both levels of organization- the individual genotype and the group (chimera) - to group living remain to be explored (Pineda-Kirch and Lehtila 2004). Clearly, this knowledge is important to understand the evolutionary maintenance of chimera formation in nature. As far as we are concerned, only Amar et al (2008) analyzed fitness consequences of fusion on both the genotype and group levels. Interestingly, they found that fusion led to opposite responses in each level: costs in growth at the genotypic level and benefits at the group level (Amar et al 2008). Furthermore, the effects of chimerism have been mostly measured as consequences in survival and growth. Although very abundant in social vertebrates, studies on the effect of group living on reproduction are still lacking for fused individuals. To reach a general picture of the overall fitness consequences of chimerism, it is important to quantify the reproductive effects of chimerism, since there might be trade-offs between levels of organization.

In seaweeds, chimerism has been documented for red (reviewed by Santelices et al 1999), green (González and Santelices 2008) and brown algae (Wernberg 2005; Vásquez et al 2008; Segovia et al 2014). Santelices and colleagues (Santelices 2004; Santelices and Aedo 2006; Santelices and Alvarado 2008; Santelices et al 2010) have intensely studied the consequences of chimerism in red algae. They have shown that spore fusion leads to benefits in growth and survival of the entity as a whole at early stages of the life cycle, yet they have found that, a short time later, fusion brings costs in growth (Santelices et al 2010). For brown algae, Wernberg (2005) documented a higher frequency of superficial fusion between holdfasts of *Ecklonia radiata* (C. Agardh) J. Agardh in more wave exposed habitats,

concluding that fusion might improve resistance to wave action. Recently, Segovia et al. (2014) reported a higher occurrence of chimeric plants of the kelp *Lessonia beteroana* Montagne in areas of low population density, which was interpreted as a benefit in more adverse conditions (i.e. those causing low population densities). Herein, we explored the reproductive effects of chimerism using the kelp *Lessonia spicata* (Suhr) Santelices as a biological model.

Lessonia spicata (González et al 2012) consists of a massive holdfast, from which up to 80 stipes emerge. Each stipe ramifies into several fronds (up to 500). Meiosis takes place on the fronds, in a tissue differentiated into a *sorus* during the reproductive period. Therefore, the reproductive investment includes the production of stipes and fronds, and the development of *sori* that replace the photosynthetic tissue of the fronds. It was previously thought that each plant constituted an individual (a genetically homogenous entity or the product of one zygote – a genotype). Yet, Vásquez et al (2008), following marked recruits, reported that *L. berteroana* holdfasts could fuse into a single macroscopic chimeric plant. González et al (2013) characterized the ultrastructural process of fusion between conspecifics in both *L. berteroana* and *L. spicata*, revealing a complete integration of tissues at the holdfast, including changes in cell morphology in the contact area (e.g. cell walls shrink and plasmodesmata develop), likely allowing cellular communication among fused genotypes (González et al 2013). Then, Segovia et al. (2014) demonstrated, through molecular analysis, the presence of plurigenotypic plants in *L. berteroana*. They found that fronds from different stipes within a single holdfast could either have different genotypes (chimeric, fused or plurigenotypic plants) or the same genotype (non-chimeric or unigenotypic plants). So far, only one genotype (Faugeron et al 2009; Tellier et al 2011) or haplotype (Tellier et al 2009) has been observed from frond

tissues', suggesting that chimerism occurs in the holdfasts, but stipes and fronds are single germ-lines. Heteroplasmy has been reported for a limited number of populations of *L. spicata*, and was attributed to the persistence of an ancient mitochondrial lineage as a nuclear transfer of mitochondrial DNA (Tellier et al 2011), excluding the possibility of interspecific hybrid or chimeric origin.

Recently, it has been shown that genetic relatedness was higher between *L. berteroana´s* genotypes sharing a holdfast than on average in the population (Segovia et al. 2014). This finding, together with the observation of a higher occurrence of chimeric kelps in low density, low quality habitats, was interpreted as some form of kin selection driving the occurrence of fusion. Nevertheless, the fitness effects of chimerism have not been explicitly studied in these kelps yet.

Here we investigated the consequences of chimerism in the kelp *L. spicata*. We hypothesize that chimerism results in direct reproductive advantages for *L. spicata*. More specifically, we examined the following scenarios: (i) individual genotypes attain higher reproductive success within chimeric plants compared with unigenotypic, non-chimeric plants, and therefore the fitness of chimeric plants is higher than that of unigenotypic plants; (ii) living within chimeras is either neutral or costly at the genotype level, but beneficial at the plant level (i.e. chimeric plants enjoy a higher probability of being reproductive). We further contrasted the previous secenarios with the possibility that (iii) no fitness benefits occur at any level of organization of chimerism. We explored these scenarios by analyzing the effect of chimerism on reproductive investment and success of the kelp *L. spicata* both at the genotype and plant levels. Moreover, we quantified the frequency of chimerism in natural populations, tested if group members are close kin, and evaluated the effects of relatedness and number of

genotypes per plant on reproduction. High within-group relatedness and/or a positive relationship between relatedness and fitness could suggest possible indirect benefits of chimerism. This study was carried out in two study sites with similar conditions to evaluate the generality of the patterns observed.

Material and Methods

Sampling and morphometric measurements

Two sites in northern Chile with similar conditions (high wave exposure, strong upwelling - following Tapia et al. 2014 - and no kelp harvesting) were chosen for sampling (Isla Damas - 29° 13´S/71° and Fray Jorge - 30° 44´S/71° 42´W). Adults of *Lessonia spicata* (Suhr) Santelices were sampled during 2009 winter when the maximum fertility is observed. In each study site, two permanent grids delimited by reference points screwed on the rock and separated by approximately 50m were placed using 50 cm x 50 cm quadrats (for a patch area of 12 to 56 m^2). The size of the grid was determined so that at least 50 *L. spicata* plants were sampled in each one, using an exhaustive sampling (i.e. including the highest kelp on the shore, to the lowest possible individuals reachable at low tide; considering though most of the width of the intertidal distribution of the kelp population). The distance between grids was defined according to a previous study that reported genetic differentiation within 20 m for *L. berteroana* (Faugeron et al 2005). Adult density was measured as the number of plants m^{-2} within grids.

To quantify the reproductive investment of *L. spicata*, we first measured holdfast diameter and counted the number of stipes of each plant in the field. Then, the central (longest) stipe of each plant was cut for posterior analysis. At the laboratory, we counted all fronds of central stipes and took a sample of 50 fronds to measure their width and length (this number was determined after plotting a saturation curve of the variance of length and width of each frond of a subset of stipes). Frond area was calculated considering their roughly rectangular shape. Similarly, the area of *sorus* per frond was estimated by measuring width and length of each dark brown area of the fronds corresponding to sexually mature tissue.

To determine the frequency of chimeric adult plants of *L. spicata*, tissue samples from fronds of five to six different stipes were sampled from each of the 50 plants in the four studied grids (FJC, FJS, IDP1 and IDP2 – see table 1). One of these frond samples corresponded to the central, longest stipe. Tissue samples were collected from the basal meristematic area of fronds. Samples were then dried in silica gel for posterior molecular analysis.

Recruits were sampled at the same sites at spring 2009. At first, grids were positioned at the same place as for the adults, using 50 x 50 cm quadrats, and a maximum of two recruits per $0.25m^2$-quadrat was collected. Then, two transects (max 30 m long) were extended parallel to the coastline, one in each side of the grid, using the same $0.25m^2$-quadrats. Again, a maximum of two recruits were collected per quadrat. Fronds of collected recruits were then dried in silica gel for posterior molecular analysis. We ended with a total of 244 and 215 adult samples for IDP1 and IDP2 respectively for grid 1 and 2 of Isla Damas, and 219 and 223 adult samples for FJC and FJS respectively for central and southern grid in Fray Jorge). A total of 155 and 213 recruits were sampled in each grid of Isla Damas and 100 and 91 at Fray Jorge.

Genetic Analyses

At the laboratory, after tissue grounding, DNA was extracted with a modified CTAB protocol with the addition of Polyvinyl Pyrolidone (PVP), following Tellier et al (2009). The pellet was eluted in 50 µL of MiliQ water, quantified by NanoDrop (NanoDrop Technologies Wilmington, Delaware, USA) and diluted to 15 ng μL^{-1}. Seven microsatellite loci (Faugeron et al 2009) were used for Fray Jorge, and only four out of ten tested were polymorphic or amplified well for Isla Damas. PCRs were performed according to Faugeron et al (2009). PCR

products were analyzed on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). Genotypes were first scored manually using Genemarker (Softgenetics LLC) and then alleles were binned with FexiBin v2 (Amos 2007).

The number of different multilocus genotypes per plant was then obtained. Genotypes that differed in at least one allele were considered different. According to our sampling scheme, each plant could present from one to six different genotypes. Plants that presented only one genotype (i.e. all samples exhibited the same genotype) were called unigenotypic or non-chimeric and plants that had more than one genotype were termed plurigenotypic or chimeric. The frequency of uni- and plurigenotypic plants was calculated for each grid. Plurigenotypic plants were considered as groups, since they harbor a collection of different genotypes.

The pairwise coefficient of relatedness *R* (Queller and Goodnight 1989) was calculated for all pairs of adult genotypes with Genalex 6.5 (Peakall and Smouse 2012), so we had intragroup (pairwise *R* values within chimeric plants) and interplant (pairwise *R* values among genotypes from different plants) relatedness.

Fitness measurements

Reproductive investment was characterized by measuring the total frond surface (i.e. the amount of vegetative tissue available for sorus to develop), sorus surface and percentage of frond surface differentiated in sorus. Counting and measuring fronds and sorus was too much time consuming, so only one estimate of reproductive investment (e.g. one measured stipe) per holdfast was available. Therefore, no plant level reproductive investment was estimated.

Genotypic reproductive success (i.e. number of assigned recruits per adult genotype) was determined through parentage analysis in each of the four studied grids. A total of 93, 98, 146 and 118 adults, and 98, 89, 104 and 179 recruits were included in the analyses of FJC, FJS, IDP1 and IDP2, respectively. Parentage assignment was done with Cervus 3.0 (Marshall et al 1998; Kalinowski et al 2007). Cervus uses a likelihood-based approach to determine the most likely parents of individuals of interest. It is based on LOD scores of potential parents, which is the likelihood of paternity of a particular individual relative to the likelihood of paternity of any alternative individuals. LOD scores were tested statistically by simulations. We carried out parent-pair analyses with unknown sex and self-fertilization allowed. Putative parents with the highest positive LOD scores and no mismatches were assigned as parents.

To correct for differences between uni- and plurigenotypic plants in the number of stipes of each genotype that could bias our comparisons of genotypic reproductive success, we standardized genotypic reproductive success by the number of stipes of each genotype within plants. Plant reproductive success was obtained through the sum of (unstandardized) genotypic reproductive success within plants. So, we ended with genotypic reproductive investment and genotypic and plant reproductive success.

Costs and benefits of chimerism in reproductive success were assessed in comparison to genotypes belonging to unigenotypic plants, following Amar et al (2008). Costs and benefits were calculated at both genotypic and plant levels as the ratio between average reproductive success of plurigenotypic (2-5 genotypes) and unigenotypic plants. Ratios >1 were interpreted as benefits in chimeras over non-chimeric plants.

Statistical Analysis

To analyze if average holdfast diameter, number of stipes, number of fronds and measures of reproductive investment (frond surface, *sorus* surface and percent frond area differentiated in *sorus*) significantly differed among grids and sites, we performed Nested ANOVAs, with sites and grids nested in sites as independent variables. Dependent variables were transformed in logarithmic or in arcsine (percentages) to meet assumptions of the test. To analyze whether chimeric plants of *L.spicata* were significantly more frequent than non-chimeric ones, we performed a multiway chi-squared test (Mantel-Haenszel test).

To evaluate kinship grouping within chimeric plants, we tested if average *R* values within groups were significantly higher than *R* values from background population (adapted from Quirici et al 2011). Briefly, we generated a distribution of average intraplant *R* values by randomly resampling with replacement a subset of observed *R* values to generate a 95% confidence interval of this background population distribution, and then compared to the average observed intragroup *R* values of each chimeric plant. Observed intragroup *R* values that fell outside the 95% confidence interval of the randomly created *R* distribution were considered significantly higher than background population.

To analyze if standardized genotypic reproductive success was related to reproductive investment, Spearman´s rank correlations were performed for each grid. Only reproductive success data from genotypes of the central stipes were used for the analysis.

The effects of the number of genotypes per plant, average plant relatedness and type of plant (chimeric versus non-chimeric) on fitness were analyzed with Generalized Linear Mixed Models (GLMMs - McCullagh & Nelder 1989) to account for both fixed and random factors on the dependent variables. The significance of random factors was tested with likelihood ratio

tests comparing the full model to those without the term of interest. First, random intercept models with a normal error structure and identity link function were fitted to evaluate the effect of the number of genotypes per plant and average plant relatedness on reproductive investment (frond surface, *sorus* surface and percent frond area differentiated in *sorus*). The number of genotypes per plant and average relatedness were used as fixed factors and sites and grids nested within sites were considered as random factors. Then, we fitted models with a normal error structure and identity link function to evaluate the effect of the number of genotypes per plant and average plant relatedness on standardized genotypic reproductive success. The number of genotypes per plant and average relatedness were considered as fixed factors and plant nested in grid, grid nested in site and site were considered as random factors.

Afterwards, we tested whether chimeric plants resulted in enhanced reproductive fitness. First, models were fitted to evaluate the effect of belonging to uni- versus plurigenotypic plants on reproductive investment. As above, a normal error structure and identity link function was used. Plant type (chimeric or non-chimeric) was considered as the fixed factor and site and grid nested in site were considered as random factors. Second, GLMMs were used to analyze reproductive success data. To analyze whether genotypes within chimeric plants reproduced more than those within non-chimeric plants, considering they possessed relatively smaller representation within plants, we fitted a model with standardized genotypic reproductive success as the response variable, plant type as the fixed factor and plant nested in grid, grid nested in site, and site as random factors. The model was adjusted with a normal error distribution structure. Third, a GLMM was adjusted to evaluate the effect of belonging to chimeric versus non-chimeric plants on plant-level reproductive success. In this case, plant reproductive success was used as the dependent variable, plant type

as the fixed factor, and grid nested in site and site as random factors. The model was adjusted with a Poisson error distribution and logarithmic link function. Fourth, to evaluate the effect of chimerism on the probability of *L. spicata* reproducing (data as 0 or 1s), we fitted two GLMMs with binomial error structure and logit link function: one for the genotypic probability of reproducing as the dependent variable and the other for the plant probability of reproducing (i.e. probability of at least one genotype reproducing within plants) in different types of plants (uni- versus plurigenotypic). Finally, we also built models to investigate the effect of the number of genotypes per plant on genotypic and plant probability of reproducing. Since the probability of reproducing within chimeric plants is likely to depend on the number of genotypes reproducing, we explored the relationship between the number of genotypes reproducing and the number of genotypes per plants for each grid. All GLMMs were adjusted with lme4 package (Bates et al 2014; functions lmer and glmer). All analyses were performed in R 3.10 (R Development Core Team 2014).

Results

Plant density, morphometric measures and reproductive investment

Table 1 shows holdfast density, morphometric measures and reproductive investment of *L. spicata* for the four grids in the two study sites. Plant density within grids was of 2.6 plants m^{-2} in FJC, 1.6 plants m^{-2} in FJS and 4.3 plants m^{-2} in both IDP1 and IDP2. Average holdfast diameter was 22.8 ± 14.2 and 20.6 ± 11.7 cm in ID grids and 28.5 ± 10.2 and 29.8 ± 13.9 cm in FJ grids, yet these values did not differ significantly among sites ($F_{1,2} = 14.38$, $p = 0.063$) or grids ($F_{2,140} = 1.34$, $p = 0.266$). Average number of stipes per holdfast ranged from 17.1 ± 13.6 in FJS to 30.0 ± 19.6 in IDP1 and was not statistically different between sites ($F_{1,2} = 0.13$, $p = 0.753$) but it was among grids ($F_{2,140} = 8.65$, $p < 0.001$). Tukey HSD test showed that IDP1 and FJS and IDPN and IDPC were significantly different ($p = 0.008$ and $p = 0.05$, respectively). Average number of fronds per stipe was higher in the two ID grids (149.9 ± 172.0 and 132.1 ± 155.3) than in FJ grids (66.0 ± 47.2 and 93.8 ± 69.2), yet a high variance within ID grids was also observed. Consequently, differences in average number of fronds per stipe were not statistically significant among sites ($F_{1,2} = 0.01$, $p = 0.922$) nor grids ($F_{2,116} = 1.31$, $p = 0.273$). Measures of reproductive investment (e.g. average frond surface, sorus surface and percent frond area differentiated in sorus) were highly variable both among and within grids. Overall, a tendency for higher average reproductive investment in Fray Jorge than in Isla Damas was observed for all variables estimated. Average frond surface was of 206.4 ± 174.9 and 341.8 ± 275.1 cm^2 in IDP1 and IDP2, respectively, while in FJC and FJS it was of 701.4 ± 419.3 and 1,134.5 ± 732.5 cm^2, respectively. Frond surface was not statistically different between sites ($F_{1,2} = 11.65$, $p = 0.076$), however it was among grids ($F_{2,116} = 6.70$, $p = 0.002$), since almost all pairs of grids were statistically different, except from FJC and FJS (p

= 0.734). Average sorus surface ranged from 17.3 ± 36.6 cm^2 in IDP1 to 385.4 ± 381.7 cm^2 in FJS. Average sorus surface was not statistically different between sites ($F_{1,2}$ = 13.14, p = 0.068), yet it was among grids ($F_{2,116}$ = 6.52, p = 0.002). Accordingly, average surface differentiated in sorus ranged from 6.2 ±9.8 % in IDP1 to 30.5 ± 17.1 % in FJS. It was not statistically different between sites ($F_{1,2}$ = 5.52, p = 0.143), yet it differed significantly among grids ($F_{2,116}$ = 6.57, p = 0.002). Significant differences were observed between FJC and IDP1 (p = 0.005), FJS and IDP1 (p < 0.001) and FJS and IDP2 (p < 0.001).

