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1 **On the advantage of sharing a holdfast: effects of density and**
2 **occurrence of kin aggregation in the kelp *Lessonia berteroana***

3

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21 **Running title:** Density and kin in kelp aggregations

22

23 **Keywords:** fusion of individuals; density dependent; genetic relatedness; kelp; kin
24 aggregation; kin selection; microsatellites

25 **Abstract**

26 Here we investigate the density-dependent and genetic relatedness that regulate the occurrence
27 of inter-individual (*genet*) fusion forming plurigenotypic organisms in the brown alga
28 *Lessonia berteroana*. Recruitment generally occurs at high densities in the intertidal, allowing
29 contact of neighbouring holdfasts as they grow and expand on the substrate. Algal density, on
30 the other hand, is regulated by the effects of herbivory and wave impact, which often lead to
31 low holdfast density. Herein, we investigated whether the occurrence of plurigenotypic
32 organisms and their genotypic composition (number of genotypes per plurigenotypic
33 organism) are density dependent and affected by kin selection in the intertidal kelp *L.*
34 *berteroana*. Four microsatellite loci were used to analyse DNA from 260 samples obtained
35 from shared and non-shared holdfasts, at two sites with high and two with low holdfast
36 density. Analyses showed that fusions forming plurigenotypic organisms are extremely
37 common. Interestingly, the frequency of fusions was higher in low-density sites, in which
38 100% of the plants had at least two genotypes while the average was 3.5. In high-density sites,
39 62% of plants were plurigenotypic, with an average of 2.8 genotypes per plant. Additionally,
40 we found that genotypes that shared a holdfast had a significantly higher genetic relatedness
41 than on average in the population, compatible with a kin structure. Density dependence and
42 kin structure suggest that the occurrence of plurigenotypic organisms is linked to
43 environmental quality, and that kin or multilevel selection may be favouring the fusion of
44 genetically related *genets*.

45 **Introduction**

46 Different units of relevance for ecological and evolutionary processes can be delineated,
47 such as the population, the family or group of related individuals, the colony, and the
48 individual organism. Because most of these units can actually be nested one into the other
49 (e.g. individuals into colonies or families, colonies into populations; see Nachtomy *et al.*
50 2002), the limits of the individual, as a basic ecological and evolutionary unit, are
51 sometimes difficult to define. In fact, individuals of many species live in groups into which
52 they find both protection against environmental stresses (e.g. predation) and closeness to
53 other individuals for reproduction. The case of clumps and colonies is particularly
54 interesting because the functional limits between the *genet*, issued from a single fertilized
55 egg, and the individual organism that is made of a mixture of different *genets*, are most
56 often **indistinguishable**. Such findings have stimulated important debate around the concept
57 and definition of the individual and the organism (e.g. Nachtomy *et al.* 2002; Santelices
58 1999; Pepper & Herron 2008). Clumps have been reported in a wide variety of taxa, some
59 of which are algal species (Santelices *et al.* 1996, 1999, 2003, Wernberg 2005; González &
60 Santelices 2008). In red algae, fusion of individuals results in a chimeric, plurigenotypic
61 organism (PO), with complete loss of individual identity (Santelices *et al.* 1996, 1999;
62 Paine 1990). This process, known as coalescence in red and green algae (not formally
63 described in brown algae), is difficult to study in natural populations because it occurs
64 mainly at early microscopic stages (e.g. spores or sporelings; Santelices *et al.* 1996, 1999;
65 Santelices & Aedo 2006). As a consequence, the factors that regulate the fusion of *genets*
66 have scarcely been explored.

67 Two non-exclusive hypotheses can be proposed to explain the occurrence of fusions

68 at the holdfast level in algal species: (1) fusions are correlated with density of recruits
69 which, during their development, grow and eventually get in such a close contact with their
70 neighbours, that they fuse together (i.e. they integrate their cell lines into a single tissue), or
71 (2) fusions are regulated by fitness differences between clumped and isolated genets. In the
72 first case, fusions are just a density-dependent process whereas selection and adaptation can
73 be invoked in the second hypothesis. Evidence based on higher survival rate of aggregates
74 compared to isolated individuals of red and brown algae suggest a selective advantage of
75 clumping (Santelices & Aedo 2006, Santelices & Alvarado 2008, Wernberg 2005). For
76 instance, holdfast aggregations of the kelp *Ecklonia radiata* have been observed in higher
77 frequency in exposed areas than in more protected ones, suggesting that aggregation
78 reduces mortality from dislodgement in exposed areas (Wernberg 2005).

