

## ORIGINAL ARTICLE

# On the advantage of sharing a holdfast: effects of density and occurrence of kin aggregation in the kelp *Lessonia berteroa*

Nicolás I. Segovia<sup>1,2</sup>, Julio A. Vásquez<sup>1</sup>, Sylvain Faugeron<sup>3,4</sup> & Pilar A. Haye<sup>1,5</sup>

1 Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte & Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, Chile

2 Doctorado en Ciencias M/ Biología y Ecología Evolutiva, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile

3 Centro de Conservación Marina, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

4 UMI 3614 Evolutionary Biology and Ecology of Algae, CNRS, Sorbonne Universités UPMC Univ. Paris 06, Pontificia Universidad Católica de Chile, Universidad Austral de Chile,

5 Interdisciplinary Center for Aquaculture Research (INCAR), Universidad de Concepción, Concepción, Chile

## Keywords

Density dependence; fusion of individuals; genetic relatedness; kelp; kin aggregation; kin selection; microsatellites.

## Correspondence

Pilar A. Haye, Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte & Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Larrondo 1281, Coquimbo, Chile.  
E-mail: phaye@ucn.cl

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## Abstract

We investigated the density-dependent and genetic relatedness that regulate the occurrence of inter-individual (genet) fusion forming plurigenotypic organisms in the brown alga *Lessonia berteroa*. Recruitment generally occurs at high densities in the inter-tidal, allowing contact of neighbouring holdfasts as they grow and expand on the substrate. Algal density, by contrast, is regulated by the effects of herbivory and wave impact, which often lead to low holdfast density. Herein, we investigated whether the occurrence of plurigenotypic organisms and their genotypic composition (number of genotypes per plurigenotypic organism) are density dependent and affected by kin selection in the inter-tidal kelp *L. berteroa*. Four microsatellite loci were used to analyse DNA from 260 samples obtained from shared and non-shared holdfasts, at two sites with high and two sites with low holdfast density. Analyses showed that fusions forming plurigenotypic organisms are extremely common. Interestingly, the frequency of fusions was higher in low-density sites, in which 100% of the plants had at least two genotypes and the average was 3.5. In high-density sites, 62% of plants were plurigenotypic, with an average of 2.8 genotypes per plant. Additionally, we found that genotypes that shared a holdfast had a significantly higher genetic relatedness than the average in the population, compatible with a kin structure. Density dependence and kin structure suggest that the occurrence of plurigenotypic organisms is linked to environmental quality, and that kin or multilevel selection may be favouring the fusion of genetically related genets.

## Introduction

Different units of relevance for ecological and evolutionary processes can be delineated, such as the population, the family or group of related individuals, the colony and the individual organism. As most of these units can actually be nested one into the other (e.g. individuals into colonies or families, colonies into populations; see

Nachtomy *et al.* 2002), the limits of the individual, as a basic ecological and evolutionary unit, are sometimes difficult to define. In fact, individuals of many species live in groups in which they find both protection against environmental stresses (e.g. predation) and closeness to other individuals for reproduction. The case of clumps and colonies is particularly interesting because the functional limits between the genet, issued from a single

fertilized egg, and the individual organism that is made of a mixture of different genets, are most often indistinguishable. Such findings have stimulated important debate around the concept and definition of the individual and the organism (e.g. Santelices 1999; Nachtomy *et al.* 2002; Pepper & Herron 2008). Clumps have been reported in a wide variety of taxa, some of which are algal species (Santelices *et al.* 1996, 1999, 2003; Wernberg 2005; González & Santelices 2008). In red algae, fusion of individuals results in a chimeric, plurigenotypic organism (PO), with complete loss of individual identity (Paine 1990; Santelices *et al.* 1996, 1999). This process, known as coalescence in red and green algae (not formally described in brown algae), is difficult to study in natural populations because it occurs mainly at early microscopic stages (e.g. spores or sporelings; Santelices *et al.* 1996, 1999; Santelices & Aedo 2006). As a consequence, the factors that regulate the fusion of genets have scarcely been explored.

Two non-exclusive hypotheses can be proposed to explain the occurrence of fusions at the holdfast level in algal species: (i) fusions are correlated with density of recruits, which, during their development, grow and eventually get into such close contact with their neighbours that they fuse together (*i.e.* they integrate their cell lines into a single tissue) or (ii) fusions are regulated by fitness differences between clumped and isolated genets. In the first case, fusions are just a density-dependent process whereas selection and adaptation can be invoked in the second hypothesis. Evidence based on higher survival rates of aggregates compared with isolated individuals of red and brown algae suggests a selective advantage of clumping (Wernberg 2005; Santelices & Aedo 2006; Santelices & Alvarado 2008). For instance, holdfast aggregations of the kelp *Ecklonia radiata* have been observed at higher frequencies in exposed areas than in more protected ones, suggesting that aggregation reduces mortality from dislodgement in exposed areas (Wernberg 2005).