Reproductive Success

Parentage analysis revealed a high assignment rate of juveniles to at least one adult genotype of the same grid. Fifty percent of the recruits sampled in FJC had at least one sampled parent in the grid. In FJS, 56.2 % of the recruits had at least one parent assigned. In Isla Damas, 48.7 % and 79.3 % of the recruits sampled in IDP1 and IDP2, respectively, were successfully assigned to an adult of the same grid. The number of adults that were assigned to at least one offspring was 30 (32%) in FJC, 43 (44%) in FJS, 50 (34%) in IDP1 and 62 (52%) in IDP2. Standardized genotypic reproductive success was highly variable within grids and ranged from 0 to 4 in FJC and FJS, 0 a 6 in IDP1 and from 0 to 13 in IDP2. Plant reproductive success (calculated as the sum of unstandardized genotypic reproductive success within plants) ranged from 0 to 5 in FJC, 0 to 8 in FJS, 0 to 8 in IDP1 and 0 to 16 in IDP2.

Frequency of chimerism

All genotyped fronds presented a maximum of two alleles per locus, suggesting that each stipe is genetically uniform. A high frequency of chimeric plants of *L. spicata* was found in the four grids studied (Table 2). The frequency of chimeric plants was over 60 % (range 61.2 – 90 %) in all grids (Table 2). Chimeric plants were significantly more frequent than non-chimeric ones in all cases (Mantel-Haenszel $X^2 = 21.27$, $p < 0.001$). The relative frequency of plants with 1-5 genotypes also changed significantly between grids and sites (Cochran-Mantel-Haenzel $M^2 = 39.08$, $p < 0.001$). In FJC two- genotype plants were the most frequent, while in FJS plants with one genotype were the most frequent. In IDP1 and IDP2, plants with five and three genotypes, respectively, were the most abundant (Table 2).

Relatedness

Overall, the distribution of average within-group R-values was skewed to higher than on average in the population, since in all but one case (one group in FJC, see Fig 1), the R-values that fell outside the 95% IC were above the superior limit, suggesting a tendency of kin aggregation within plants. Yet, average relatedness within groups was not statistically different from background population in many cases. This was particularly evident in FJC and IDP2, where only 9 to 13% of the groups had an R-value that fell outside the 95% IC of the population (Fig. 1). In FJS and IDP1, a higher frequency of chimeric plants consisted of close kin (30 and 49%, respectively; Fig. 1).

Reproductive investment versus reproductive success

We did not find evidence that higher genotypic reproductive investment leads to increased standardized reproductive success in *L. spicata*. Overall, genotypic reproductive success was not significantly correlated with frond surface, sorus surface, nor frond surface differentiated in sorus (r_s ranged from -0.35 to 0.16 - Table 3). The only exception was the significant negative correlation between genotypic reproductive success and frond surface found in FJS (r_s = -0.35, p = 0.01).

The effect of the number of genotypes per plant and relatedness on reproduction

Reproductive investment was not significantly affected by the number of genotypes within plants (Frond surface: parameter estimate ± SE = -0.01 ± 0.03, t = -0.33, p = 0.75; Sorus surface: -0.05 ± 0.06, t = -0.95, p = 0.38; Frond surface differentiated in sorus: -0.01 ± 0.01, t = -0.48, p = 0.64 – Table 4). Moreover, the effect of intraplant relatedness on reproductive investment was not statistically significant (Frond surface: 0.13 ± 0.07, t = 1.73, p = 0.13; Sorus surface: 0.12 ± 0.15, t = 0.80, p = 0.46; Frond surface differentiated in sorus: 0.03 ± 0.04, t = 0.85, p = 0.42 – Table 4). Also, genotypic reproductive success was neither significantly affected by the number of genotypes per plant (0.06 ± 0.06, t = 1.04, p = 0.486) or by relatedness (-0.17± 0.18, t = -0.96, p = 0.384). Interestingly, a positive significant relationship between the number of genotypes per plant and plant reproductive success was found (0.35 ± 0.07, z = 4.66, p < 0.001). Conversely, while the probability of plants reproducing (i.e. presenting at least one reproductive genotype) was significantly affected by the number of genotypes per plant (-0.10 ± 0.08, z = -1.19, p = 0.232), the probability of genotypes reproducing was not (0.79 ± 0.18, z = 4.29, p = <0.001 - Table 4).

The effect of chimerism on reproduction

On average, reproductive investment tended to be higher in non-chimeric plants, (Fig. 2). Yet, models did not reveal a significant effect of chimerism on any of the three measures of reproductive investment (Frond surface: parameter estimate ± SE = 0.127 ± 0.062, t = 2.081, p = 0.105; Sorus surface: 0.237 ± 0.118, t = 2.021, p = 0.113; Frond surface differentiated in sorus: 0.05 ± 0.03, t = 1.82, p = 0.142 – Table 4). A significant effect of grids to the variance in reproductive investment was found (Frond surface; $X^2 = 6.51$, p = 0.011; Sorus surface: $X^2 = 6.19$, p = 0.013; Frond surface differentiated in sorus: $X^2 = 4.62$, p = 0.031).

Standardized genotypic reproductive success in non-chimeric versus chimeric plants was of 0.05 ± 0.04 versus 0.41 ± 0.08 in FJC, of 0.16 ± 0.04 versus 0.46 ± 0.08 in FJS, of 1.08 ± 0.57 versus 0.47 ± 0.08 in IDP1 and of 1.13 ± 0.56 versus 1.45 ± 0.24 in IDP2, respectively (Fig. 3a – see Fig S2 for comparison to unstandardized data). Overall, genotypic reproductive success was not significantly affected by the type of plant (non-chimeric versus chimeric) (parameter estimate ± SE = 0.12 ± 0.20, t = 0.61, p = 0.64 –Table 4). Variance among grids contributed significantly to the variance in genotypic reproductive success ($X^2 = 17.46$, p < 0.001). Plurigenotypic plants produced more offspring than unigenotypic ones. Average plant reproductive success within chimeric and non-chimeric plants was of 0.30 ± 0.21 and 1.59 ± 0.26 in FJC, of 0.58 ± 0.13 and 1.73 ± 0.31 in FJS, of 1.27 ± 0.56 and 1.79 ± 0.30 in IDP1, and of 1.94 ± 0.60 and 6.23 ± 0.81 in IDP2, respectively (Fig. 3b). A significant effect of chimerism on plant reproductive success was found (parameter estimate ± SE = 1.00 ± 0.19, z = 5.33 , p < 0.001 – Table 4). Variance between grids contributed significantly to the variance in plant reproductive success ($X^2 = 19.77$, p < 0.001), yet variance between sites did not (X^2

=0.0007, p = 0.98). When we plotted the ratio between average reproductive success within chimeric and non-chimeric plants in function of the number of genotypes per plant, no clear pattern of genotypic fitness effects of chimerism was observed due to differences among grids (Fig. 4a). Yet, a clear tendency towards benefits of chimerism was observed at the plant level, despite differences among grids (Fig. 4b).

The probability of a genotype reproducing was not significantly affected by the type of plant it belonged (-0.31 ± 0.29, z = -1.08, p = 0.28) nor by the number of genotypes per plant (-0.10 ± 0.08, z = -1.19, p = 0.23) (Table 4). Yet, chimeric plants showed a significantly higher probability of successfully reproducing than non-chimeric plants (1.08 ± 0.36, z = 3.04, p = 0.002) (Table 4, Fig. 5). Accordingly, the number of genotypes reproducing within plants increased significantly with the number of genotypes per plant (0.33 ± 0.06, z = 5.71, p < 0.001). The slopes of the observed relationship were not significantly different from the ones expected based on the probability of a genotype reproducing in each grid (FJC: t_6 = -0.95, p > 0.05; FJS: t_6 = -0.27, p > 0.05; IDP1 t_6 = -0.49, p > 0.05, IDP2: t_6 = -1.67, p > 0.05 - Fig. S2).

Discussion

Chimerism in natural populations

Although it is widely recognized that several kinds of organisms form chimeras, their frequency in natural populations is poorly known (Pineda-Krch and Lehtila 2004), mainly due to the difficulty of distinguishing solitary individuals (or colonies) from chimeras in the field. Using molecular analysis techniques, we found that the majority of *L. spicata* plants sampled in four grids from two sites in northern Chile were chimeric (range 61-90 %). Accordingly, González et al (2013) reported that 71.4 % of *L. spicata* plants were chimeric. This demonstrates that group living is common in natural populations of this kelp. It is also frequent within populations of its sister species *L. berteroana* (González et al 2013, Segovia et al 2014). Rates of natural chimerism are comparatively much lower in other marine populations: 3-5 % in the coral *Acropora millepora* (Puill-Stephan et al 2009), 3 % in the anemone *Urticina felina* (Mercier et al 2011), 8-14 % in the ascidian *Botryllus schlosseri* (Ben-Shlomo et al 2001, Ben-Shlomo et al 2008), 3-61 % in the ascidian *Diplosoma listerianum* (Sommerfeldt et al 2003). Considering that the frequency of chimerism in a given environment is thought to reflect both the likelihood of encounters between compatible conspecifics and the relative costs and benefits of forming chimeras (Hart and Grosberg 1999), the high frequency of chimerism in adults of *L. spicata* suggests that encounters between compatible individuals in natural populations are usual and that costs are low, differently from what is observed in most marine invertebrates, except from *D. listerianum* (see below).

Relatedness within chimeras

Relatedness within chimeric plants of *L. spicata* was variable. Although an overall tendency towards high relatedness values was observed within groups, in many cases average relatedness within groups of *L. spicata* was similar to background relatedness. This is in contrast with the findings of Segovia et al (2014), who reported that most chimeric plants of *L. berteroana* were composed of individuals more related to themselves than to the rest of the population. Yet, our result does not necessarily imply that chimeras are not composed by kin, since biased relatedness estimates due to spatial genetic structure could have underestimated within-group relatedness in *L. spicata*. Relatedness estimates are calculated in relation to a reference population (i.e. background population), which is assumed to be large and with random mating (Wang 2011). However, when this assumption is not met (i.e. background population is genetically structured), relatedness coefficients will probably be underestimated (Wang 2011) and the detection of a further structure within groups will be less likely unless it is too marked. This could explain our result, since kin structure (i.e. a negative relationship between pairwise relatedness and geographic distance) is expected to occur within local populations of *L. spicata* as a result of limited spore dispersal (Faugeron et al 2005, Tellier et al 2009). In this case, fusion might occur between closely related conspecifics, despite similar average within- and between-plant relatedness values. We are going to investigate this fiurther in a forthcoming paper on the fine-scale genetic structure of *L. spicata* (Chapter 2).

Even so, the high variability in within-group relatedness suggests that fusion in *L. spicata* may occur between less related individuals in some cases. A similar finding has been reported for the colonial ascidian *Diplosoma listerianum* (Bishop and Sommerfeldt 1999). In

contrast, high within-group relatedness has been reported in several marine invertebrates including the corals *Stylophora pistillata* (Amar et al 2008) and *Acropora millepora* (Puill-Stephan et al 2009), the hydrozoan *Hydractinia symbiolongicarpus* (Grosberg et al 1996) and the colonial ascidian *Botryllus schloserii* (Rinkevich 2005). All of these species share a similar characteristic: the presence of an effective kin recognition system that only allows successful fusion to occur among close kin, otherwise a reaction rejection is triggered. It has been proposed that the evolution of such effective kin recognition systems in invertebrates has been driven by the severe costs of fusing with unrelated conspecifics in those species, mainly resultant from cell parasitism- i.e. competition between genetically different cells (Buss 1982; Aanen et al 2008; Brusini et al 2013). Cell parasitism is present in species in which fusion involves the development of a common "circulatory" system that allows the passage of cell lineages from one individual to the other (the so-called cytomictical chimeras). In contrast, the possibility of cell parasitism is absent in those species that form "sectorial chimeras", since no common vascular system exists, allowing genotypes to maintain their integrity. Our results indicate that *L. spicata* forms sectorial chimeras, since no evidence of more than two alleles per microsatellite locus was found (which would indicate the presence of more than one genotype). Apparently, stipes maintain their genotypic identity after holdfast fusion. This suggests that the costs of fusion with non-kin in *L. spicata* are comparatively lower than in some invertebrates. The same has been proposed for *D. listerianum*, in which the exchange of cell lineages is precluded due to the absence of a common vascular system after chimera formation (Bishop and Sommerfeldt 1999). Moreover, the high frequency of chimerism in natural populations of *L. spicata* and *D. listerianum* compared to that of other marine organisms seems to support that costs of chimerism in these species are low.

Chimerism effects on reproductive success

Chimerism is thought to be adaptive if fitness benefits related to group living exceed its costs (Buss 1982). Studies on the costs and benefits of fusion have compared the fitness of solitary individuals to that of the whole chimeric entity (but see Amar et al 2008). The simultaneous analysis of the effects of fusion at both the individual and the group levels is important though, since contrasting responses might appear at each level. Here we evaluated the fitness effects of group living in *L. spicata* by comparing genotypic reproductive investment and success between uni- and plurigenotypic plants. We also assessed potential effects of group living at total plant reproductive success. Our results were consistent between sites, despite some variability among grids. We did not find clear evidences of direct benefits on genotypic reproductive investment or reproductive success related to group living. In fact, genotypic reproductive investment tended to be higher in non-chimeric plants (although not statistically significant). A potential explanation for this is that in the case of *L. spicata*, fusion means sharing a holdfast with conspecifics, which might involve a potential decrease in the relative number of stipes per genotype (and hence fronds), that could lead to a lower reproductive investment per genotype. Genotypic reproductive investment did not correlate with genotypic reproductive success though. Some limitations of our sampling scheme may have influenced the result observed. These include: i) the low number of stipes (5-6) sampled from each holdfast (average number of stipes per holdfast ranged from 17.1 ± 13.6 to 30.0 ± 19.6), and ii) the high variance observed among genotypes, that could have decreased the power of our statistical tests. Regardless, a possible lack of correspondence between reproductive investment and success in *L. spicata* is not surprising, considering that in kelps (Laminariales), several other factors may influence a genotype´s reproductive success

including spore survival, spore settlement, gametophyte survival, fertility of the gametophyte, distance between male and female gametophytes for effective fecundity, and survival of microscopic sporophyte (Schiel and Foster 2006).

Overall, chimerism did not significantly improve genotypic reproductive success or probability of reproducing. This suggests that, in general, there are no direct genotypic benefits in reproductive success related to chimerism in *L. spicata*. Yet, there are also no costs (except for IDP1). It has been suggested that since chimerism involves an immediate increase in size, one of its benefits would be enhanced reproductive output, given that fecundity increases with size in many marine species (Buss 1982, Santelices et al 1999). Yet, few studies have explicitly evaluated the consequences of chimerism on reproduction and the only one that did, reported that fusion between colonies of the coral *Stylophora pistillata* decreased the overall reproductive output of fused colonies (Rinkevich and Loya 1985). As far as we are concerned, no study has investigated reproductive effects of chimerism at the genotype level, possibly due to methodological difficulties. Here we demonstrated that an estimate of the reproductive effects of chimersim is possible using molecular markers and parentage analysis.

Differently from what we observed for genotypes, we found clear benefits in reproductive success at the level of the entire plant. Average reproductive success was higher in chimeric than in non-chimeric plants in all grids. Moreover, chimeric plants exhibited a higher probability of producing offspring than non-chimeric plants. Therefore, our results point towards benefits in reproductive success related to chimerism in *L. spicata*, as a result of plant level positive effects. This pattern is a product of more individuals reproducing in chimeric plants. The number of individuals that reproduce within a plant increased with group

size (the number of genotypes per plant) in all four grids with a rate similar to the one expected by chance. It demonstrates that more than one genotype can successfully reproduce within groups and that chimeric plants have a higher probability of bearing reproductive genotypes than non-chimeric ones.

This study points that the importance of chimerism to reproductive success in *L. spicata* relies on allowing the coexistence of a higher number of individuals (and potential reproducers and mates), if compared to a scenario where only unigenotypic plants occurred. Considering that self-reproduction is possible in *L. spicata*, chimerism may also play an important role in promoting outcrossing, as observed in aggregations of the barnacle *Pollicipes elegans* (Plough et al 2014). Future studies addressing inbreeding rates, inbreeding depression and the effect of chimerism in promoting outcrossing in *L. spicata* could provide support for this proposition.

The adaptive value of chimerism

It still remains puzzling how chimerism is evolutionary maintained in so many clades, since it might involve severe costs such as rejection and death of some partners (Rinkevich 2005). Considering that in the majority of species studied, successful chimerism occurs between close relatives, it has been proposed that indirect fitness benefits might counteract possible direct costs (Rinkevich 2011). Yet, in some other species costs related to cell parasitism and rejection are negligible (Bishop and Sommerfeldt 1999, Brusini 2013), and although fusion with relatives can occur, direct benefits may also exist. This seems to be the case of *L. spicata*.

The high frequency of chimerism detected in natural populations of *L. spicata* suggests that group living might be adaptive for this kelp. Here we showed that despite fused genotypes did not produce a higher number of offspring, chimeric plants as a whole contributed with a higher number of offspring than non-chimeric plants, since more than one genotype eventually reproduced and therefore the probability of a chimeric plant being reproductive was higher. Hence, this study points towards advantages of chimeric over non-chimeric plants. In this sense, our findings suggest the potential for natural selection also acting at levels higher than the genotype (i.e. a multilevel selection context). Selection at the level of the chimera could be favoring the maintenance of chimerism in natural populations of *L. spicata*. Several other studies on chimeras have also pointed toward this direction (e.g. Rinkevich 2005, Amar et al 2008, Folse and Roughgarden 2010, McIntire and Fajardo 2011). Moreover, although relatedness within groups was not higher than between plants, we cannot rule out the possibility that groups are composed by kin as a result of population viscosity (Hamilton 1964). In this case, indirect genotype-level benefits related to fusion with conspecifics could also be accrued (i.e. kin selection could also have a role in the maintenance of chimerism). More studies are needed to build a general picture on the current adaptive value of chimerism in *L. spicata*.