Based on field observations of tagged sporophytes, Vásquez *et al.* (2008) and Rodríguez *et al.* (2014) showed that fusions of individuals forming a PO might be recurrent in the kelp *Lessonia berteriana* Montagne (formerly *Lessonia nigrescens*, González *et al.* 2012). This species dominates the low inter-tidal rocky shores of Chile and southern Peru (Hoffmann & Santelices 1997) and its structure consists of a massive holdfast attached to the rocky substrate. A variable number of stipes emerge from the holdfast. Stipes have branches and each branch carries one frond distally. Holdfasts grow vertically and horizontally leading to contact between neighbours that progressively grows, resulting in their tissues mixing and forming a single unit that externally mimics a single organism (Vásquez *et al.* 2008). A simple expectation from this

growth pattern is that the rate at which genets fuse increases with density (Rodríguez *et al.* 2014). However, if fusions confer some kind of advantage under stressful environmental conditions, then the occurrence of fusions should increase when the environment moves away from optimal conditions for the species.

To test these predictions, we quantified the number of different genets (*i.e.* multilocus microsatellite genotypes) per plant (stipes and fronds of a single holdfast) of *L. berteriana* under two contrasting densities of holdfasts in one natural population.

In *L. berteriana*, density variability, spatial distribution, establishment of new recruits, mortality rates and growth patterns are regulated mainly by herbivory, wave impact and intra-specific competition for hard substrate (Ojeda & Santelices 1984; Santelices & Ojeda 1984). The strengths of these three selective agents differ in association with distance between holdfasts. For instance, herbivory increases with distance between holdfasts (Vásquez & Santelices 1990), which in turn reduces the net recruitment of *L. berteriana* (Ojeda & Santelices 1984; Camus 1994). These patterns result in a general negative relationship between kelp coverage and herbivore abundance (Broitman *et al.* 2001), and pinpoint herbivory as a strong density-dependent selective pressure on *L. berteriana*. Wave impact is another major cause of mortality in benthic algae (Dayton *et al.* 1975; Vadas *et al.* 1990) that is considered a selective agent driving morphological and physiological adaptation (Vásquez 1992; Blanchette 1997; Martínez & Santelices 1998). Wave impact also imposes a high dynamic pressure that can result in dislodgement or removal of settled plants (Vadas *et al.* 1990, 1992). In close holdfast proximity, the interaction between water motion and the shape of the stipes and fronds produces a wave movement known as the whiplash effect (WE, *sensu* Dayton 1975). Owing to the WE, high holdfast density is beneficial for kelp populations, allowing the persistence of new recruits (Ojeda & Santelices 1984). Vásquez (1995) showed that when the distance between holdfasts exceeds 2 m, the WE is reduced (*i.e.* the environment is less protective), increasing herbivory. Thus, both herbivory and the WE have density-dependent effects in both inter-tidal and subtidal *Lessonia* species (e.g. *Lessonia trabeculata*, Vásquez 1992; Vásquez & Buschmann 1997) that lead to greater survival of plants at high holdfast density.

High holdfast density implies intra-specific competition for substrate and light, limiting the settlement of new recruits (Santelices & Ojeda 1984; Andrew & Viejo 1998; Steen & Scrosati 2004). In this context, fusions between conspecifics may be seen as a way to increase individual density without increasing substrate occupancy and to avoid intra-specific competition. This kind of strategy has

been found more frequently between kin than unrelated individuals (Gerlach *et al.* 2007; Lizé *et al.* 2012). For example, plants effectively experience strong social interactions and kin recognition to varying physiological and morphological responses depending on the identity of the neighbour (de Kroon 2007; Biedrzycki *et al.* 2010; Wu *et al.* 2013). However, as Hamilton's (1964) rule predicts, fusions may be an adaptive trait only if the benefits outweigh the costs, and close contact amongst individuals within a holdfast is likely to induce strong competition amongst them (Novoplansky 2009). This cost can probably be reduced if fusion occurs amongst relatives, so that benefits are expressed in terms of inclusive fitness (*i.e.* the direct contribution of each genotype to the next generation's gene pool plus their indirect contribution through the progeny of their relatives). Therefore, if fusion between relatives enhances survival rate, then positive kin selection is a likely explanation (File *et al.* 2013). In this context, fusions between relatives in *L. berteroa* would be more frequent in low-quality environments (*i.e.* low density) in which isolated individuals (unigenotypic organisms) may not persist.

The goal of this study was to shed light onto the factors that regulate the fusion of genets into a single organism, by assessing genetic diversity of holdfasts at low and high plant densities and the genotypic relatedness within plants in the kelp *L. berteroa*.