Here we have evaluated the effect of chimerism at one life-cycle stage and on one fitness component. It is possible that selective pressures that favor being chimeric are stronger at other stages of the life-cycle of *L. spicata* and/or in other fitness components like growth and survival. For example, fusion between conspecifics at early ontogenetic stages might enhance individual growth and probability of survival, when mortality rates by herbivory and wave action are high (Martínez and Santelices 1998). It is well documented that fusion at early

life-cycle stages increases survival probabilities of red algae (Santelices and Aedo 2006; Santelices and Alvarado 2008). Also, the high frequency of chimerism reported for juvenile plants of *L. berteroana* in low density habitats suggests survival benefits related to chimerism in adverse conditions (Segovia et al 2014). Therefore, studies on the fitness effects of fusion in early stages in the life-cycle of *L. spicata* are required. Moreover, although the analysis of the effects of kinship on group living relies on the estimation of inclusive fitness, quantifying inclusive fitness is a difficult task (Grafen 1982, Lukas et al 1996). Future studies cultivating sporophyte clones in the laboratory could provide opportunity for comparing survival probabilities of solitary genotypes, genotypes fused to themselves (clones), fused genotypes produced by selfing (chimeras with high relatedness) and fused genotypes produced with different levels of outbreeding (chimeras with variable relatedness), while controlling for the effect of the genotype. Finally, the recent description of the existence of cellular connection between fused genotypes of *L. spicata* (González et al 2013), potentially allowing for the interchange of molecules among members of the chimera, opens an avenue of research possibilities to explore the occurrence of cooperative and competitive interactions among group members in this species.

Aknowledgements

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Figure Legends|

Figure 1. Frequency distribution of average intragroup relatedness of chimeric plants of *L. spicata* for each grid. Black bars indicate the 95% confidence interval of background population relatedness. FJC = Fray Jorge Centro, FJS = Fray Jorge Sur, IDP1 = Isla Damas Patch 1, IDP2 = Isla Damas Patch 2.

Figure 2. Genotypic reproductive investment of *L. spicata* in uni-and plurigenotypic plants. Points represent averages ± standard errors. FJC = Fray Jorge Centro, FJS = Fray Jorge Sur, IDP1 = Isla Damas Patch 1, IDP2 = Isla Damas Patch 2.

Figure 3. Genotypic (a) and plant reproductive success (b) of *L. spicata* in uni- and plurigenotypic plants. Points represent averages ± standard errors. FJC = Fray Jorge Centro, FJS = Fray Jorge Sur, IDP1 = Isla Damas Patch 1, IDP2 = Isla Damas Patch 2.

Figure 4. Costs or benefits of chimerism on reproductive success of genotypes (a) and plants (b) for groups of 2-5 genotypes. Values represent ratios between average reproductive success within chimeric plants and non-chimeric plants. Values >1 were considered as benefits and values <1 were considered as costs. FJC = Fray Jorge Centro, FJS = Fray Jorge Sur, IDP1 = Isla Damas Patch 1, IDP2 = Isla Damas Patch 2.

Figure 5. The effect of chimerism on plant probability of reproducing for each grid. Bars depict the proportion of uni (Uni) and plurigenotypic (Pluri) plants that reproduce (Yes) and do not reproduce (No). FJC = Fray Jorge Centro, FJS = Fray Jorge Sur, IDP1 = Isla Damas Patch 1, IDP2 = Isla Damas Patch 2.

Table 1. Adult holdfast density (number.m^{-2}) and morphometric variables of the kelp *Lessonia spicata* averaged in each grid. Standard deviation in parenthesis. FJC = Fray Jorge Centro, FJS = Fray Jorge Sur, IDP1 = Isla Damas Patch 1, IDP2 = Isla Damas Patch 2.

	FJC	**FJS**	**IDP1**	**IDP2**
Plant density [N m^{-2}]	2.6	1.6	4.3	4.3
Holdfast diameter [cm]	28.5 (± 10.2)	29.8 (± 13.9)	22.8 (± 14.2)	20.6 (± 11.7)
Number of stipes	28.8 (± 23.2)	17.1 (± 13.6)	30.0 (± 19.6)	21.7 (± 22.2)
Number of fronds[a]	66.0 (± 47.2)	93.8 (±69.2)	149.9 (± 172.0)	132.1 (± 155.3)
Frond surface[ab] [cm^2]	701.4 (± 419.3)	1,134.5 (± 732.5)	206.4 (± 174.9)	341.8 (± 275.1)
Sorus surface[ab] [cm^2]	158.8 (± 189.4)	385.4 (± 381.7)	17.3 (± 36.6)	32.7 (±59.4)
% Reproductive tissue[ab]	20.4 (± 16.1)	30.5 (± 17.1)	6.2 (±9.8)	10.6 (±14.0)

[a]Measured in the central (longest) stipe of each holdfast.

[b]Reproductive investment

Table 2. Frequency of chimeric and non-chimeric plants of *Lessonia spicata*. Values are expressed as counts and relative frequencies (percentages in parentheses). FJC = Fray Jorge Centro, FJS = Fray Jorge Sur, IDP1 = Isla Damas Patch 1, IDP2 = Isla Damas Patch 2.

	FJC	**FJS**	**IDP1**	**IDP2**
Total number of plants	42	49	50	49
Non-chimeric plants	10 (23.8)	19 (38.8)	5 (10.0)	18 (36.7)
Chimeric plants (2-5 genotypes)	32 (76.2)	30 (61.2)	45 (90.0)	31 (63.3)
Plants with 2 genotypes	20 (47.6)	16 (32.6)	4 (8.0)	9 (18.4)
Plants with 3 genotypes	6 (14.3)	9 (18.3)	13 (26.0)	12 (24.5)
Plants with 4 genotypes	5 (11.9)	5 (10.2)	11 (22.0)	6 (12.2)
Plants with 5 genotypes	1 (2.4)	0	17 (34.0)	4 (8.2)

Table 3. Spearman´s rank correlation between standardized genotypic reproductive success (SGRS) and investment. Reproductive investment consists of frond surface, sorus surface and frond surface differentiated in sorus. FJC = Fray Jorge Centro, FJS = Fray Jorge Sur, IDP1 = Isla Damas Patch 1, IDP2 = Isla Damas Patch 2.

Grid	Variables	r_s	p
FJC	SGRS and frond surface	-0.06	0.73
	SGRS and sorus surface	0.07	0.68
	SGRS and frond surface differentiated in sorus	0.02	0.90
FJS	SGRS and frond surface	**-0.35**	**0.01**
	SGRS and sorus surface	-0.11	0.47
	SGRS and frond surface differentiated in sorus	0.16	0.29
IDP1	SGRS and frond surface	-0.02	0.91
	SGRS and sorus surface	0.04	0.82
	SGRS and frond surface differentiated in sorus	0.05	0.79
IDP2	SGRS and frond surface	-0.09	0.57
	SGRS and sorus surface	0.12	0.43
	SGRS and frond surface differentiated in sorus	0.16	0.32

Table 4. Generalized linear mixed models (GLMM).

Variable	Estimate	SE	t-or z-value[b]	p-value
Reproductive Investment				
Frond Surface				
Model 1				
Type of plant[a]	0.13	0.06	2.08	0.105
Model 2				
Number of genotypes	-0.01	0.03	-0.33	0.749
Relatedness	0.13	0.07	1.73	0.133
Sorus Surface				
Model 1				
Type of plant[a]	0.24	0.12	2.02	0.113
Model 2				
Number of genotypes	-0.05	0.06	-0.95	0.380
Relatedness	0.12	0.15	0.80	0.456
Frond Surface Differentiated in Sorus				
Model 1				
Type of plant[a]	0.05	0.03	1.82	0.142
Model 2				
Number of genotypes	-0.01	0.01	-0.48	0.641
Relatedness	0.03	0.04	0.85	0.418
Reproductive Success				
Genotypic Reproductive Success				
Model 1				
Type of plant[a]	0.12	0.20	0.61	0.645
Model 2				
Number of genotypes	0.06	0.06	1.04	0.486
Model 3				
Relatedness	-0.17	0.18	-0.96	0.384
Plant Reproductive Success				
Model 1				
Type of plant[a]	1.04	0.14	7.60	<0.001
Model 2				
Number of genotypes	0.35	0.07	4.66	<0.001
Model 3				
Relatedness	-0.22	0.31	-0.70	0.484
Probability of Reproducing				
Genotypes				
Model 1				
Type of plant[a]	-0.31	0.29	-1.08	0.279
Model 2				
Number of genotypes	-0.10	0.08	-1.19	0.232
Plants				
Model 1				
Type of plant[a]	1.20	0.36	3.31	<0.001
Model 2				
Number of genotypes	0.79	0.18	4.29	<0.001

[a]Type of plant refers to non-chimeric or chimeric.

[b]t values correspond to GLMMs with normal distribution of errors and z values correspond to models with non-normal distribution of errors.

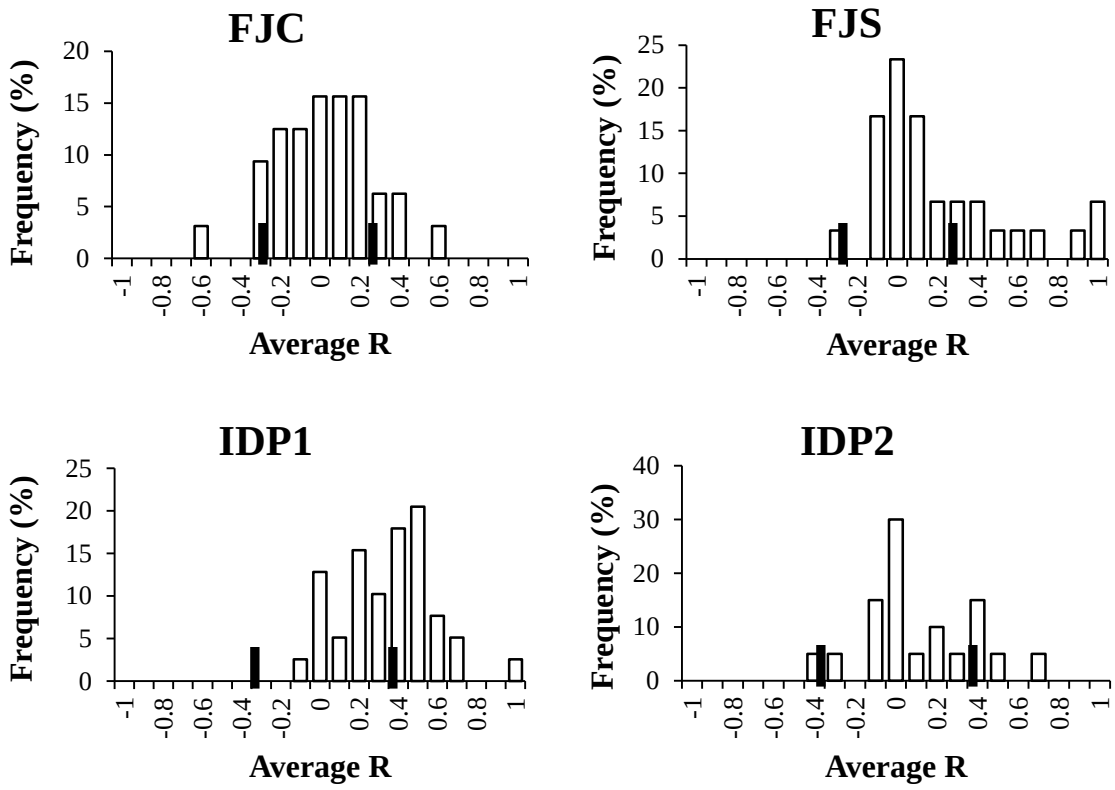
FJC
Frequency (%)
Average R
FJS
Frequency (%)
Average R
IDP1
Frequency (%)
Average R
IDP2
Frequency (%)
Average R

Figure 1.

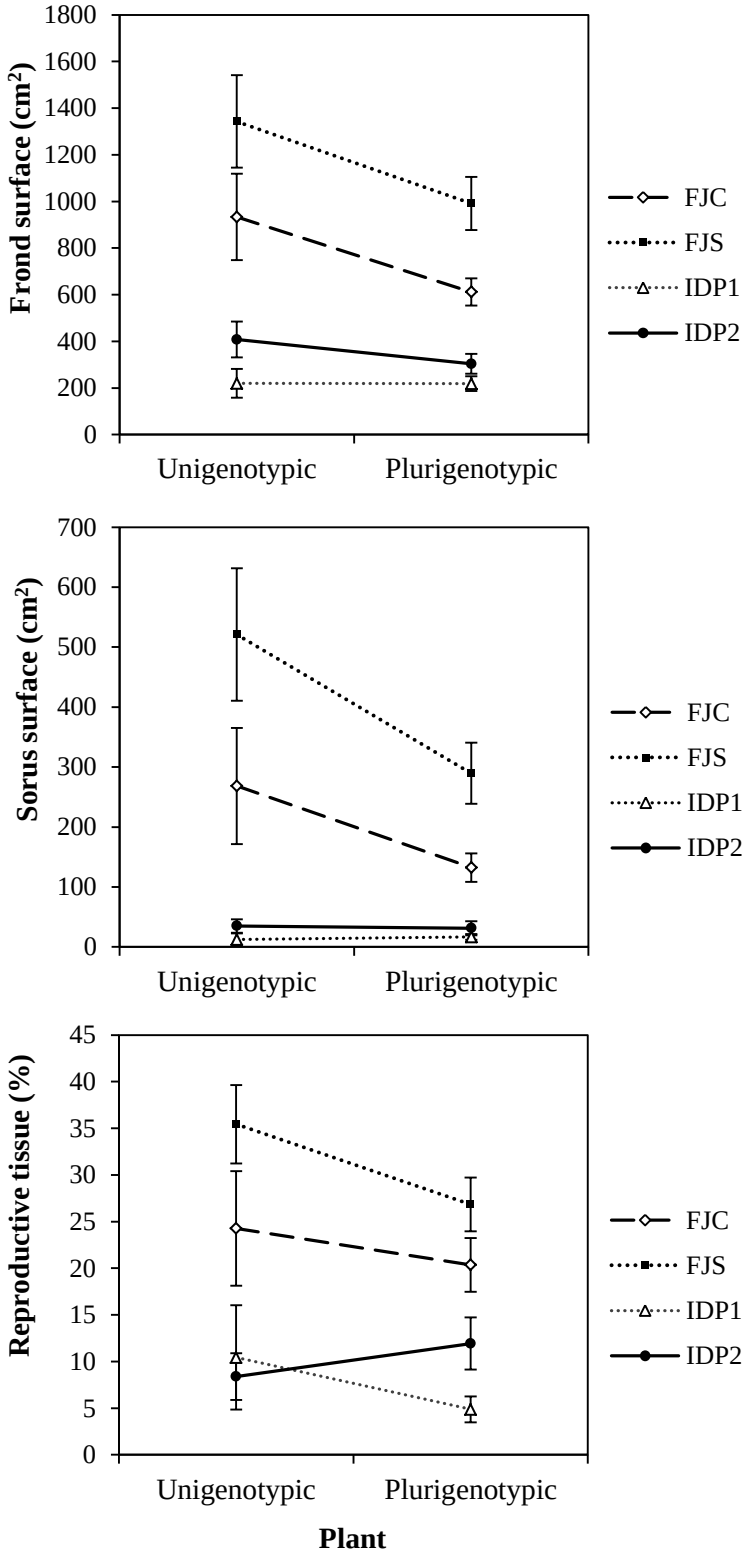
Frond surface (cm²)
1800
1600
1400
1200
1000
800
600
400
200
0
Unigenotypic
Plurigenotypic
FJC
FJS
IDP1
IDP2
Sorus surface (cm²)
700
600
500
400
300
200
100
0
Unigenotypic
Plurigenotypic
FJC
FJS
IDP1
IDP2
Reproductive tissue (%)
45
40
35
30
25
20
15
10
5
0
Unigenotypic
Plurigenotypic
FJC
FJS
IDP1
IDP2
Plant

Figure 2.