## Material and Methods

### Sampling, field measurements and DNA extraction

Tissues of *Lessonia berteroa* were sampled in an extensive inter-tidal kelp bed in Lagunillas, Chile (30°06' S, 71°22' W) on areas showing no evidence of recent kelp harvest. In order to test for density dependence, 12 plants of different sizes were sampled in two sites of low holdfast density (LD: 0.5–1 holdfasts·m<sup>-2</sup>) and two of high holdfast density (HD: 5–8 holdfasts·m<sup>-2</sup>). We use the term plant to refer to the organism identifiable macroscopically on the shore, and which may be made of either a single or multiple genets.

From each of the 48 sampled plants, six tissue samples were collected each from a different stipe selected at random, except for some small plants (*i.e.* fewer than six stipes). In these cases, the samples were taken from all the available stipes (at least five). Fresh tissue samples of 2 cm in diameter were collected from the base of fronds avoiding reproductive tissue and epiphytes. Collected pieces were dried using paper towel, placed in sealed individual bags with silica gel and stored at room temperature until DNA extraction. DNA was extracted from 30–40 mg of dried powdered tissue using a slightly modified version

of the cetyltrimethyl ammonium bromide DNA extraction adding polyvinyl pyrrolidone in order to remove polyphenols (Martínez *et al.* 2003). Extracted DNA was quantified in a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and kept at –20 °C.

To account for the effect of plant size on the frequency of fusions, three plant-to-plant morphometric values were recorded for each sampled plant: maximum length, holdfast diameter and number of stipes. In brown algae, growth is highly related to microhabitat conditions; thus, kelp size is not necessarily related with age. In order to consider genet-to-genet morphology, *i.e.* differences within the plant, for each sampled stipe, we recorded the length, diameter and number of dichotomies.

### Informative microsatellite loci determination

To evaluate which loci were informative for the study, 10 DNA samples from different plants were randomly picked to explore eight microsatellite loci available for *Lessonia berteroa* (Faugeron *et al.* 2009). PCRs were performed in a final volume of 20 µl with: 1.5 ng DNA, 2 mM of 10× buffer, 0.6 mM dNTP mix, 1.8 mM MgCl<sub>2</sub>, 0.2 mM of each primer, 0.075 mg·ml<sup>-1</sup> of bovine serum albumin and 0.2 U *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania). Temperature cycling consisted of an initial soak of 3 min at 95 °C, then 10 touchdown cycles at 95 °C for 45 s, 60–63 °C for 45 s and an extension of 45 s at 72 °C. Following this, 30 cycles at 95 °C for 45 s, 50 °C for 45 s, 72 °C for 45 s and a final extension of 72 °C for 7 min were performed. Amplicons were genotyped in an ABI Prism 3700 (Applied Biosystems, Foster City, CA, USA) and the electropherograms were visualized using GENEMARKER v. 1.81 (SoftGenetics, State College, PA, USA).

The main criteria for considering loci as informative were the degree of polymorphism in the local population and PCR amplification success. After checking for polymorphism, genotypes were analysed in MICROCHECKER 2.2 (Van Oosterhout *et al.* 2004) to determine the probability of null alleles. The probability of finding the same multilocus genotype by chance twice in each site of each density was calculated with GENEALX v. 6 (Peakall & Smouse 2006). It was considered a sufficient number of informative loci when each of the 10 analysed DNA samples had different multilocus genotypes.

### Multilocus genotype data analyses

Any holdfast with more than one multilocus genotype was considered a PO. The effects of size and density were analysed using generalized linear mixed models (GLMMs) with Poisson errors implemented in the lme4 package in the software R (R Core Team 2013). GLMMs are

appropriate for non-normal data influenced by fixed and random effects (Crawley 2005; Bolker *et al.* 2008; Grueber *et al.* 2011). In each model, effects of plant morphometric values (MV) (holdfast diameter, total length and total number of stipes) and density (low and high, denoted as LD and HD, respectively) in the occurrence of POs, and their interaction, was evaluated using each of the three MV as co-variables with the site (St) nested in density. The correlation between plant length and holdfast diameter is well known in *Lessonia* (Santelices 1982; Vázquez & Santelices 1984; Vázquez 1991); however, the correlation of these variables and the number of stipes is not consistent and only occurs when reproductive stipes are considered (Vázquez 1991). Finally, differences in the number of genotypes between densities were evaluated in a single GLMM using density (D) as categorical variable following the same structure as the previous models.

Strictly, site is a random effect because all levels of that factor are selected as a random sample from all possible levels (sites) that could be included in the study area (spatial pseudoreplication). So additionally, as an exploratory analysis of the relative importance of the factors in the models, according to the Akaike information criterion (AIC), the site effect was evaluated as an explanatory variable with fixed effects, and in interaction with variables that e tGLMMs showed to have a significant effect on the number of genotypes.

Lastly, principal component analyses (PCAs) and lineal discriminant analysis (LDA) were carried out with the log-transformed data of the three genet-to-genet variables (length, diameter of the stipe and number of dichotomies) and the three plant-to-plant MV variables (holdfast diameter, total length, number of stipes). The objective of these analyses was to observe possible differences in both densities considering genet-to-genet variance as a way to include the morphological differences within the plant and plant-to-plant differences between densities. Both analyses were carried out using the MASS package, and the 95% confidence ellipses were calculated with the ELLIPSE package, both available in the CRAN R project (R Core Team 2013).

#### Relatedness amongst fused genets

To investigate the occurrence of kin aggregations within a PO, we tested whether genotypes sharing a holdfast were genetically closer than on average in the population. This was achieved by comparing the average pairwise coefficient of relatedness *R* (Ritland 1996) of each plant with the average inter-plant pairwise *R*. The upper limit of the one-tailed 95% confidence interval for inter-plant *R*-values for each site was determined by bootstrapping in R (R Core Team 2013) 10,000 values of mean *R* amongst 10 randomly sampled (with replacement) pairwise *R*-values

obtained from pairs of genotypes from different plants. Observed intra-plant mean *R*-values that were higher than the 95% limit were considered to represent a significantly higher genetic relatedness than the background population.

## Results

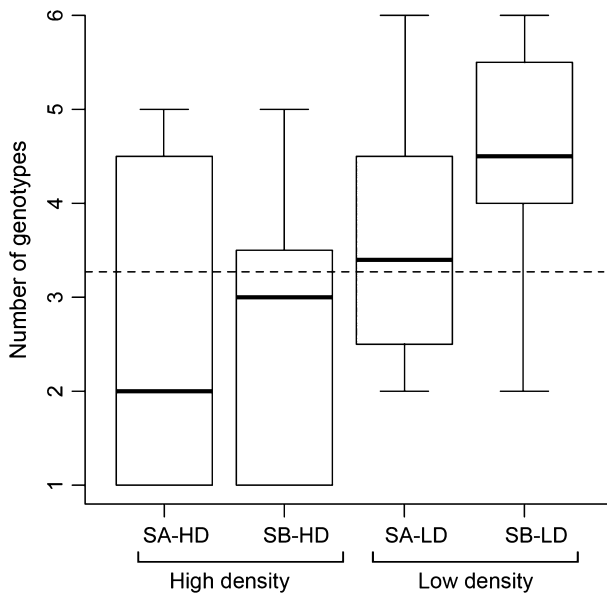
### Informative microsatellite loci identification

Of the eight explored microsatellite loci, four (Less1T11, Less2D22, Less2D25 and Less2D26) were polymorphic enough to unequivocally identify each of the 10 preliminary sampled individuals, and thus were selected to characterize all samples. The probability of finding the same multilocus genotype twice by chance using these four loci was  $9.07 \times 10^{-5} \pm 0.0004$  and  $0.001 \pm 0.020$  for LD and HD, respectively.

### Fusion of individuals in *Lessonia berteriana*

Of the 260 tissue samples analysed, from 48 plants, 150 corresponded to different individuals based on multilocus genotypes, which correspond to a total of 39 plurigenotypic organisms (81.3% of the sampled holdfasts). There was an average of 3.17 multilocus genotypes per plant, of a maximum of six that could be detected with the sampling scheme. Only 18.5% of the sampled holdfasts showed only one multilocus genotype. By contrast, 22.9% showed at least five different multilocus genotypes, including small kelps (*i.e.* all five stipes with a different multilocus genotype; Fig. 1).

The evaluation of the effects of plant-to-plant MV using GLMMs revealed that neither total length nor holdfast diameter influenced the number of genotypes per holdfast (df: 41,  $P = 0.1130$  and  $0.1891$ , respectively), even when interacting with density (df: 41,  $P = 0.0970$  and  $0.0879$ , respectively) (Table 1). Interestingly, the number of genotypes varied significantly with the number of stipes per holdfast (df: 41,  $P = 0.00721$ ) with a significant effect of density in this model (df: 41,  $P = 0.00261$ ), although the interaction of density with the MV was not significant (df: 41,  $P = 0.15932$ ) (Table 1). The effect of the size, particularly with the number of stipes as the response variable was significant only at HD, whereas at LD there was no relationship (Fig. 2). Moreover, holdfast density had a significant effect on the average number of genotypes per holdfast (df: 41,  $P = 0.0036$ ; Table 1). At LD 100% of the holdfasts were plurigenotypic, whereas only 62.5% were at HD. The average number of genotypes was also greater at LD, with 3.54 genotypes per holdfast, *versus* 2.79 genotypes per holdfast at HD. This suggests that plant density



**Fig. 1.** Box-plot of numbers of multilocus genotypes per plant detected at two sites (SA and SB) with high (SA-HD and SB-HD) and two with low holdfast densities (SA-LD and SB-LD) in *Lessonia berteroana*. The dashed horizontal line indicates the global average of multilocus genotypes.