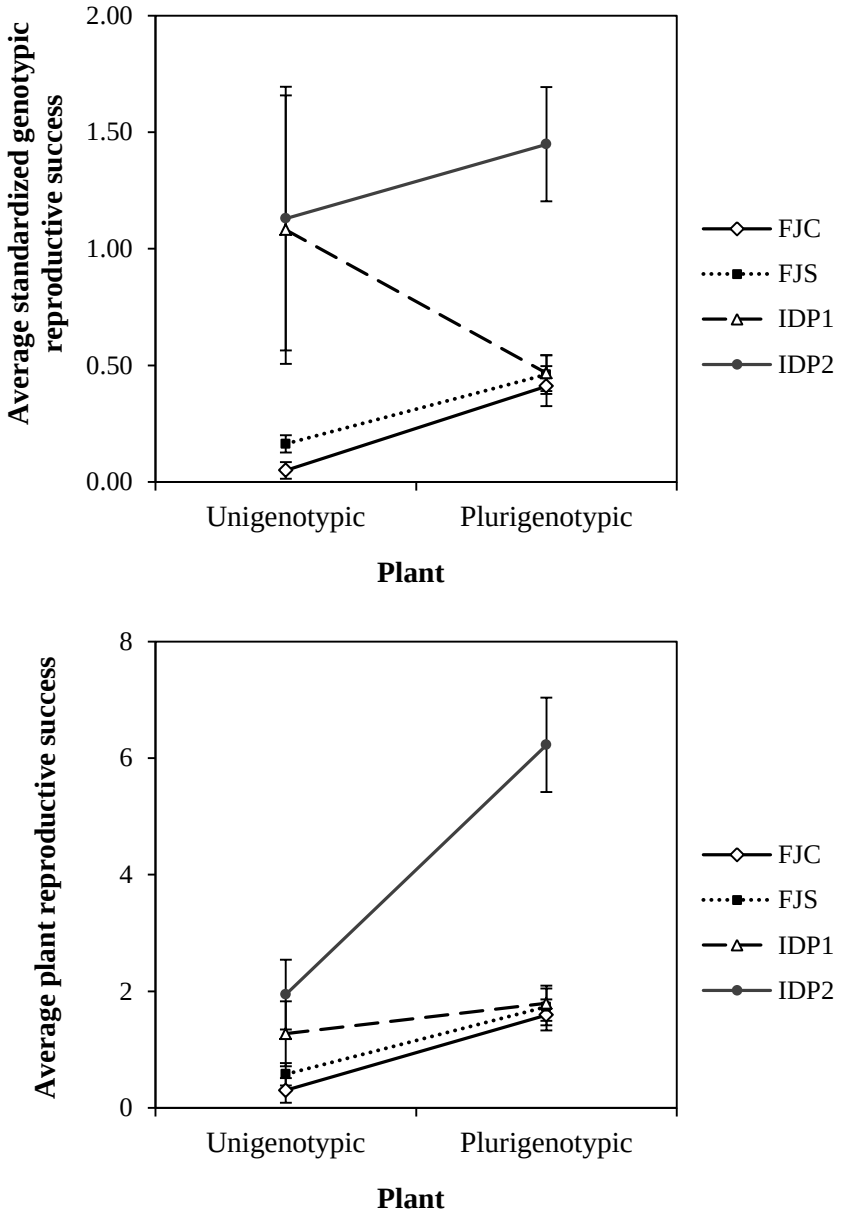
Average standardized genotypic reproductive success
2.00
1.50
1.00
0.50
0.00
Unigenotypic
Plurigenotypic
Plant
FJC
FJS
IDP1
IDP2
Average plant reproductive success
8
6
4
2
0
Unigenotypic
Plurigenotypic
Plant
FJC
FJS
IDP1
IDP2

Figure 3.

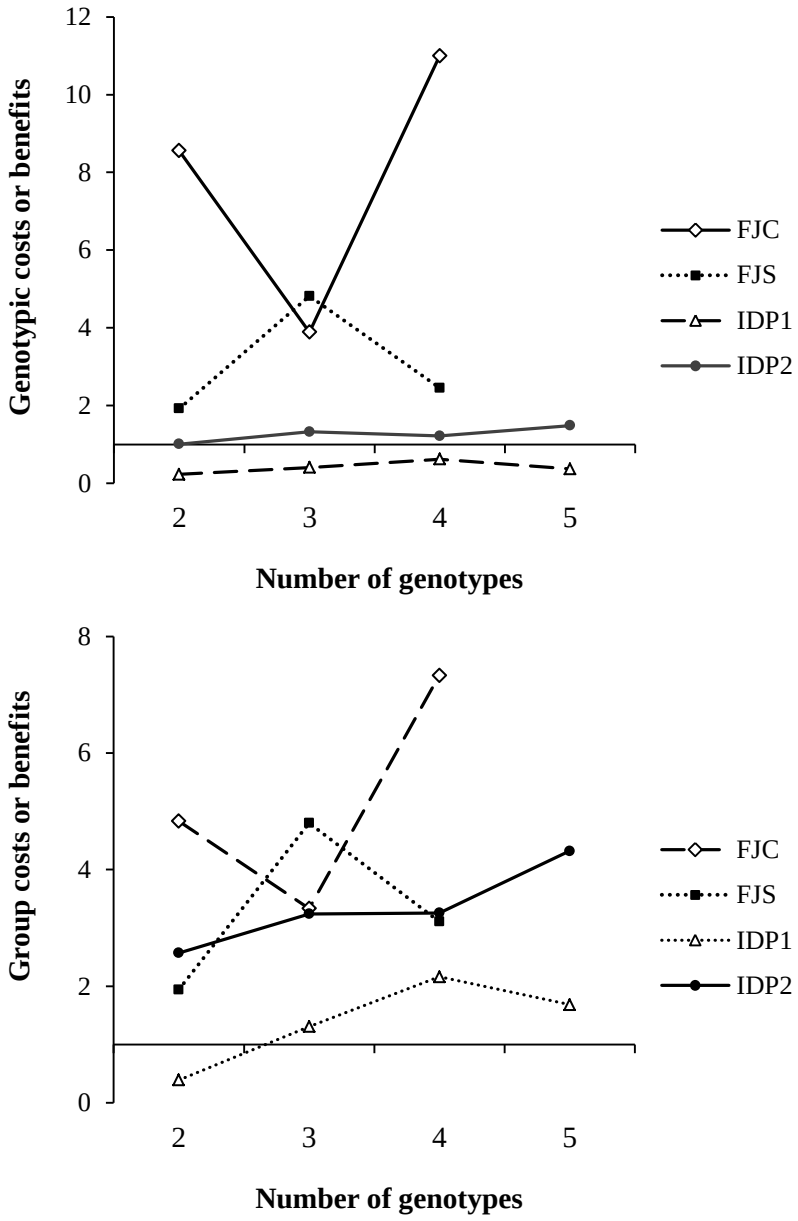
Genotypic costs or benefits
12
10
8
6
4
2
0
2
3
4
5
Number of genotypes
FJC
FJS
IDP1
IDP2
Group costs or benefits
8
6
4
2
0
2
3
4
5
Number of genotypes
FJC
FJS
IDP1
IDP2

Figure 4.

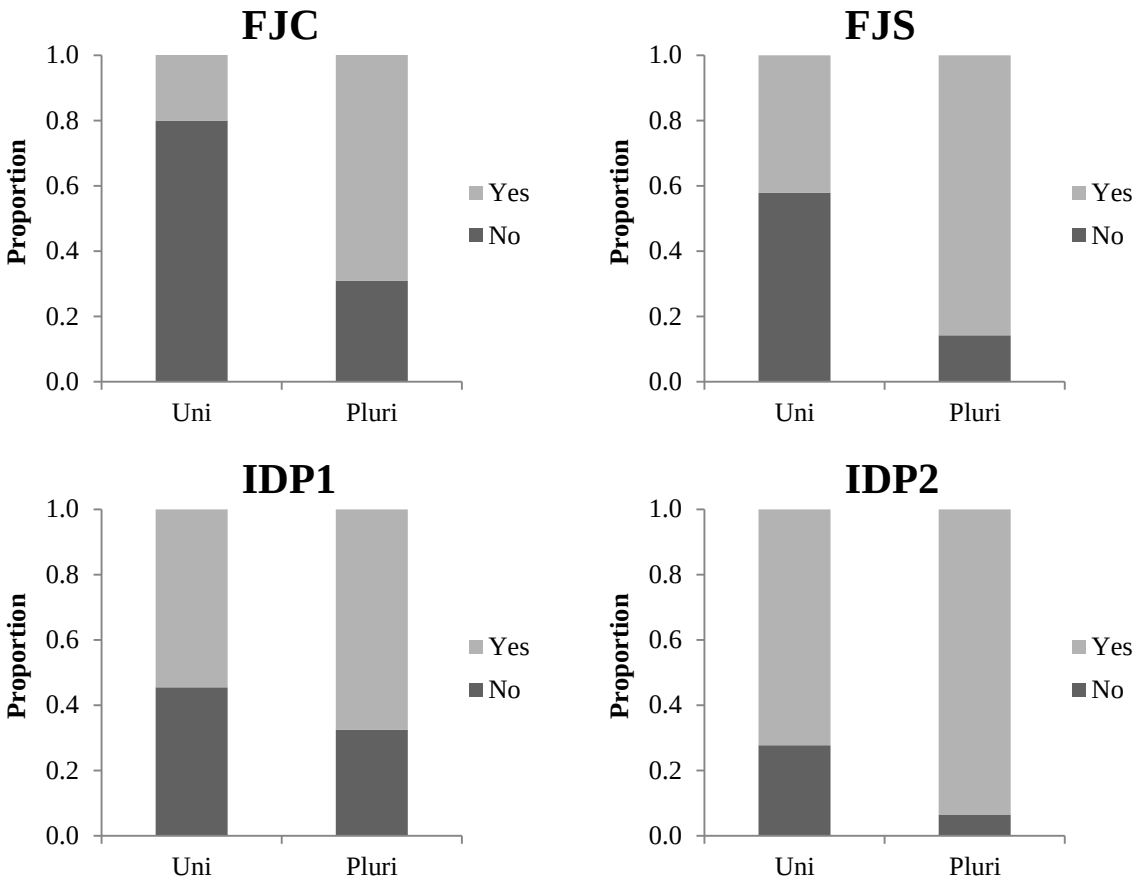

Figure 5.

CHAPTER 2

LIMITED DISPERSAL, FINE-SCALE GENETIC STRUCTURE, AND KIN GROUP FORMATION IN THE KELP *Lessonia spicata*

Abstract

Limited dispersal and non-random mating are predicted to lead to fine-scale genetic structure mainly due to kin structure (the spatial aggregation of relatives). This can have important ecological and evolutionary consequences, especially in sessile group living species, since groups can be formed by relatives even if individuals gather indiscriminately. Here we studied the fine-scale genetic structure of the kelp *Lessonia spicata*, an ecologically and economically important species of the Chilean low intertidal that has poor dispersal ability and forms chimeric holdfasts. Parentage assignment and spatial autocorrelation analyses allowed evaluating: i) small-scale dispersal kernels (<100m); ii) the presence of genetic structure within local populations; and iii) the relationship between local genetic structure and kin group formation in *L. spicata*. Dispersal kernels revealed that recruitment events mainly occurred at short distances from parental source (14-40 m) in both study sites. Dispersal kernel was more leptokurtic in Isla Damas, though. Accordingly, recruit kin structure was observed in Isla Damas, but not in Fray Jorge. We found concordance between the presence of kin structure in recruits and significant adult within-group relatedness, suggesting that fine-scale genetic structure leads to kin group formation within populations of *L. spicata*. Our study demonstrated that local recruitment and kin structure may drive SGS in *L. spicata*. In this context, the high occurrence of chimeras in natural populations seems to result from the fusion of highly inbred individuals, potentially providing benefits in terms of inclusive fitness.

Introduction

Dispersal is a central theme in ecology and evolution, since it influences all levels of biological organization (Broquet and Petit 2009). Locally, dispersal influences the spatial distribution of individuals and genes (Ouborg et al. 1999), the social interactions among individuals (Clobert et al 2001) and it affects local adaptation (Sanford and Kelly 2011).

In the marine realm, several studies have demonstrated the occurrence of genetic structure (i.e. the non-random distribution of genotypes) at various spatial scales, despite previous thought that marine populations were widely connected through a well-mixed propagule pool. Moreover, it has been shown that many species display spatial genetic structure (SGS) at small scales, even those with long distance dispersal potential (e.g. Iacchei et al 2013), contrary to the prediction of an inverse relationship between larval duration in the plankton and population genetic differentiation. Oceanographically and biologically driven patterns of collective dispersal of related individuals (e.g. Veliz et al 2006, Bernardi et al 2012), larval retention near parental source (e.g. Jones 2005, Planes et al 2009, Christie et al 2010) and high stochastic individual variance in reproductive success (Hedgecock 1994) are the main mechanisms responsible for reducing effective dispersal in relation to potential dispersal (Selkoe et al 2010, Broquet et al 2013). Direct methods of parentage and spatial autocorrelation analyses applied to recruit cohorts have been particularly useful for the detection of SGS in these organisms. These findings highlight that local-scale processes are more important in structuring marine populations than usually thought.

While less expected in species with long distance dispersal potential, fine-scale genetic structure is predicted to occur frequently in species with limited dispersal ability, especially those in which adults are sessile, like plants and macroalgae. The most common cause of such

fine-scale genetic structure is the formation of kin structure (i.e. close proximity of related individuals resulting in a negative relationship between pairwise relatedness and geographic distance) (Vekemans and Hardy 2004). The intensity and extent of local SGS will depend on other factors including mating system (Vekemans and Hardy 2004), population density and abiotic conditions (Byers and Pringle 2006).

Because ecological interactions occur primarily among neighbors in populations of sessile organisms, kin structure in turn will result in ecological interactions among relatives (Heywood 1991) and possibly in kin group formation, even if the absence of kin recognition systems (Hamilton 1964). This can have evolutionary consequences, since a high level of relatedness within groups represents opportunity for the action of kin selection on social traits. So, characterizing SGS at local scales may shed light into the mechanisms that underlie the evolution and maintenance of group living.

The interplay between limited dispersal, local genetic structure and sociality has been little explored in sessile species, probably due to the underestimation of sociality in these organisms. In sessile species, propagules (spores, pollen, seeds, larvae, etc.) constitute the dispersive phase. So, life-history characteristics related to propagule dispersal and settlement patterns will play a key role in shaping the social environment of individuals. A common form of group living in sessile marine organisms is chimerism (i.e. fusion of individuals originated from different reproductive events generating a genetic heterogeneous entity or group). Chimerism has been documented in several marine invertebrates (Buss 1982; Rinkevich 2005; Amar et al 2008; Puill-Stephan et al 2012) and macroalgae (Santelices et al 1999; Wernberg 2005; González and Santelices 2008; Segovia et al. 2014). The occurrence of kin groups have been shown to be frequent in some species that form chimeras as the ascidia *Botryllus*

schlosserii (Ben Shlomo et al 2008) and the coral *Acropora millepora* (Puil Stephan et al 2012), and their formation has been attributed to the aggregated settlement of kin larvae and to rejection responses when non-kin fuse. However, the influence of pure local kin structure (only produced by limited dispersal) on kin-group formation has not been evaluated in marine organisms that form chimeras yet, despite its potential importance. Marine macroalgae represent suitable models for the study of the interplay between limited dispersal, SGS and group living given: their limited dispersal ability (Santelice 1990), the presence of SGS at very fine-scales (Faugeron et al 2001, Engel et al 2004, Kusumo et al 2006, Krueger-Hadfield 2013), the occurrence of inbreeding (Raimondi et al 2004, Johansson et al 2013), and the possibility of group living (Santelices et al 1999, Segovia et al 2014). Hence, here we evaluated the fine-scale genetic structure within populations of the kelp *Lessonia spicata* and investigated its influence in kin group formation.

L. spicata - formerly called *L. nigrescens* (González et al 2012) - is one of the most ecologically and economically important species in the low intertidal of the Southeastern Pacific coast (Santelices et al 1980, Santelices & Ojeda 1984, Vasquez 2008). It ranges from central Chile ($29^{o}03´S$) to Chiloe Island (41^{o} 38´S) (Tellier et al 2009), where it usually forms dense stands in exposed rocky shores. It has recently been demonstrated that *L. spicata´*s holdfasts are frequently chimeric (i.e. composed of different genotypes due to interholdfast fusion) (González et al 2013). As all Laminariales, *L. spicata* has a heteromorphic life-cycle, in which the macroscopic form is the diploid sporophyte and the microscopic form is the haploid gametophyte (Hoffmann & Santelices 1997). During reproduction, vegetative tissues of blades differentiate in sporangia, which are grouped in sori. Flagelated spores produced by meiosis (meiospores) are then released in the water column. As they settle on the substrate,

spores germinate and develop into either male or female, morphologically different, gametophytes. Male gametophytes produce motile gametes that are attracted by pheromones to the egg (Müller 1981), which is sessile and remains attached to the female gametophyte. Fertilization occurs on the female gametophyte, and the zygote originates the sporophyte that develops in the same place. Sperm dispersal is negligible since male and female gametophytes must be very close to enable successful reproduction, because pheromone attraction occurs at a scale < 1mm (Boland et al 1983). Spore dispersal is thought to be of a few meters, because *Lessonia* spores usually settle 12 hours after being released (Ávila et al 1985) and only remain viable for a maximum of 24 hours (Parada 2001). On the other hand, other processes like fragment dispersal, could lead to greater dispersal distances. Fragment dispersal can occur during storms that dislodge the whole plant or some stipes that may bear sori. If these stipes are carried to a suitable settling site, spores might be released and fecundation might occur. Full adult individuals are sometimes observed been transported by currents along sandy shores, allowing movement along tens to hundreds of meters (S Faugeron personal observation) but this long distance dispersal is likely to be rare. So, dispersal in *Lessonia* mainly relies on meiospores.

Considering *Lessonia´*s life-history characteristics, dispersal should be mainly observed at short distances. Indeed, high genetic differentiation between populations and regions has been reported from indirect evidence of a phylogeographic study on *L. spicata* and *L. berteroana* (sister species, formerly grouped in *L. nigrescens*) showing that populations had few haplotypes shared among populations, and only between neighbor populations (Tellier et al 2009). At local scales, sandy beaches as small as 1.5km-long represent effective barrier to dispersal (Tellier et al 2011). Faugeron et al (2005) detected significant genetic differentiation

of *L. berteroana* at scales of 20 m and showed that an artificial interruption of the otherwise continuous distribution (due to copper mine pollution) for a few generations was sufficient to generate strong genetic differentiation, evidencing that dispersal is drastically limited by distances of a few tens of kilometers. Moreover, Martínez et al (2003) attributed the limited recolonization rate of *L. berteroana* after an ENSO event that caused massive mortality in Northern Chile to its restricted dispersal capacity. Within populations, a kin structure within chimeric holdfasts was observed for *L. berteroana* (Segovia et al 2014), evidencing a substructure of the genetic diversity at very short spatial scales. Although a trend of high within-holdfast relatedness was observed in *L. spicata,* the pattern (e.g. higher relatedness within than among holdfasts) was not always statistically significant (Chapter 1). This lack of statistical discrimination between within- and among-holdfast relatedness could be related to the presence of kin structure within the population as a whole.

If, as previous studies suggest, spore dispersal is limited, recruitment will occur close to parental source and related individuals will be found in close proximity leading to kin structure at fine scales. As a consequence, related individuals may fuse, promoting kin group formation. In this case, we expect that both within-population kin structure and high relatedness within groups are likely to occur. In this context, our aim here was to investigate the relationship between limited dispersal, fine-scale genetic structure and group living in *L. spicata,* using direct methods of parentage assignment and spatial autocorrelation analyses. Specifically, we predicted that if dispersal is limited to few meters from the parental source, as Faugeron et al (2005) suggested: i) the dispersal kernel would be leptokurtic even at scales of <100 m, as a result of the greater frequency of recruits near the location of their parents; ii) pairwise relatedness between recruits would be inversely related to the distance between them

(because of kin structure); and iii) high intra-group relatedness would be observed where within-population kin structure occurred.

Materials and Methods

Sampling

A spatially explicit scheme was used to sample adults and recruits. Two sites in Chile, separated by ~200 km, were chosen for sampling (Isla Damas - 29° 13´S/71° 31´ W and Fray Jorge - 30° 44´S/71° 42´W). Both sites are areas of high wave exposure, strong upwelling and no kelp harvesting. Adults of *L. spicata* were sampled during 2009 winter, when maximum fertility is observed. In each study site, two grids separated by 60 m were placed using 50 cm x 50 cm quadrats (for a patch area of 12 to 56 m^2). The size of the grid was determined so that at least 50 *L. spicata* holdfasts were sampled in each one, using an exhaustive sampling (i.e. including the highest kelp on the shore, to the lowest possible individuals reachable at low tide; considering thus most of the width of the intertidal distribution of the kelp population). To evaluate diversity within holdfasts, five frond samples were taken from different mature stipes from each holdfast. Vegetative tissue samples were collected from the basal meristematic area of fronds and samples were then dried in silica gel for posterior molecular analysis. The position of all adult samples was mapped with x y coordinates. Recruits (plants with holdfast diameter < 5 cm) were sampled at the same sites at spring. At first, grids were positioned at the same place as for the adults using 50 cm x 50 cm quadrats and a maximum of two recruits per quadrat was collected. Then, two transects (max 30 m long) were extended parallel to the coastline, one in each side of the grid, using the same 0.25 m^2 quadrats. Again, a maximum of two recruits were collected per quadrat. Recruit sampling reflects their natural distribution in the area. Collected fronds were then dried in silica gel for posterior molecular analysis. The position of all recruit samples was mapped. With this sampling scheme, we

ended with two grids of adults and two larger grids (grids +transects) of recruits from each site (see Fig. 1). A total of 244 and 215 adults were sampled from each grid of Isla Damas (in IDP1 and IDP2) and 219 and 223 from each grid of Fray Jorge (in FJC and FJS). 155 and 213 recruits were sampled from Isla Damas and 100 and 91 from Fray Jorge.

Genetic Analysis

At the laboratory, after tissue grounding, DNA was extracted with a modified CTAB protocol with the addition of Polyvinyl Pyrolidone (PVP), following Tellier et al (2009). The pellet was eluted in 50 µL of MiliQ water, quantified by NanoDrop (NanoDrop Technologies Wilmington, Delaware, USA) and diluted to 15 ng μL^{-1}. Seven microsatellite loci (Faugeron et al 2009) were used for Fray Jorge, and only four out of ten tested were polymorphic or amplified well for Isla Damas. PCRs were performed according to Faugeron et al (2009). Uncertainties were resolved by re-amplifications. PCR products were analyzed on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). Genotypes were first scored manually using Genemarker (Softgenetics LLC) and then alleles were binned with FexiBin v2 (Amos 2007).

The numbers of alleles, observed and expected heterozygosity were calculated for adults and recruits from each grid with Arlequin (Excoffier et al 2005). Null alleles were checked with Microchecker (van Oosterhout et al 2004). Heterozygote deficit (F_{is}) within grids was tested by performing 10000 randomizations in Fstat (Goudet, 2001), and significance levels were obtained after sequential Bonferroni correction for multiple comparisons. To infer genetic differentiation within sites based on allele frequency

differences, we first calculated pairwise F_{st} between adults and between recruits from different grids within sites. Then, pairwise F_{st} were also calculated to evaluate the differentiation between adults and recruits from the same site. Calculations and statistical tests were done with ARLEQUIN (Excoffier et al 2005) after 10000 permutations.

Parentage analysis

To determine parent-offspring pairs, parentage assignment was done for each site with Cervus 3.0 (Marshall et al 1998; Kalinowski et al 2007). Cervus uses a likelihood-based approach to determine the most likely parents of individuals of interest. It calculates the likelihood of paternity of any individual relative to the likelihood of paternity of alternative individuals. LOD scores are tested statistically by simulations. We carried out parent-pair analyses with unknown sex and self-fertilization allowed. Adults with the highest positive LOD scores and no mismatches were assigned as putative parents. We first assigned parents to recruits sampled in the same grid (i.e. FJC, FJS, IDP1 and IDP2). Then we pooled the adults within sites and analyzed FJ adults as potential parents for FJC and FJS recruits and ID adults as potential parents for recruits from IDP1 and IDP2 (noted hereafter FJC (FJ), FJS (FJ), IDP1 (ID) and IDP2 (ID), respectively). After removing repeated genotypes within adult holdfasts and genotypes with missing data, we ended with a total of 93, 98, 146 and 118 adults, and 98, 89, 104 and 179 recruits that were included in the analyses of FJC, FJS, IDP1 and IDP2, respectively.

Parent-offspring distance distribution and dispersal kernel

To estimate the most frequent dispersal distances from parental source, we first generated frequency distributions of observed parent-offspring Euclidean distances, after parentage analysis. Distributions were statistically compared with Kolmogorof-Smirnoff. Then, we adjusted dispersal kernels for each distribution. A dispersal kernel is a probability density function that depicts the relative frequency of offspring at different distances from parental individuals, hence its shape may reveal where recruitment events are more frequent. We fitted three types of probability density functions (Exponential, Weibull and Lognormal) to the observed data. The three functions have a common property of presenting a fat tail at the end of the curve and they differ primarily in the near tail (close to parental source). These functions have been shown to fit well to empirical data (Greene et al 2004, Nathan et al 2012).

Model parameters were estimated by maximum likelihood, and AIC (Akaike´s Information Criterion) was used to select the best fitting model for each distribution. The goodness of fit of selected models was determined by correlating observed to predicted relative recruit frequencies at given distances from the parental source through Pearson´s product moment correlation coefficient. Average dispersal distances and percentiles were estimated from the best fit model. All analyses were performed with the package fitdistrplus (MASS) in R (R Development Core Team 2012).

Pairwise relatedness and geographic distance

To test the prediction that short distance dispersal leads to kin structure, we regressed pairwise relatedness coefficients (Queller and Goodnight 1989) for recruits on geographical distance for the two study sites. Average pairwise relatedness values were calculated for each of 20 distance classes with averages that ranged from 1.7 to 95.3 m in Fray Jorge and from 1.2 to 81.3 m in Isla Damas. To analyze if the regression slope was significantly different from 0, 10000 permutations of locations on individuals were carried out to create a null distribution. P- values were then determined as the fraction of this distribution greater than the observed slope. All analyses were done with SPAGeDi 1.3 (Hardy and Vekemans 2002). Then, to inspect with more detail how pairwise relatedness varied at the smallest scale (within quadrats), we plotted the frequency distribution of pairwise relatedness among recruits sampled at the same quadrats for Fray Jorge and Isla Damas.

Adult relatedness at different scales

To analyze kin structure at different local scales in adults, we analyzed adult relatedness within holdfasts and within grids for Fray Jorge and Isla Damas. We calculated average intra-holdfast pairwise relatedness in adults and average pairwise relatedness among all adults in the grid, using all the adults from the site as the reference population. Then, to test if average pairwise related values were significantly different from 0, resampling procedures were carried out, since parametric tests could give false significant results due to the elevated number of pairwise estimates. Briefly, we generated distributions of average intra-holdfast and intra-grid relatedness values by randomly resampling a subset (N = 30) of observed

relatedness values for each grid for 1000 times. The cases in which the 95% confidence interval of the randomly created relatedness distributions did not include 0 were considered significant. Resampling was done in R 2.14 software (R Development Core Team 2012).

Then we pooled the data from IDP1 and IDP2 and FJC and FJS and calculated average pairwise relatedness within holdfasts, within quadrats, at distances lower than 2m, greater than 2m (but within grids), and between grids (distances > 40m). We also analyzed whether average relatedness values were significantly different from 0, as above.

Results

Genetic diversity

Microsatellite amplification success and polymorphism changed greatly from Fray Jorge to Isla Damas. Few alleles were shared by individuals from the two sites. In general, genetic diversity was similar between adults and recruits within sites and global expected heterozigosity was around 0.65 in both sites (Table 1). Specifically, within Fray Jorge grids, the number of alleles, observed and expected heterozigozity and F_{is} values were similar between adults and recruits (Table 1). Both adults and recruits from FJC and FJS showed a large and significant heterozygote deficiency for loci LESS2D22 ($F_{is} > 0.8$) and LESS1T3 ($F_{is} > 0.3$). This could have been caused by null alleles. We then reanalyzed the data without these loci and observed that average heterozigosity presented the same tendency as before, so the loci were kept. Both adults and recruits from Fray Jorge grids presented significant average heterozygote deficiency. Adults and recruits presented a similar number of alleles for all loci, except for one case in which the adults presented a higher number of alleles (11 alleles in adults versus 7 in recruits in locus LESS1T4 in FJC) and two cases in which the number of alleles was higher in recruits (7 alleles in adults versus 10 in recruits in locus LESS1T4 in IDP1 and 7 alleles in adults versus 11 in recruits in locus LESS1T17 in IDP2). Heterozygosity among loci was variable for both Isla Damas´ grids. While both adults and recruits from IDP1 presented significant average heterozygote deficit, in IDP2 neither adults nor recruits did (Table 1).

Genetic differentiation

Genetic differentiation between adults from different grids was low but statistically significant, and approximately seven times greater in ID than in FJ (F_{st} = 0.0757, p <0.0001 versus F_{st} = 0.0098, p = 0.0039, respectively). Regarding the recruits, while no evidence of genetic differentiation was found in FJ (F_{st} = 0.0002, p = 0.0611), genetic differentiation was high between ID´s recruits (F_{st} = 0.13, p <0.0001). The comparison of allele frequencies between adults and recruits within sites can shed light into possible parent-offspring relations and the origin of recruit cohorts. When we compared adults to recruits, we found that recruits from FJC and FJS were not significantly genetically differentiated between FJC and FJS adults. In ID a different picture was observed. While no significant genetic differentiation was found between IDP2 recruits and adults (F_{st} = 0.0001, p = 0.4549), genetic differentiation between IDP1 adults and both IDP1 and IDP2 recruits was significant (F_{st} = 0.0333, p <0.0001 and F_{st} = 0.0731, p <0.0001, respectively). Also, IDP2 adults were genetically differentiated from IDP1 recruits (F_{st} = 0.1324, p < 0.0001).

Parentage assignment

Parentage analysis revealed that, 63 out of 93 (68 %) adults from FJC were excluded as potential parents for recruits sampled in the same grid, while 55 out of 98 (56 %) adults were excluded in FJS. Out of the 146 adults sampled in IDP1, 96 (66 %) were excluded as putative parents for offspring sampled in IDP1 and 56 out of 118 (48 %) adults from IDP2 were not assigned to recruits in the grid. Conversely, parentage analysis revealed that in FJC, 30 (32 %) adults contributed to offspring production in the grid, while in FJS, 43 (44 %) adults were

successfully assigned to offspring sampled in the grid. In Isla Damas, the number of adults that had at least one offspring in the grid was 50 (34 %) in IDP1 and 62 (52 %) in IDP2. When we pooled adults and recruits from different grids and repeated the analyses, the number of sampled adults excluded as putative parents slightly decreased to 54 (58%) in FJC, 51 (52%) in FJS and 94 (64%) in IDP1, while in IDP2 it increased to 63 (53%).

For 50 % of the recruits sampled in FJC, at least one parent was found in the grid (Table 2). In FJS, 56.2 % of the recruits had at least one parent assigned (Table 2). When we pooled FJC and FJS adults, the frequencies of recruits with at least one collected parent changed to 63.3 % in FJC and 67.4 % in FJS. In Isla Damas, 48.7 % and 79.3 % of the recruits sampled in IDP1 and IDP2, respectively, were successfully assigned to a parent in the first analyses (Table 2). When we pooled ID adults, 56 % of the recruits sampled in IDP1 and 81.6 % of the ones sampled in IDP2 had at least one parent assigned to.

Parent-offspring distance distributions

Frequency distributions of observed parent-offspring distance of *L. spicata* for the four grids are shown in Fig. 2. Overall, the distributions were right-skewed, since the majority of *L. spicata* recruits were found at shorter distances from their parents, despite differences between sites and between grids within sites (Fig. 2). Distributions from FJ and ID were significantly different in most cases, except in the comparison between FJC and IDP1 (Kolmogorov-Smirnoff: $D = 0.190$, $p = 0.174$). FJS was the grid where dispersal at relative longer distances was more frequent (i.e. the distribution presented a fatter tail). Indeed, while at least 70 % of the recruits from FJC, IDP1 and IDP2 were found up to 15 m from their parents (Fig 2a, e ,f),

in FJS this fraction was 46 % (Fig. 2b). When we plotted the frequency distribution of parent-offspring distances for FJC and FJS considering all FJ adults as potential parents, we observed some events of longer distance dispersal, as expected (Fig. 2c and 2d). This reflects that there were recruits sampled in FJC whose parent(s) belonged to FJS and vice-versa. Specifically, approximately 36 % of the recruits sampled in FJC (Fig. 2c) and 24 % of those sampled in FJS had their parent located in the other grid (Fig. 2d).). IDP1 and IDP2 frequency distributions of parent-offspring distances to all ID adults showed that 13 % of the recruits sampled in IDP1 had a parent from IDP2 (Fig 2g), while 24% of the ones sampled in IDP2 had a parent sampled in IDP1 (Fig 2h).

Model fitting - dispersal kernel

The curves of fitted models – the dispersal kernels – are depicted in Fig. 2 (a-h). According to AIC, both the Negative Exponential and the Weibull functions performed equally well in almost all cases (Table 3). Indeed, the Weibull reduces to an Exponential when parameter shape = 1.0. Pearson´s correlation coefficient between observed and predicted data ranged between 0.36 and 0.81 (Table 3). Estimated average recruitment distances were approximately 10m in both IDP1 and IDP2, and 15 and 18m in IDP1 (ID) and IDP2 (ID), respectively. In FJ, estimated average recruitment distances were 11m in FJC and 15 m in FJS, considering only the parents within the grid as potential parents, and 30 and 25 m, respectively, considering all FJ adults as putative parents. Dispersal kernels predicted that 75 % of the recruits were found within 15.62 and 20.35 m in FJC and FJS and within 40.91 and 35.92 m from parental source in FJC (FJ) and FJS (FJ), respectively. In Isla Damas, dispersal

kernels predicted that 75 % of the recruits were found within 13.34 and 14.19 m in IDP1 and IDP2 and within 21.31 and 25.52 m from parental source in IDP1 (ID) and IDP2 (ID), respectively.

Relatedness

The spatial autocorrelation analysis revealed a low and statistically non-significant relationship between recruit pairwise relatedness and geographic distance in Fray Jorge ($b = 0.0008$, $p = 0.746$) (Fig. 3). In Isla Damas, though, we observed a slightly negative and statistically significant ($b = -0.07$, $p < 0.001$) relationship (Fig. 3). Interestingly, pairwise relatedness between recruits sampled in the same quadrat was highly variable. It ranged from -0.45 to 1 in Fray Jorge and from -0.5 to 1 in Isla Damas, although clearly in Isla Damas a greater frequency of recruits found in the same quadrat presented positive relatedness coefficients.

Relatedness structure in adults was also notably different in Fray Jorge and Isla Damas. In FJC and FJS average intra-holdfast ($R = 0.06 \pm 0.034$ and $R = 0.06 \pm 0.046$, respectively) and intra-grid relatedness ($R = 0.02 \pm 0.004$ and $R = -0.01 \pm 0.004$, respectively) were not significantly different from 0. On the other hand, in IDP1 average intra-holdfast relatedness was high ($R = 0.28 \pm 0.029$) and significantly different from 0. Average intra-grid relatedness ($R = 0.01 \pm 0.003$) was not statistically significant though. In IDP2, both average intra-holdfast relatedness ($R = 0.20 \pm 0.034$) and intra-grid relatedness ($R = 0.12 \pm 0.004$) were significantly different from 0. When we pooled FJC with FJS data and IDP1 with IDP2, we observed that in FJ average relatedness values were concentrated around 0 at all distances,

despite a tendency for decreasing values with distance (Fig. 4). Instead, in ID, average intra-holdfast ($R = 0.22 \pm 0.02$) relatedness was clearly higher than average relatedness within quadrats, within grids, and between grids, and was the only value statistically different from 0 (Fig. 4).

Discussion

Here we investigated the interplay between dispersal, fine-scale genetic structure, and kin group formation in the kelp *L. spicata* using direct methods of parentage and spatial autocorrelation analyses. Despite notable differences between sites, our results pointed that self-recruitment and kin structure can produce fine-scale genetic structure for this kelp. Kin structure, in turn, seems to lead to the formation of kin groups. We discuss below our results.