**Table 1.** Summary of the generalized linear mixed models using morphometric values of the plants. The table shows the results of comparing high (H) versus low (L) holdfast density.

Model	Estimate	SE	Z-value	P-value
Ng = D × number of stipes + St(D) (AIC: 35.79)				
Intercept	0.601	0.191	3.137	>0.001
Density (H – L)	0.780	0.259	3.010	0.003*
Number of stipes	0.018	0.006	2.687	0.007*
Density H – L × number of stipes	-0.018	0.013	-1.407	0.159
Ng = D × total length + St(D) (AIC: 37.96)				
Intercept	0.527	0.297	1.772	0.076
Density (H – L)	0.609	0.367	1.660	0.097
Total length	0.003	0.002	1.585	0.113
Density H – L × total length	-0.001	0.002	-0.376	0.706
Ng = D × holdfast diameter + St(D) (AIC: 38.14)				
Intercept	0.558	0.322	1.730	0.083
Density H – L	0.622	0.364	1.706	0.087
Holdfast diameter	0.028	0.021	1.313	0.189
Density H – L × holdfast diameter	-0.011	0.023	-0.466	0.641

Ng = number of genotypes; D = density; St = sites; AIC = Akaike information criterion.

\*Significant values (P < 0.05).

explains the observed data better than MV; a marginal association between number of stipes and number of genotypes could be observed only at HD (Fig. 2).

**Table 2.** Summary of the generalized linear mixed model testing the effect of density (D) on the number of genotypes (Ng).

Model	Estimate	SE	Z-value	P-value
Ng = D + St(D) (AIC: 22.2, df = 5, k = 3)				
Intercept	0.9328	0.1280	7.286	<0.001*
Density	0.4535	0.1637	2.769	0.006*

St = sites; AIC = Akaike information criterion; k = number of variables.

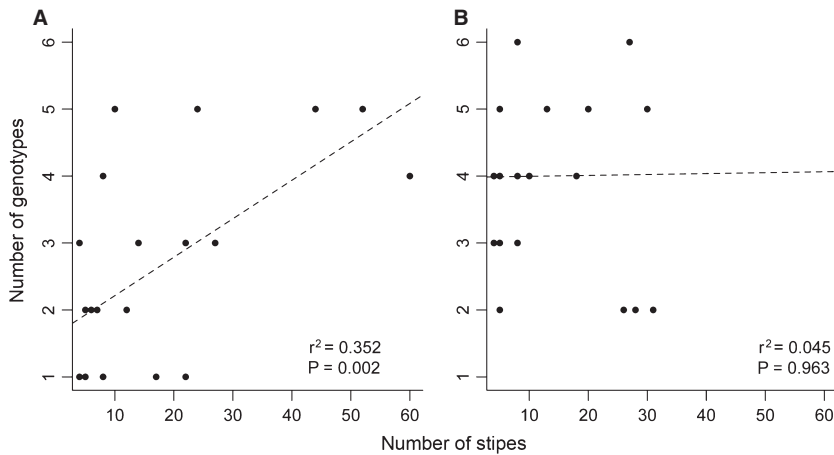
\*Significant values (P < 0.05).

Exploring the relative weights of each factor in the models considering the site (St) as a fixed predictor, the model of the interaction of size and density showed low values in terms of relative weights (according to the AICs, Table 2), suggesting that density and size did not act together as explanatory variables on the occurrence of POs and the number of genotypes per holdfast. Site-to-site density variations seem to explain the differences between the number of POs and number of genotypes per PO in each model (Fig. 1).