Dispersal restricted to few meters

According to our prediction, the majority of recruits (75%) were found close to parental source (within 13-41 m) in both sites Fray Jorge and Isla Damas. The leptokurtic dispersal kernels found at the fine scale evaluated here (~100m) demonstrate that self-recruitment occurs frequently within *L. spicata* populations. Dispersal kernels from Isla Damas were clearly more leptokurtic than the ones from Fray Jorge, showing that the scale of recruitment events may vary between sites. It is important to highlight that despite methodological issues (few loci, null alleles) we found concordance between the shape of the dispersal kernels and F_{st} estimates, indicating that our results are robust. Also, our results are consistent with previous studies that reported a high level of genetic structure of *L. berteroana* and *L. spicata* (Faugeron et al 2005, Tellier et al 2009), formerly grouped as *L. nigrescens*. Our findings do not imply though that recruitment events of *L. spicata* only occur at short distances. Recruitment at longer distances (km) can also eventually take place. For example, Martínez et al (2003) estimated that *L. berteroana* recolonized empty areas after an ENSO

event at rates of approximately 3 km per year. If our sampling scale was larger we would have probably found fatter-tailed more leptokurtic dispersal kernels.

Dispersal kernels were built on effective dispersal estimation (e.g. parent-offspring distance), not on propagule (i.e spores) dispersal estimation. Successful recruitment depends not only on successful dispersal, since propagules or individuals may not be able to establish themselves or survive in the new location after arrival (Gaylord et al 2006, Reed et al 2006). In kelps, the direct relationship between successful dispersal and recruitment is even less straightforward, because fertilization occurs after spore dispersal, settlement, survival and sexual maturation of the gametophytic stage (Reed et al 2006). In this sense, our findings of predominant local recruitment in *L. spicata* can be explained by a high incidence of local spore retention (< 40 m) and maybe an increased probability of recruiting beneath the parental source. The prevalence of short distance spore dispersal (10s to 100m) has also been shown in spore dispersal kernels developed for the giant kelp *Macrocystis pyrifera* (Gaylord et al 2006). Spore dispersal has been reported to be even more limited in the intertidal annual kelp *Postelsia palmaeformis* (1-3 m) (Kusumo et al 2006). The pattern we found for *L. spicata* is likely the result of a combination of biological and environmental processes. There are several life-history characteristics that restrict *L. spicata´*s ability of dispersal: i) short duration of spore viability (24 h); ii) intertidal habitat (dependency on intertidal cycles for spore release) and iii) absence of floating structures. Moreover, successful fertilization requires male and female gametophytes to be very close (<1mm), so the probability of successful recruitment decreases with distance from propagule source due to spore dilution (Reed et al 1997). Abiotic factors including habitat continuity (hindered by the presence of sandy beaches separating rocky shores) (Alberto et al 2010) and both regional and local oceanographic features may

also affect spore dispersal and recruitment success of *L. spicata* at local scales. Indeed, the differences in dispersal kernels found for Isla Damas compared to Fray Jorge are probably related to particular microscale oceanographic processes (i.e. local coastal currents), considering that both sites are upwelling centers. However, both sites were consistent in showing dispersal mostly restricted to few tens of meters.

Kin structure and group living

Limited dispersal is supposed to result in the spatial aggregation of related individuals which lead to a negative relationship between relatedness and geographic distance (kin structure) (Vekemans and Hardy 2004). Consistent with dispersal kernels, the spatial autocorrelation analysis also revealed a marked difference between Isla Damas and Fray Jorge. Pairwise relatedness among recruits decreased significantly with distance in Isla Damas, while no relationship was observed for Fray Jorge. This indicates the presence of SGS due to kin structure in Isla Damas but not in Fray Jorge, at least at the scale of our study. Moreover, in Isla Damas average pairwise relatedness between recruits sampled at the same quadrats (distance = 0) was significantly higher than expected by chance. Also, the distribution of pairwise recruit relatedness within quadrats was clearly right-skewed in ID, despite the great variability observed. The variance in the magnitude of F_{st} values agreed with the patterns observed in kin structure analysis, also suggesting that gene flow is more restricted in ID than in FJ. We cannot rule out the possibility of SGS in Fray Jorge, though. It is possible that if our sampling scale had been greater we would have found significant SGS in Fray Jorge as well.

Apart from limited dispersal, the fine-scale genetic structure within populations of *L. spicata* is probably influenced by mating system. While outbreeding is promoted in self-incompatible species, the possibility of selfing can increase inbreeding rates even further than limited dispersal only. Thus, self-compatible species are expected to exhibit SGS at smaller scales than outbred species (Vekemans and Hardy 2004). Self-reproduction can occur in kelps if fertilization takes place between gametes produced by gamethophytes originated from spores produced by the same sporophyte (e.g. Raimondi et al 2004, Barner et al 2011). Here we found significant F_{is} estimates for adults and recruits from almost all grids analyzed, suggesting inbreeding.

Although the interplay between SGS and sociality has been extensively studied in terrestrial organisms, less attention has been given to the relationship between SGS and social interactions in the sea (Kamel and Grosberg 2013). Here we investigated the relationship between limited dispersal, kin structure and fusion among kin. We found concordance between the presence of kin structure in recruits and significant adult within-group relatedness, suggesting that fine-scale genetic structure resultant from limited dispersal can lead to kin group formation within populations of *L. spicata*. In ID, where kin structure was found for the recruit cohort analyzed, adult average relatedness within holdfasts was statistically greater from 0. The pattern was less clear in FJ adult relatedness, consistent with the absence of kin structure obtained for the recruits. Our results agree with theoretical predictions that state that population viscosity (limited dispersal) can lead to kin group formation in social species, even if grouping occurs indiscriminately, due to the close proximity of relatives (Hamilton 1964; Queller 1994). Other studies on chimeras have shown that fusion with kin could result from aggregated kin propagule settlement and allorecognition

systems (e.g. Hart and Grosberg 1999; Ben-Shlomo et al 2008; Puill-Stephan et al 2012). Here we demonstrated that it can be the result of limited dispersal as well.

The evidence of kin group formation found here suggests kin selection might have played a role on the evolution and maintenance of chimerism in *L. spicata*. It could be argued that limited dispersal coupled to mating system creates proper ecological conditions for the evolution of kin-selected social behavior in *L. spicata*. Therefore, there could be a feedback structure between the evolution of dispersal and kin group formation, mediated by the fitness effects of living in kin groups and of inbreeding (Gandon 1999) , and the relative strength of other selective pressures that act on dispersal. Future studies on the fitness effects of kinship (Griffin and West 2002) and of inbreeding are necessary to evaluate this possibility. Yet, since no fitness costs related to chimerism have been reported for *L. spicata* yet (see chapter 1), we cannot rule out the possibility of direct fitness benefits of group living.

In conclusion, we found leptokurtic dispersal kernels, revealing that recruitment events mainly occurred at short distances from parental source (14-40 m) and pointing to the prevalence of short distance spore dispersal (10s to 100m) in *L. spicata*, in accordance with F_{st} estimates and previous studies. Moreover, we observed significant kin structure within one of the studied sites, which corresponded to kin group formation at the same site, illustrating the role of limited dispersal on kin group formation in this kelp. Consequently, this study suggests kin selection may play a role on maintaining chimerism in natural populations of *L. spicata*, via inclusive fitness benefits of fusing with kin, in addition to possible direct fitness benefits. Further studies are needed to quantify the fitness consequences of living in kin- versus non-kin groups.

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Figure legends

Figure 1. Map of sampled adults and recruits of *L. spicata* at (a) Fray Jorge and (b) Isla Damas. FJC = Fray Jorge Centre; FJS = Fray Jorge South; IDP1 = Isla Damas Patch 1 and IDP2 = Isla Damas Patch 2.

Figure 2. Frequency distribution of observed parent-offspring distance of *L. spicata* with fitted dispersal kernels assuming Weibull (—), Exponential (– –) and Lognormal (·····) functions. a) FJC recruits with FJC adults as potential parents; b) FJS recruits with FJS adults as potential parents; c) FJC recruits with FJ (FJC + FJS) adults as potential parents; d) FJS recruits with FJ (FJC + FJS) adults as potential parents; e) IDP1 recruits with IDP1 adults as potential parents; f) IDP2 recruits with IDP2 adults as potential parents; g) IDP1 recruits with ID (IDP1+ IDP2) adults as potential parents and h) IDP1 recruits with ID (IDP1+ IDP2) adults as potential parents.

Figure 3. The relationship between pairwise relatedness and geographic distance of *L. spicata* for the two studied sites. The solid line depicts average pairwise relatedness coefficients in different distance classes. The dashed line represents the 95 % confidence interval built after 10000 permutations of spacial locations on individuals. X axis is on logarithmic scale.

Figure 4. Adult average pairwise relatedness values as a function of spatial distances. Data are average ± SE (standard error). SE is invisible in some cases due to the great number of observations. Distances greater than 40m correspond to between-grid values.

Table 1. Genetic characteristics of of *L. spicata´*s adults and recruits from four grids in Chile. N = total number of genotyped individuals ; Na = number of alleles; Ho = observed heterozigosity; He = expected heterozigosity; Fis = estimate of heterozygote deficiency (significant values after Bonferroni correction are in bold).

		Fray Jorge				Isla Damas			
		FJC		FJS		IDP1		IDP2	
Locus	Parameter	Adults	Recruits	Adults	Recruits	Adults	Recruits	Adults	Recruits
LESS 2D22	N	93	98	98	89	146	104	118	179
	N_a	3	3	4	4	6	5	8	9
	H_o	0.087	0.051	0.061	0.101	0.524	0.663	0.603	0.670
	H_e	0.456	0.505	0.531	0.511	0.675	0.672	0.713	0.717
	F_{is}	**0.810**	**0.899**	**0.885**	**0.803**	**0.223**	0.013	0.154	0.065
						-	-	-	-
LESS 2D25	N	93	98	98	89				
	N_a	18	17	15	16	-	-	-	-
	H_o	0.785	0.765	0.765	0.843	-	-	-	-
	H_e	0.869	0.835	0.840	0.861	-	-	-	-
	F_{is}	0.098	0.084	0.089	0.021	-	-	-	-
						-	-	-	-
LESS 2D1	N	93	98	98	89				
	N_a	8	8	7	7	-	-	-	-
	H_o	0.559	0.602	0.612	0.663	-	-	-	-
	H_e	0.563	0.597	0.609	0.655	-	-	-	-
	F_{is}	0.007	-0.008	0.005	-0.013	-	-	-	-
						-	-	-	-
LESS 1T3	N	93	98	98	89				
	N_a	18	17	16	19	-	-	-	-
	H_o	0.570	0.622	0.612	0.562	-	-	-	-
	H_e	0.890	0.892	0.899	0.903	-	-	-	-
	F_{is}	**0.361**	**0.303**	**0.320**	**0.379**	-	-	-	-
LESS 2D26	N	93	98	98	89	146	104	118	179
	N_a	4	3	4	4	7	6	6	5
	H_o	0.419	0.479	0.536	0.528	0.651	0.635	0.653	0.603
	H_e	0.442	0.449	0.500	0.464	0.685	0.699	0.682	0.686
	F_{is}	0.052	-0.070	0.073	-0.138	0.050	0.093	0.043	0.121

Table 1. Continued

LESS 1T9	N	93	98	98	89	-	-	-	-
	N_a	12	12	12	10	-	-	-	-
	H_o	0.761	0.622	0.663	0.708	-	-	-	-
	H_e	0.840	0.851	0.833	0.813	-	-	-	-
	F_{is}	0.095	**0.270**	**0.204**	0.130	-	-	-	-
LESS 1T17	N	-	-	-	-	146	104	118	179
	N_a	-	-	-	-	10	10	7	13
	H_o	-	-	-	-	0.363	0.288	0.373	0.369
	H_e	-	-	-	-	0.660	0.596	0.418	0.458
	F_{is}	-	-	-	-	**0.450**	**0.517**	0.108	**0.196**
Global	N	93	98	98	89	146	104	118	179
	N_a	10.6	9.6	9.286	9.571	7.500	7.75	6.5	7.75
	H_o	0.499	0.504	0.512	0.523	0.569	0.548	0.570	0.599
	H_e	0.630	0.646	0.656	0.638	0.682	0.703	0.627	0.630
	F_{is}	**0.208**	**0.213**	**0.218**	**0.169**	**0.166**	**0.221**	0.090	0.050

Table 2. Parentage analysis results for *L. spicata* recruits are shown for Fray Jorge Centre (FJC), Fray Jorge South (FJS), Isla Damas Patch 1 (IDP1) and Isla Damas Patch 2 (IDP2). Analysis was first carried out considering only the adults in the grid as possible parents (parents in the grid) and then considering all the adults in the site (parents in the site). UC/UC = both parents uncollected, UC/C = one parent uncollected and C/C = both parents collected.

		parents in the grid			parents in the site		
Grid	N	UC/UC (%)	UC/C (%)	C/C (%)	UC/UC (%)	UC/C (%)	C/C (%)
FJC	98	50.0	45.9	4.1	36.7	55.1	8.2
FJS	89	43.8	44.9	11.2	32.6	52.8	14.6
IDP1	104	41.3	37.5	21.2	20.2	35.6	44.2
IDP2	179	20.7	38.0	41.3	18.4	26.8	54.7

Table 3. Model fit for the probability distributions of dispersal distances for *L. spicata* recruits. Parameters for four functions (Exponential, Weibull and Lognormal) were estimated by maximum likelihood. AIC = Akaike Information Criterion. The models with the best goodness of fit are in bold. r = Pearson´s correlation coefficient between observed and predicted values.

Population	Function	Model Parameters		AIC	r
FJC	Exponential	rate = 0.089		384.79	
	Weibull	shape = 1.337	scale = 12.234	380.25	0.71
	Lognormal	meanlog = 2.071	sdlog = 0.914	384.76	
FJC (FJ)[a]	**Exponential**	rate = 0.034		642.19	0.49
	Weibull	shape = 0.98	scale = 29.268	644.14	0.54
	Lognormal	meanlog = 2.79	sdlog = 1.20	646.10	
FJS	**Exponential**	rate = 0.068		451.75	0.31
	Weibull	shape = 1.224	scale = 15.611	450.49	0.36
	Lognormal	meanlog = 2.240	sdlog = 1.110	463.02	
FJS (FJ)[a]	**Exponential**	rate = 0.04		682.73	0.46
	Weibull	shape = 1.047	scale = 26.373	684.47	
	Lognormal	meanlog = 2.703	sdlog = 1.241	697.98	
IDP1	**Exponential**	rate = 0.104		550.38	0.61
	Weibull	shape = 1.12	scale = 10.018	550.74	0.72
	Lognormal	meanlog = 1.77	sdlog = 1.243	576.24	
IDP1 (ID)[a]	**Exponential**	rate = 0.0651		576.80	0.75
	Weibull	shape = 0.884	scale = 14.437	576.77	0.80
	Lognormal	meanlog = 1.445	sdlog = 1.445	590.50	
IDP2	**Exponential**	rate = 0.099		1527.11	0.61
	Weibull	shape = 1.067	scale = 10.366	1527.74	0.70
	Lognormal	meanlog = 1.756	sdlog = 1.35	1602.59	
IDP2 (ID)[a]	**Exponential**	rate = 0.054		1882.83	0.68
	Weibull	shape = 0.934	scale = 17.954	1882.62	0.75
	Lognormal	meanlog = 2.254	sdlog = 1.379	1921.37	

[a] Distributions generated considering FJ (FJC+FJS) and ID (IDP1 + IDP2) adults as potential parents.

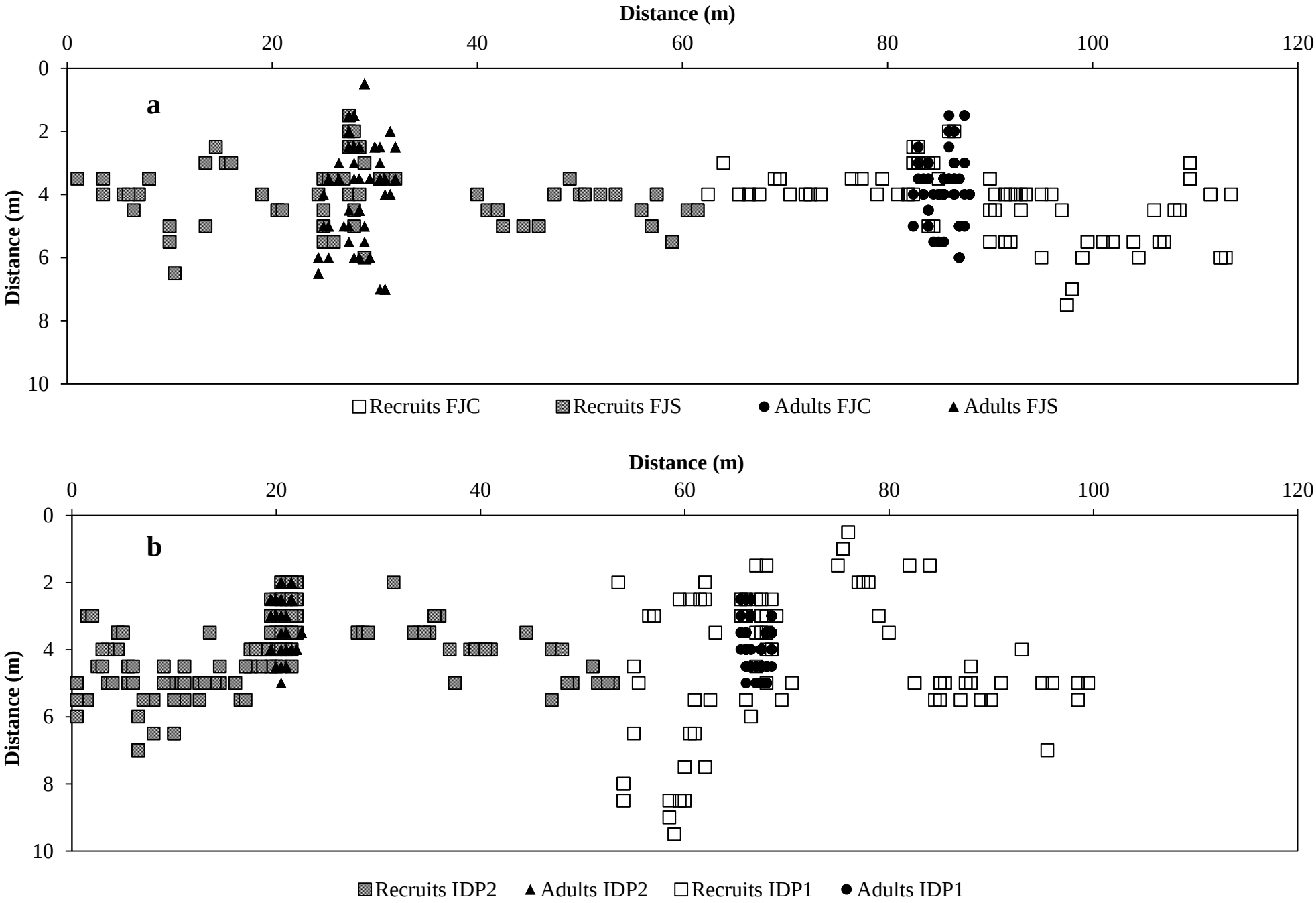
Distance (m)
0
20
40
60
80
100
120
Distance (m)
0
2
4
6
8
10
a
Recruits FJC
Recruits FJS
Adults FJC
Adults FJS
Distance (m)
0
20
40
60
80
100
120
Distance (m)
0
2
4
6
8
10
b
Recruits IDP2
Adults IDP2
Recruits IDP1
Adults IDP1

Figure 1.