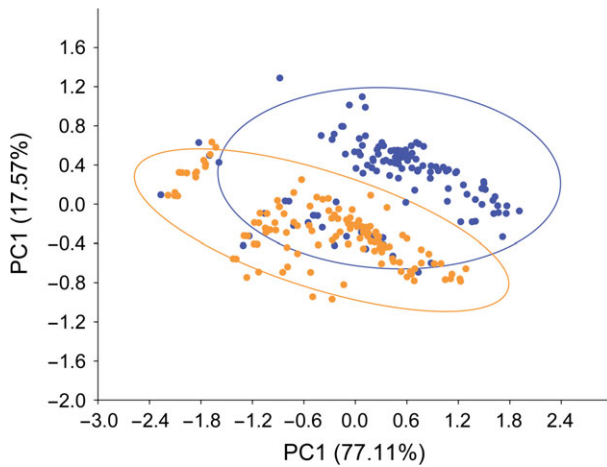
In the PCA analyses, using genet-to-genet morphological variables, the first two axes explained 95.67% of the total variance. The first axis was composed of the three variables that had similar loadings, and the second axis mainly of stipe diameter, suggesting that the three genet-to-genet variables are associated with morphological variation between densities. The PCA showed two groups with an overlap of a few genets that were significantly differentiated (one-way ANOVA;  $F_{1,258} = 52.59$ ,  $P < 10^{-12}$ ; Fig. 3). In this analysis, the results showed that genets at LD are in general smaller than at HD. The trend is more evident for stipe diameter, which tends to be thinner at LD than HD. The LDA approach, with one canonical discriminant function (*i.e.* two classes: HD and LD), showed that stipe diameter was the variable with the highest discriminant coefficient. This discriminant function was able to correctly assign 89.7% of the samples to their density. Wrong predictions were in almost all cases (except for one) for HD samples assigned as LD. By contrast, using plant-to-plant MV, the PCA showed only one undistinguishable group and the LDA could correctly assign the plant to the density of origin in 47.9% of cases.

**Relatedness analyses**

From a total of 39 POs, 15 at HD and 24 at LD, 30 (*i.e.* 77%) displayed an intra-plant mean relatedness significantly higher than expected given the inter-plant pairwise relatedness in the population (Table 3). From these 30 holdfasts, 66.6% occurred at HD and 79.16% at LD. There was strong heterogeneity in intra-plant relatedness: site A in HD (SA-HD) had the lowest number of plants



**Fig. 2.** Relationship between the number of multilocus genotypes per plant and the number of stipes at high (a) and low (b) holdfast density in *Lessonia berteroana*.



**Fig. 3.** Bivariate plot of scores from principal components 1 (PC1) and 2 (PC2) of a principal components analysis of the 260 sampled genets of *Lessonia berteroana* and three genet-to-genet morphological values of stipes (length, diameter and number of dichotomies). Blue points are low density and orange points are high density; the 95% confidence ellipses for each group are shown.

(three out of seven) with significantly higher mean relatedness than the population level, whereas every PO in site B (SB-HD) displayed a significantly higher intra-plant mean  $R$  (Table 3). At LD, where relatedness is more common, most intra-holdfast pairwise  $R$ -values were significant, with only two and three POs from SA-LD and SB-LD, respectively, with values within the expected range of inter-plant values.

## Discussion

### Occurrence of plurigenotypic holdfasts

Our results indicate that the formation of POs in *Lessonia berteroana* is a very frequent phenomenon. Thirty-nine of

the 48 analysed plants had at least two different genets. As for a number of red and green coalescing algae (Santelices *et al.* 1999, 2003), fusion of *L. berteroana* at the holdfast level results in a single macroscopic organism in which the different genets are indistinguishable (Rodríguez *et al.* 2014). The average number of genotypes per holdfasts was surprisingly high, with 11 out of 39 POs bearing at least five genotypes. Together with the observation that fusions were more frequent in LD than in HD areas, the results indicate that the occurrence of fusions is not a simple consequence of holdfast proximity. Therefore, fusions do not occur during the plants' ontogeny, but instead as a consequence of reduced space when plants grow and the increased distance between plants. Rodríguez *et al.* (2014) showed that the coalescence in *L. berteroana* is a continuous process in which recruits may fuse with other isolated recruits or groups, or even with adults and senescent plants.

This has been proved to explain the early benefits of coalescence in microscopic stages of red algae (Santelices *et al.* 1996; Santelices & Aedo 2006), which show a positive relationship between the number of coalescing spores and the probability of survivorship (Santelices *et al.* 1999; Santelices 2001, 2004). Small plants (*i.e.* fewer than five stipes) of *L. berteroana* were found at both densities, but at LD every small plant is formed of up to five multilocus genotypes, giving further support for the early selection of POs against unigenotypic organisms at LD. By contrast, high densities seem to result from the survival of both uni- and plurigenotypic organisms, at least during the early stages of kelp growth.

There are three main ways of coalescing at the microscopic stages of kelps: (i) aggregated settlement of spores within a few mm<sup>2</sup>, producing a single PO that is observable with the naked eye; (ii) recruitment of spores on top of established holdfasts; and (iii) vegetative ramification of the female gametophyte that then produces multiple

**Table 3.** Estimates of intra- and inter-plant pairwise relatedness R-values. The number of sampled plants with more than one genotype is given in parentheses after each R-value.