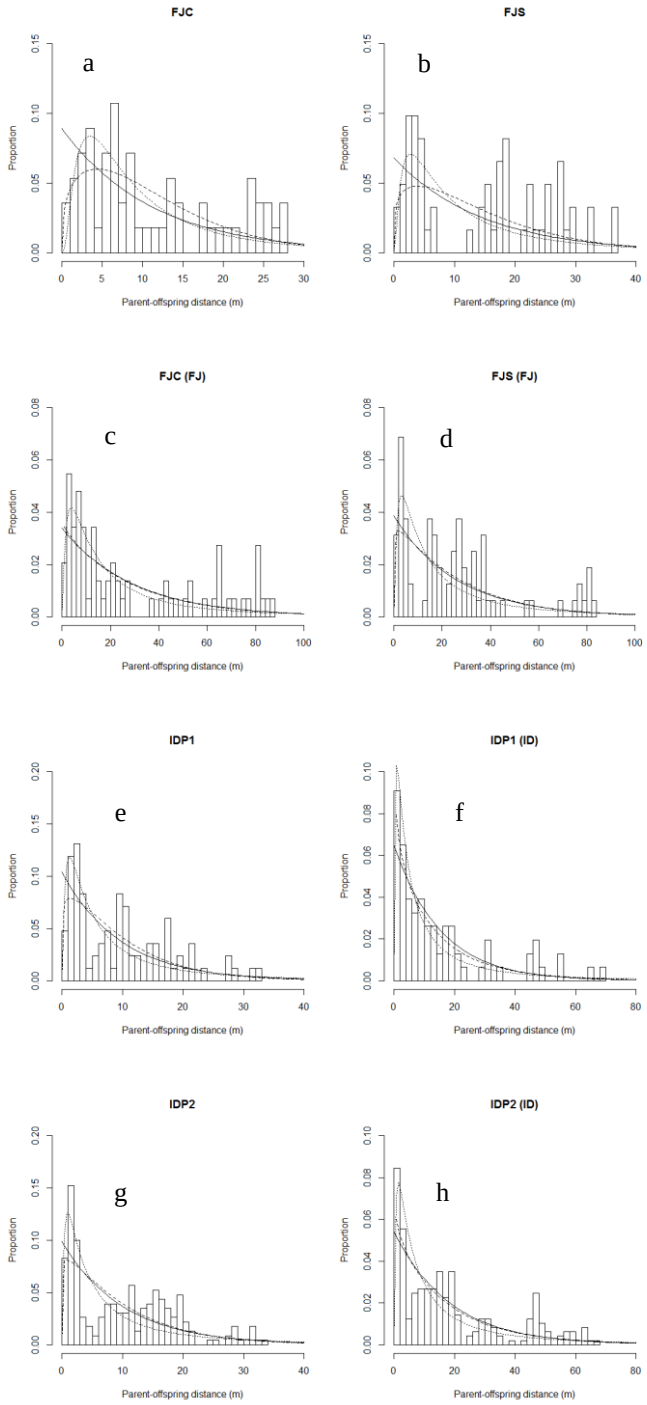
FJC
a
FJS
b
FJC (FJ)
c
FJS (FJ)
d
IDP1
e
IDP1 (ID)
f
IDP2
g
IDP2 (ID)
h
Proportion
Parent-offspring distance (m)

Figure 2.

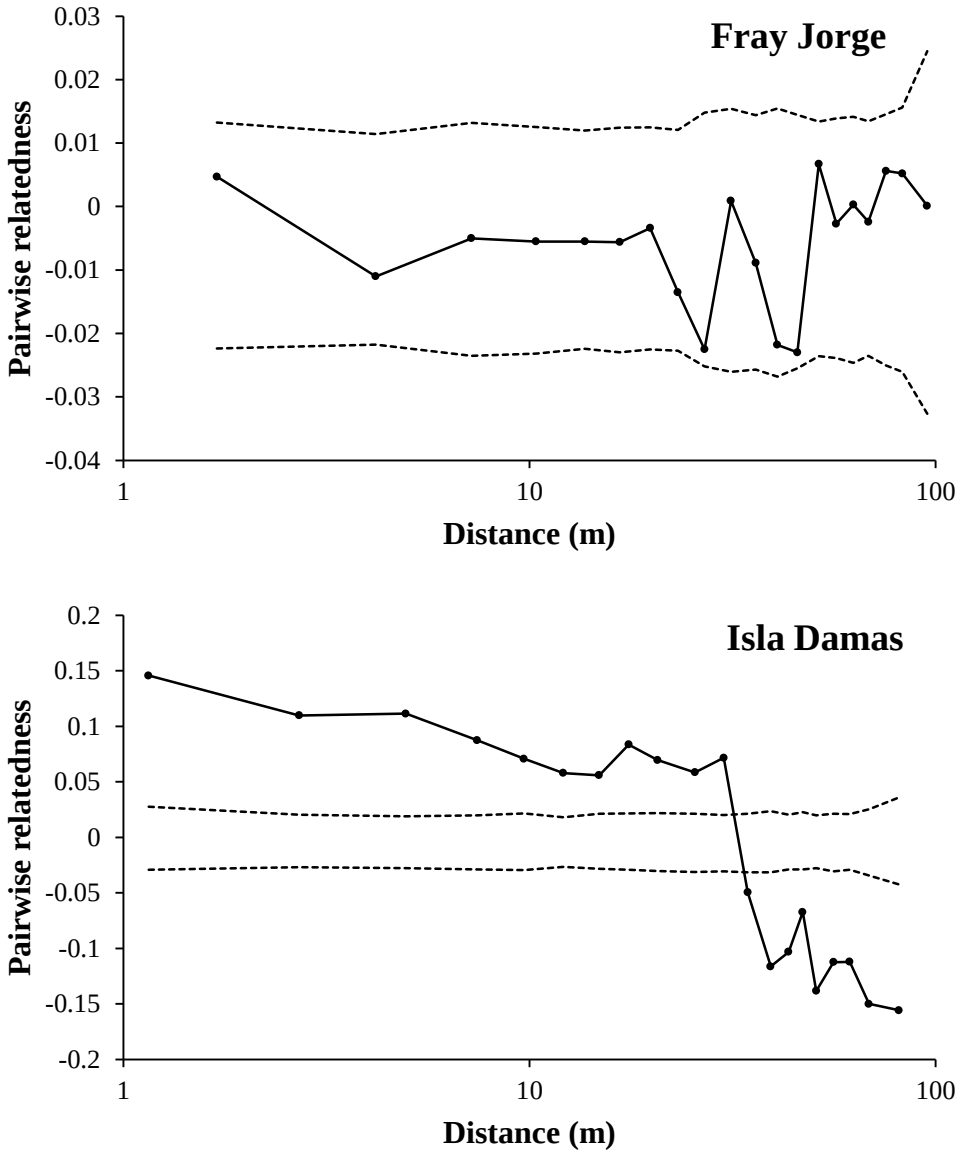
Fray Jorge
Pairwise relatedness
0.03
0.02
0.01
0
-0.01
-0.02
-0.03
-0.04
1
10
100
Distance (m)
Isla Damas
Pairwise relatedness
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
1
10
100
Distance (m)

Figure 3.

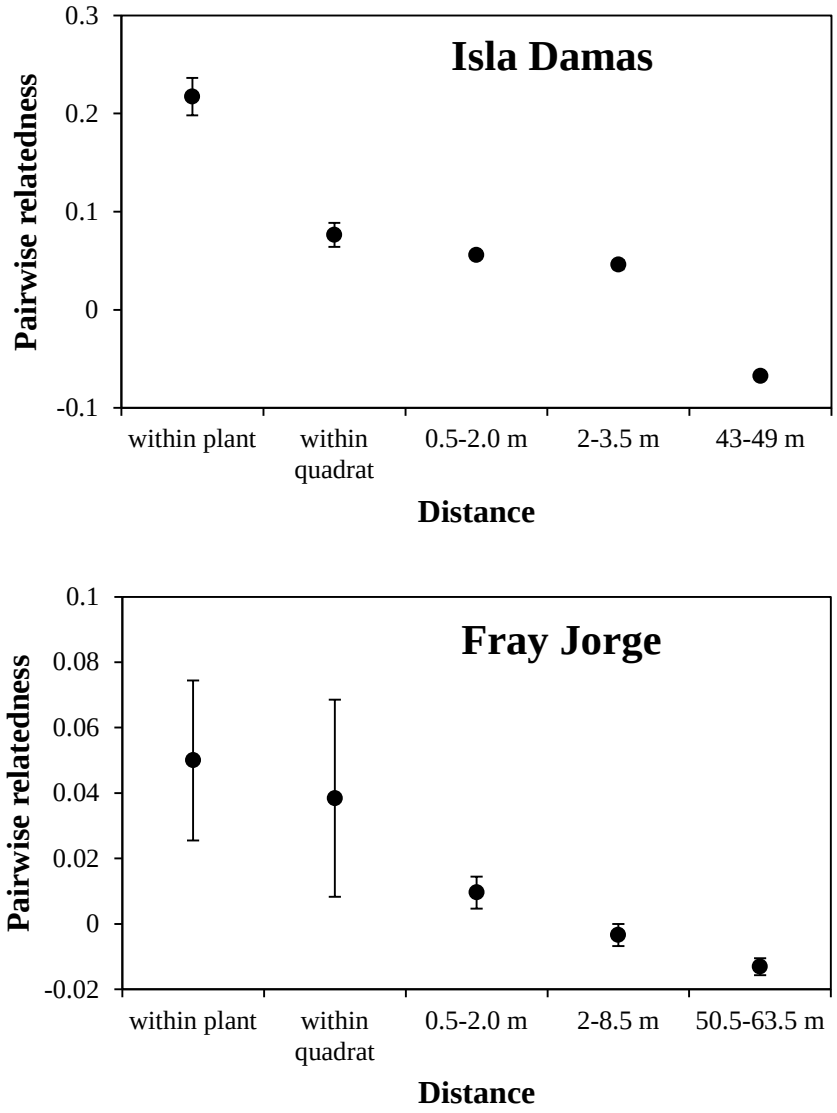
Isla Damas
Pairwise relatedness
0.3
0.2
0.1
0
-0.1
within plant
within quadrat
0.5-2.0 m
2-3.5 m
43-49 m
Distance
Fray Jorge
Pairwise relatedness
0.1
0.08
0.06
0.04
0.02
0
-0.02
within plant
within quadrat
0.5-2.0 m
2-8.5 m
50.5-63.5 m
Distance

Figure 4.

FINAL DISCUSSION

Understanding the adaptive value of group living requires addressing questions like: What are the costs and benefits related to sociality? How likely are relatives to encounter each other in natural populations? What is the degree of relatedness within groups? In this thesis I took the challenge of answering these questions for the kelp *Lessonia spicata*, whose individuals may fuse originating chimeric plants, to gain insights into the selective advantages of chimerism, a peculiar, yet widespread, type of group living. Using molecular markers, I quantified the frequency of chimerism in natural populations of *L. spicata*, evaluated the consequences of fusion between conspecifics in reproductive success and also investigated the interplay between dispersal, fine-scale genetic structure and kin group formation within populations.

As any type of group living, chimerism involves both benefits and costs, and its adaptive value depends on whether fitness benefits exceed the costs (Buss 1982). Previous studies have shown that chimerism may lead to costs including intraspecific competition, that can be present in the form of cell parasitism (Stoner and Weissman 1996; Pancer et al 1995; Stoner et al 1999; Rinkevich 2002), and disease transmission (Velicer and Vos 2009). Demonstrated benefits related to chimerism include synergistic complementation (Buss 1982), protection from stressful environmental conditions (Wernberg 2005; McIntire and Fajardo 2011), increased probability of finding mates (Pietsch 2005; Høeg and Lutzen 1995 – see Rinkevich 2011), enhanced ability to responding to environmental changes due to genetic diversity (Rinkevich and Yankelevich 2004) and increased size of chimeras (Foster et al 2002,

Amar et al 2008), that can lead to an enhanced probability of survival (Raymundo and Maypa 2004; Santelices and Alvarado 2008), earlier onset of reproduction (Santelices et al 1999) and higher intra- and interspecific competitive ability (Buss 1981).

Most studies carried out in the marine realm have evaluated the fitness effects of chimerism comparing size, growth and/or survival of chimeras as a whole to solitary individuals (or colonies), at early stages of the life-cycle. Even though these studies have provided interesting information on chimerism, little is known about what happens in late stages of the life-cycle, whether there are trade-offs among levels of organization (genotypes versus groups – see Amar et al 2008) and most importantly, what are the consequences of chimerism on reproductive success, a fundamental fitness component. Here I estimated reproductive success through parentage assignment analysis. To my knowledge, this study is the first to explicitly explore the effects of fusion with conspecifics in reproductive success at both the genotypic and group levels.

Major findings of this thesis

This thesis shows that despite an observed tendency for decreased genotypic reproductive investment within chimeric plants of *L. spicata*, genotypic reproductive success in non-chimeric plants was either lower, higher or similar to that in chimeric plants, depending on the grid evaluated (Chapter 1). No general pattern regarding the relationship between genotypic reproductive success and chimerism was detected. This suggests that there are no direct costs in reproductive success related to chimerism in *L. spicata*. Yet, there are also no benefits. Interestingly, benefits were found at the plant level: chimeric plants presented a higher probability of being reproductive and produced more offspring than non-chimeric

plants. More than one individual reproduced within chimeric holdfasts and the number of individuals that reproduced within plants influenced total group reproductive success. Although this seems intuitive, it reveals that reproduction is not concentrated in only one genotype within chimeric plants, which means that, although competition may occur among group members, direct benefits may be gained by more than one group member. So, the results of this thesis indicate that chimerism in *L. spicata* may provide benefits in reproductive success by allowing the coexistence of a higher number of genotypes (and potential reproducers and mates), if compared to a scenario where only unigenotypic plants occurred. Moreover, our findings of higher reproductive success of chimeric over non-chimeric plants suggest the potential for natural selection also acting at levels higher than the genotype (i.e. a multilevel selection context). Selection at the level of the chimera could be favoring the maintenance of chimerism in natural populations of *L. spicata*. Several other studies on chimeras have also pointed toward this direction (e.g. Rinkevich 2005, Amar et al 2008, Folse and Roughgarden 2010, McIntire and Fajardo 2011). Hence, studies on chimerism explicitly using a multilevel selection perspective seem to be promising.

The study of population genetic structure allows characterizing how likely relatives are to encounter each other in natural populations (Strassmann and Queller 2011). This knowledge is of fundamental importance for chimeric species since it helps to determine the probability of kin group formation in nature and can complement fitness effects analysis. This thesis shows that limited dispersal (probably coupled with mating system) can produce fine-scale genetic structure (< 40 m) within populations of *L. spicata*, since it leads to the spatial aggregation of relatives (i.e. kin structure), favoring kin group formation (Chapter 2). This is consistent with

theoretical predictions that state population viscosity can promote kin group formation (in our case fusion with relatives), even in the absence of kin recognition, but simply because related individuals tend to settle close to each other (Hamilton 1964). Although I have only quantified direct fitness effects here, the high relatedness within chimeric plants reported in this study (especially for Isla Damas) suggests that chimerism can also lead to inclusive fitness benefits in *L. spicata*. Therefore it is possible that kin selection might also have played an important role on the evolution and maintenance of chimerism in *L. spicata*. Inclusive fitness estimation remains to be done however. This is a challenging experiment, but feasible in kelps given their complex life-cycle and the possibility to clone the haploid phase and produce genetically identical diploids that can be manipulated to grow alone or to form chimera with others issued from controlled crosses. It is therefore theoretically possible to empirically quantify the indirect benefits a genotype receives due to increased fitness of its relatives (*versus* non-relatives) in chimeras, in addition to its fitness when living alone.

Chimerism in the marine realm

The process of chimerism in marine invertebrates and algae has some similarities and one major difference. While the great majority of invertebrates can potentially suffer from cell parasitism, seaweeds do not face this risk, since their rigid cell walls prevent cell mobility (Buss 1982; Bruisini 2013), hampering the intermixing of cells following fusion among conspecifics. Therefore, most invertebrates experience much higher costs related to chimerism than algae (see Bishop and Sommerfeldt 1999 for an exception). This probably explains the notable disparities between chimerism frequency in populations of most invertebrates and algae. The frequency of natural chimerism reported for most marine invertebrates is below

15% (Ben-Shlomo et al 2001, 2008; Puill-Stephan et al 2009; Mercier et al 2011 but see Bishop and Sommerfeldt 1999), whereas the frequencies reported here ranged from 60 to 90%, consistent with the values reported by González et al (2013) and Segovia et al (2014). Natural rates of chimerism are still unknown in red algae, yet they are predicted to be much higher than that of invertebrates.