	SA-HD	SB-HD	SA-LD	SB-LD
Intra-plant pairwise R-values	0.046* (10)	0.215* (5)	0.058* (37)	0.065* (1)
	−0.006 (11)	0.094* (7)	0.030 (38)	0.094* (3)
	0.023 (24)	0.624* (8)	0.318* (39)	0.077* (4)
	0.124* (27)	0.233* (15)	0.058* (40)	0.174* (6)
	0.027 (28)	0.026* (16)	0.046* (41)	0.049* (13)
	0.024 (32)	0.300* (17)	0.892* (42)	0.033 (22)
	0.211* (33)	0.088* (19)	0.071* (43)	0.041 (29)
		0.360* (20)	−0.014 (44)	0.172* (31)
			0.077* (45)	−0.059 (34)
			0.283* (46)	0.084* (35)
			0.478* (47)	0.058* (39)
			0.941* (48)	0.338* (50)
One-tailed 95% confidence interval for inter-plant pairwise R-values	−0.123 to 0.029	−0.149 to 0.020	−0.169 to 0.046	−0.144 to 0.042

SA = site A; SB = site B; HD = high density; LD = low density.

\*Values significantly higher than expected from one-tailed 95% ( $P < 0.05$ ) confidence interval of mean inter-plant pairwise R-values.

eggs (Avila *et al.* 1985) that may be fertilized by different males. The present study was not designed to discriminate amongst these possibilities. However, two major findings of this study allow inferring on the causes of the fusions. On the one hand, aggregated settlement should vary amongst sites and be negatively correlated to population density in order to explain the higher occurrence of POs at LD than at HD. On the other hand, multiple paternities in a single ramified female gametophyte should create half-sib relatedness, increasing the degree of genetic relatedness amongst genotypes within a plant, compared with the population. Alternatively, both aggregated and non-aggregated settlements occur everywhere, but only closely settled gametophytes survive and reproduce. These points are discussed in the following sections.

#### Fusions of individuals as an environmentally mediated process

The occurrence of intra-specific fusion in *Lessonia berteriana* appears to be strongly correlated with environmental quality, as well as in other kelps (Malm & Kautsky 2004; Wernberg 2005). Indeed, differences in population density of *L. berteriana* result from environmental quality, which is heterogeneous, and optimal conditions favour high densities. However, the negative correlation between holdfast density and occurrence of POs strongly suggests that POs have certain advantages over non-POs in sub-optimal conditions, *i.e.* in low-density areas. Uni-genotypic organisms only occur at HD, accounting for 75% of the small plants with fewer than five stipes,

suggesting that optimal environmental conditions allow recruitment and survival of the different kinds of organisms (uni- and plurigenotypic), as opposed to LD. Similar results were obtained by Malm & Kautsky (2004) for *Fucus vesiculosus*, in which the proportion of fused individuals was greater in wave-exposed areas. In addition, interestingly, our ordination analyses showed that plant density could be effectively differentiated according to the morphology of the stipes. As suggested by Novoplansky (2009), plant morphology can be modified as a response to competition in contrasting densities.

Our results suggest that the quality of the environment for *L. berteriana* influences both the occurrence and composition of the POs and stipe-to-stipe intra-plant morphology. High density implies intra-specific competition for substrate and light. Concordantly, a positive correlation between mortality of recruits and plant density has been reported for other algae (*e.g.* of the genus *Fucus*, Steen & Scrosati 2004). As the presence of adult conspecifics inhibits recruitment (Santelices & Ojeda 1984), fusion poses an additional challenge to each individual genotype, by potentially adding intra-plant competition on top of intra-population competition. A precise evaluation of intra-specific competition between genets within and between plants is necessary to further understand the balance between environmental quality and intra-specific competition.

Finally, our results add complexity to demographic studies in *L. berteriana* that thus far have considered the whole plant as the individual entity (*e.g.* Ojeda & Santelices 1984; Santelices & Ojeda 1984). Our finding suggests that rather than the whole plant, it is important to con-

sider the stipes, which would take into account the possible differences amongst genets within a clump.

### Kin aggregation

An important result of this study is the observation that genotypes sharing a holdfast were more genetically related than on average in the population. This kin aggregation is not expected under the paradigm of stochastic spore settlement (*i.e.* with no choice of settlement site). However, kin aggregation is increasingly being detected in coastal species, including sessile (Veliz *et al.* 2006) and mobile invertebrates (Selkoe *et al.* 2006), providing evidence that mixing of propagules in the water column is less extensive than previously considered.