If chimerism can be so costly for invertebrates, why and how has it been maintained by natural selection in so many taxa? It has been proposed that strong selective pressures acting at early stages of the life-cycle that favor chimeric over non-chimeric entities, coupled to indirect fitness benefits (Buss 1982; Grosberg and Quinn 1986; Rinkevich 2011) have driven the evolution and maintenance of chimerism in marine invertebrates. Studies revealing higher size (Rinkevich and Shapira 1999; Amar et al 2008) and probability of survival (Raymundo and Maypa 2004; Puill-Stephan et al 2012) of chimeras, early in life, support the first hypothesis. In addition, the presence of an effective kin recognition system that only allows successful fusion to occur among closely related individuals has been suggested as an evidence of kin selection (Buss 1982; Grosberg and Quinn 1986). Finally, the evidence of aggregated kin propagule settlement found for several species (e.g. Ben-Shlomo et al 2008; Puill-Stephan et al 2012), has been pointed as a mechanism that enhances the probability of kin encounters in natural populations of marine invertebrates. So, in most marine invertebrates chimerism seems to have been maintained by kin selection. The only exception reported until present is the ascidian *Diplosoma listerianum*, in which no possibility of cell parasitism exists, colonies fuse with non-kin, natural chimerism frequencies are comparatively high and direct fitness benefits are expected to occur (Bishop and Sommerfeldt 1999).

In marine algae, it is likely that, similarly to what happens in marine invertebrates, strong selective pressures that act upon early life-cycle stages (herbivores, wave action, desiccation, and competition) select for chimerism. The study of Santelices and Alvarado (2008) that revealed higher size and probability of survival of chimeric sporelings of red algae support this hypothesis. Although it has been demonstrated that mortality rates by herbivory and wave action are higher at early ontogenetic stages in *L. spicata* (Martínez and Santelices 1998 - as *L. nigrescens*), no studies have evaluated the effect of chimerism in survival yet. Fitness effects of chimerism seem to depend on environmental conditions as well. Chimeric kelps occur more frequently in stressful environments, suggesting that it may confer survival advantages in adverse conditions (Wernberg 2005; Segovia et al. 2014).

Despite the fact that seaweeds do not suffer from cell parasitism, and therefore are likely to face less costs than marine invertebrates, other types of costs related to chimerism may be present. Few costs have been reported for seaweeds so far, perhaps due to the tendency of measuring the effects of chimerism only in the chimera as a whole. Costs in individual growth were detected within chimeric red algae after 60 days of spore fusion (Santelices et al 2010). Concerning reproduction, here we found a slight tendency for decreased genotypic reproductive investment within chimeric plants, yet no overall evidence of costs was observed in genotypic reproductive success related to chimerism in *L. spicata*. Chimeric plants exhibited a higher probability of reproducing and produced more offspring than unigenotypic plants, and the increase in reproductive success was essentially additive. Eventual benefits seem to arise from the increase in local density of reproductive individuals, as holdfast density and size are independent of the number of genotypes therein. We propose that chimerism may also play an important role in promoting outcrossing (reducing inbreeding) in *L. spicata*.

Given that self-reproduction is possible in *L. spicata* and that inbreeding is likely to occurr in natural populations of this kelp, chimerism might foster outcrossing by increasing the proximity between gametophytes produced from different genotypes. If more outcrossed genotypes have higher chances of surviving than more inbred ones (i.e. inbreeding depression), chimerism is expected to enhance survival probabilities. Future studies addressing inbreeding rates, inbreeding depression and the effect of chimerism in promoting outcrossing in *L. spicata* and in other seaweed species could provide support for this proposition.

More studies are needed to determine the fitness effects of chimerism at the genotype and plant levels in marine algae. Our findings of chimeric plants being more productive than non-chimeric plants suggest the potential for natural selection also acting at levels higher than the genotype (i.e. a multilevel selection context). Selection at the level of the chimera could be favoring the maintenance of chimerism in natural populations of *L. spicata*. Several other studies on chimeras have also pointed toward this direction (e.g. Rinkevich 2005, Amar et al 2008, Folse and Roughgarden 2010, McIntire and Fajardo 2011). Therefore, studies on chimerism framed in a multilevel selection perspective might be promising. Moreover, the recent description of the occurrence of cellular contact among group members (González et al 2013) following fusion in *L. spicata* opens an interesting possibility to identify possible cooperative or competitive (cheating) behaviors of genotypes within groups. Some genotypes could behave opportunistically by taking advantage of molecules produced by partners (e.g. photosynthates) without incurring the cost of producing them. Finally, the observation that not all genotypes present in *Lessonia´*s holdfasts are represented in the stipes of the same plant (Alejandra González, unpublished data) points to the presence of complex interactions among

genotypes, resembling those described for the amoeba *D. discoideum* (Strassman and Queller 2011), that deserve further detailed investigation.

This is the first study that related population genetic structure with kin group formation in seaweeds. This thesis shows that kin group formation can occur in *L. spicata*, as a result of limited dispersal, a prerequisite for possible indirect fitness benefits related to chimerism in *L. spicata*. It could be hypothesized that limited dispersal coupled to mating system creates proper ecological conditions for the evolution of kin-selected social behavior in *L. spicata*. Yet, since no direct costs in reproduction related to chimerism have been found for *L. spicata*, specific studies on the fitness effects of kinship are strongly needed to evaluate this possibility (Griffin and West 2002). Within-group relatedness is likely to be high in several other seaweed species that present limited dispersal abilities and/or group settlement, as reported for some red algae (Faugeron et al 2001, Santelices and Aedo 2006, Krueger-Hadfield 2013). Hence, indirect benefits related to chimerism may also be present in many other marine algae.

In conclusion, this study has generated important knowledge on group living in *L. spicata* that complement previous studies on chimerism in kelps (Wernberg 2005; González et al 2013; Segovia et al 2014) and in red algae (Santelices et al 2004; Santelices and Alvarado 2008, Santelices et al 2010). Several lines of evidence seem to indicate that chimerism is an adaptive strategy in seaweeds: i) it is common in several species of green, red and brown algae (Santelices et al 1999; Wernberg 2005; González and Santelices 2008; Segovia et al. 2014); ii) it confers selective advantages at early life-cycle stages (Santelices et al 2004; Santelices and Alvarado 2008); iii) its frequency seems to be related to stressful conditions (Wernberg, 2005;

Segovia et al 2014); iv) it enhances the number of potential reproducers (this study) ; v) it probably occurs mostly among kin, suggesting the possibility of indirect fitness benefits within groups (this study - remains to be evaluated in other species). Yet, several interesting questions still need to be addressed to reach a complete understanding of the ecological and evolutionary success of chimerism in seaweeds. These include:

- Is fusing with kin more advantageous than fusing with non-kin?
- What is the rate of inbreeding in natural populations of seaweeds? Does it lead to inbreeding depression? Does it decreases with chimerism frequency?
- Does selection also act at the level of chimeras?
- Do fitness effects of chimerism vary with environmental conditions?
- What happens, biochemically speaking, after two individuals fuse? Is there a transport of molecules (e.g. products of photosynthesis) between them? Is it uni- or bi-directional?
- Are differences in genotypic composition of holdfasts and stipes within chimeric plants frequent in natural populations? How do they affect genotypic and plant fitness?

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SUPLEMENTARY MATERIAL

GLOSSARY

Allorecognition: the ability to recognize non-self, usually determined by high polymorphic loci. Common in marine invertebrates.

Cell parasitism: competition between genetically different cell lineages. Cell parasitism is present in species in which fusion involves the development of a common "circulatory" system that allows the passage of cell lineages from one individual to the other (the so-called cytomictical chimeras). It can be divided in somatic (competition for position in the soma) and germ cell (competition for the germ line) parasitism.

Chimera: a group of genetically different genotypes fused to each other forming a single macroscopic entity.

Direct methods (of measuring dispersal): methods that estimate contemporary dispersal events, including the assignment of individuals to at least one of their parents (Parentage Analysis) or to their population of origin (Population Assignment) and spatial autocorrelation analysis – see indirect methods.

Dispersal: the movement of individuals or propagules from the places where they were produced to other locations (where they breed). Any movement that leads to gene flow.

Effective dispersal: the actual distance an individual or propagule travels.

Fronds: Flattened, leaflike structures of algae, where most of the photosynthetic activity takes place.

Heteroplasmy: The presence of multiple distinct mitochondrial genome sequences within an individual.

Holdfast: the massive structure that adheres the alga into the substrate, from where stipes emerge.

Holdfast fusion: fusion between two or more algal holdfasts generating a chimeric plant.

Indirect methods (of measuring dispersal): methods that estimate dispersal through the analysis of genetic differentiation among populations.

Multilevel selection: A generalization of group selection that states that natural selection can act at more than one level of organization.

Potential dispersal: the potential distance an individual or propagule can travel based only on its biological characteristics (without taking into account restrictions).

Propagule: a structure capable of being propagated or acting as an agent of reproduction, propagating an organism to the next stage of its life cycle. Examples include spores, pollen, seeds, larvae, etc. The propagule is usually distinct from the parental organism.

Sedentary organism: an organism that has restricted movement.

Sessile organism: an organism that lives attached to a surface and cannot move.

***Sorus* (plural *sori*)**: A cluster of sporangia. In kelps, sori are dark brown, visible structures that develop mostly on fronds.

Spores: unicellular propagules produced by *sporangia* formed in sporophytes. After settling in a substrate, spores germinate and develop into gametophytes.

Stipe: A stalk or stemlike structure present in an algae.

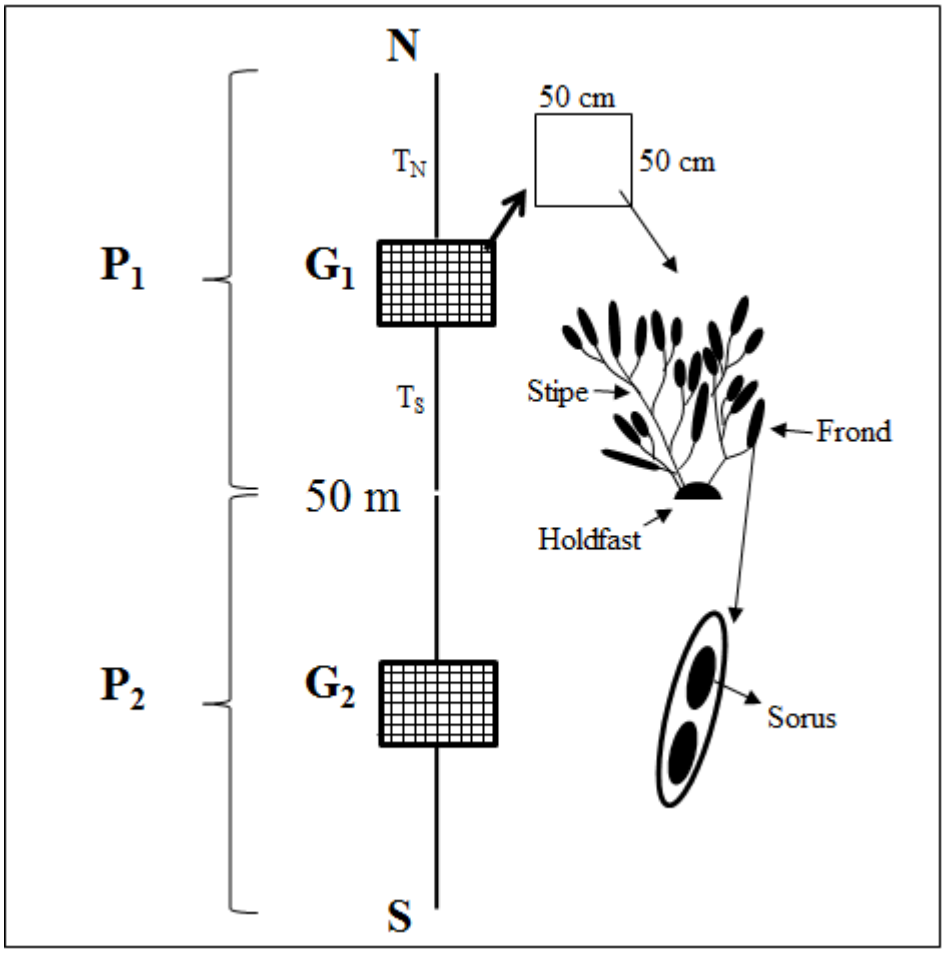

Figure S1. Schematic representation of the sampling design used in each of the two study sites. G_1 and G_2 refer to grid 1 and 2, respectively. T_N and T_S correspond to transect North and South. Adults were sampled with quadrats (50 x 50 cm) within grids and recruits were sampled with quadrats both within grids and within transects. Main morphological parts of *L. spicata* (holdfast, stipe and fronds) are depicted in the scheme and a detail of a frond showing a *sorus* is illustrated.

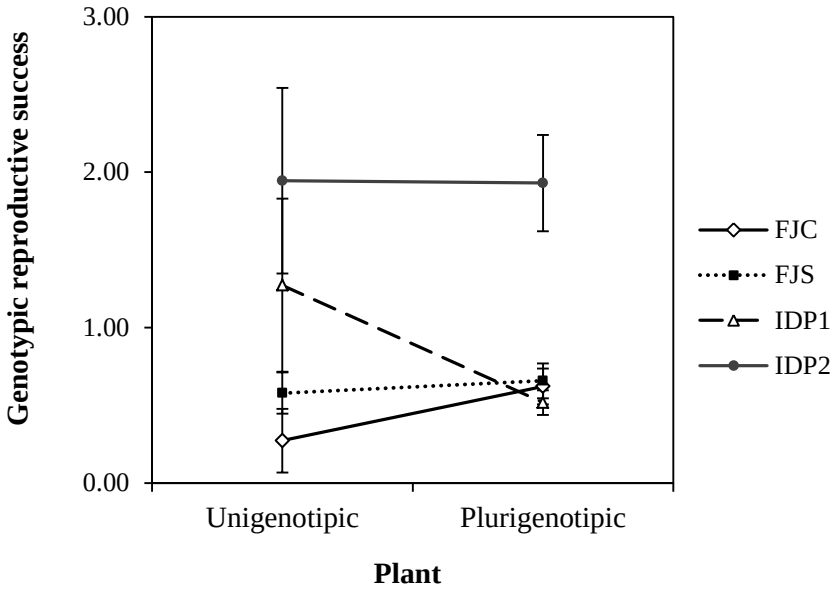

Figure S2. Unstandarized genotypic reproductive success in unigenotypic and plurigenotypic plants. Points and bars represent averages ± standard errors.

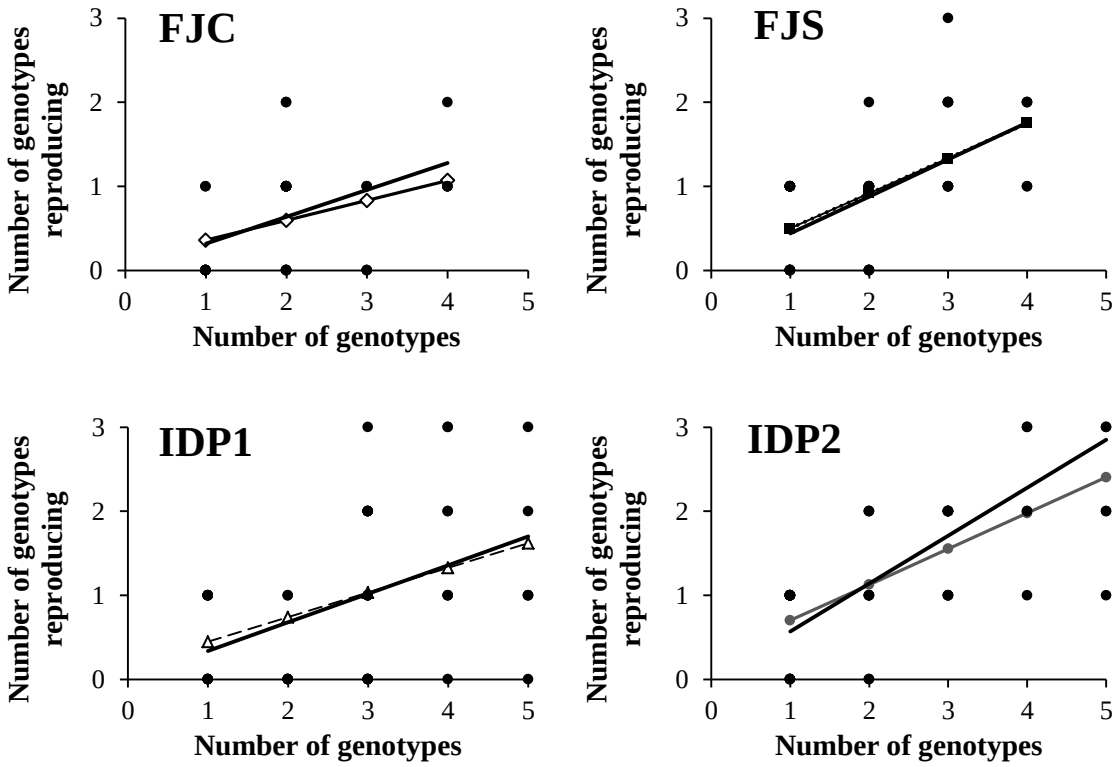

Figure S3. Number of genotypes that reproduce as a function of the number of genotypes per plant. The line with the markers represents the fitted regression to observed data. Solid black lines depict the expected relationship based on the observed probability (p) of genotypes reproducing within each grid (FJC: p = 0.32; FJS: p = 0.44; IDP1: p = 0.34; IDP2: p = 0.57).