At least three non-exclusive hypotheses could explain this strong trend, each as post-settlement processes. First, higher *R*-values can result from fusions between sporophytes sharing the same mother and multiple fathers (siblings or half-siblings). Vegetative ramification and multiple egg production of the female gametophyte is a common process in Laminariales (Muñoz *et al.* 2004; Nelson 2004). To determine whether genotypes do correspond to full or half-sibs, a higher number of loci than studied here would be required in order to reduce the large sampling variance that lowers the precision of estimated relatedness (Lynch & Ritland 1999). Second, dispersal and settlement may be non-random. Kin-structured dispersal (the joint dispersal of seeds or juveniles that come from the same family or the same mother) is an example of process that leads to kin aggregation in the adult stage. It explains some cases of small-scale genetic structure in plants (Torimaru *et al.* 2007) and genetic patchiness in marine invertebrates (Johnson & Black 1982). Such dispersal modes have been reported in some seaweeds that bear unitary reproductive organs like the cystocarp in red algae, which can release bunches of spores surrounded by their maternal mucilage that keeps the spores together until settlement (Aedo 2007). This phenomenon seems unlikely to apply to kelps, which release motile spores individually that can hardly maintain proximity with their relatives because of the turbulence of the coastal waters where they are released.

The third hypothesis argues that dispersal and settlement are random but survival of the young sporophytes is determined by the genetic relatedness of the surrounding individuals. If fusions between genets are environmentally mediated, it is possible that aggregation between relatives provides higher benefits to the PO than non-kin aggregates would. This scenario is possible whenever intra-plant competition is reduced by the genetic relatedness of individuals sharing the holdfast. This is particularly noteworthy because it opens up a potential role of

selection in determining the occurrence of kin aggregations within plants. It is not necessarily kin selection as it is known for social animal species, particularly because the existence of high relatedness amongst interacting individuals is not by itself sufficient evidence that kin selection is the driving force (Griffin & West 2002). In the case of *L. berteriana*, genotypes within a kin aggregate may have a higher survival up to the adult stage than those within non-kin aggregates, as evidenced by the predominance of POs in LD areas, suggesting that kin aggregation brings fitness advantages over random interaction. In this context, every life history trait that favours the fusion between relatives to form a PO should be selected for, including traits at the group level (File *et al.* 2013). Such multi-level selection (Wilson 1997) on group-living traits in kelps is an interesting and testable hypothesis that emerges from the pattern observed in this study.

### Perspective: fusion of individuals as an adaptive strategy

Traditionally, *Lessonia berteriana* has been considered as a unitary, asexual species (*sensu* Santelices 1982), wherein each plant corresponds to one individual (*e.g.* Ojeda & Santelices 1984; Santelices & Ojeda 1984). Here, we have shown that one organism, made up of a single holdfast and a variable number of stipes and fronds can be composed of one or more individuals, and large kelps are most likely colonies of different genets. As several other inter-tidal species of red and green algae share this fusion capacity, it is possible that the phenomenon of coalescence in algae is an adaptation to highly stressful and heterogeneous environments such as the marine inter-tidal rocky shore. Direct benefits (*i.e.* higher survival, protection of the recruits) to the individual genet when integrated within a PO lead to the question whether fusion or coalescence is an adaptive strategy. This may be difficult to answer because it requires characterization of the traits of the PO that influence the individual genet, as well as the relative contribution of the traits of the PO and of the individual genet to the fitness of the genet. It also requires measurement of the exact costs and benefits of being part of a PO. So far, we have shown that the number of stipes of the PO is correlated with the number of genotypes, and that at LD stipes tend to be thinner than HD. The number of stipes is a good indicator of the reproductive potential for *L. berteriana* (Santelices & Ojeda 1984), and therefore the observed morphological differences could mean fitness differences in terms of reproductive success.

At the individual level, a higher number of stipes in a PO may indicate that the trade-off between sharing the holdfast and the opportunity to produce external structures (the stipes) is reduced, as opposed to a situation in



which the number of stipes was fixed by external factors. The predominance of POs in LD areas suggests that benefits largely exceed the eventual costs of sharing a holdfast, despite the plants tending to be smaller in LD than in HD areas. Better evaluation of these trade-offs (e.g. by quantifying the number of stipes that each genotype is able to produce within a shared holdfast, compared with a solitary holdfast) would allow an exact assessment of these costs and benefits at the individual level.

Santelices (1999) showed that genetic homogeneity and uniqueness as well as physiological autonomy of algae, are relevant criteria to define an individual. In this context, the limits of an individual, as a functional basic unit of organization, may vary according to the questions addressed, the approach developed and the model organism (Pepper & Herron 2008). For example, ramets, clones, colonies and clumps can be defined as individual organisms, although none of these would fit into the more traditional unitary organism concept (Santelices 1999). Although an important debate is still ongoing around universal definitions of individual and organism (Nachtomý *et al.* 2002; Santelices 1999; Pepper & Herron 2008 among others), the ecological and evolutionary implications of different ways of organization have received relatively little attention. The propensity of most species to form groups has been considered as an adaptation that may maximize the inclusive fitness of the individual genotype. The adaptation of these concepts traditionally used in social animals to non-animal models is a most challenging perspective of the study of plurigenotypic organisms.

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