79 Based on field observations of tagged sporophytes, Vásquez *et al.* (2008) and
80 Rodríguez *et al.* (2014) showed that fusions of individuals forming a PO might be recurrent
81 in the kelp *Lessonia berteroana* Montagne (formerly *L. nigrescens*, González *et al.* 2012).
82 This species dominates the low intertidal rocky shores of Chile and southern Peru
83 (Hoffmann & Santelices 1997) and its structure consists of a massive holdfast attached to
84 the rocky substrate. A variable number of stipes emerge from the holdfast. Stipes have
85 branches and each branch carries one frond distally. Holdfasts grow vertically and
86 horizontally leading to contact between neighbours that progressively grown and their
87 tissues mix forming a single unit that externally mimicks a single organism (Vásquez *et al.*
88 2008). A simple expectation from this growth pattern is that the rate at which *genets* fuse
89 increases with density (Rodríguez *et al.* 2014). However, if fusions confer some kind of
90 advantage under stressful environmental conditions, then the occurrence of fusions should

91 increase when the environment moves away from optimal conditions for the species.

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

95 In *L. berteroana*, density variability, spatial distribution, establishment of new
96 recruits, mortality rates, and growth patterns are regulated mainly by herbivory, wave
97 impact and intraspecific competition for hard substrate (Ojeda & Santelices 1984;
98 Santelices & Ojeda 1984). The strength of these three selective agents differs in association
99 with distance between holdfasts. Net recruitment of *L. berteroana* is reduced in the
100 presence of herbivores (e.g. chitons and urchins) (Camus 1994; Ojeda & Santelices 1984),
101 while distance between holdfasts enhances herbivory (Vásquez & Santelices 1990). This
102 patterns result in a general negative relationship between kelp coverage and herbivore
103 abundance (Broitman *et al.* 2001), and pinpoint herbivory as a strong density dependent
104 selective pressure on *L. berteroana*. Wave impact is another major cause of mortality in
105 benthic algae (Dayton *et al.* 1984; Vadas *et al.* 1990) that is considered a selective agent
106 driving morphological and physiological adaptation (Vásquez 1992; Blanchette 1997;
107 Martínez & Santelices 1998). Wave impact also imposes a high dynamic pressure that can
108 produce dislodgement or removal of settled plants (Vadas *et al.* 1990, 1992). In close
109 holdfast proximity, the interaction between water motion and the shape of the stipes and
110 fronds produce a wave movement known as the Whiplash Effect (WE, *sensu* Dayton 1994).
111 Because of the WE, high holdfast density is beneficial for the kelp populations, allowing
112 the persistence of new recruits (Ojeda & Santelices 1984). Vásquez (1995) showed that
113 when distance between holdfasts exceeds 2 m, the WE is reduced (i.e. environment is less

114 protective), increasing herbivory. Thus, both herbivory and WE have density-dependent
115 effects in both intertidal and subtidal *Lessonia* species (e.g. *L. trabeculata*, Vásquez 1992;
116 Vásquez & Buschmann 1997) that lead to greater survival of plants at high holdfast density.

117 High holdfast density implies intraspecific competition for substrate and light,
118 limiting the settlement of new recruits (Santelices & Ojeda 1984; Andrew & Viejo 1998;
119 Steen & Scrosati 2004). In this context, fusions between conspecifics may be seen as a
120 way to increase individual density without increasing substrate occupancy and to avoid
121 intraspecific competition. This kind of strategy has been found more frequently between
122 kin than unrelated individuals (Gerlach *et al.* 2007, Lizé *et al.* 2012). For example, plants
123 effectively experience strong social interactions and kin recognition to varying
124 physiological and morphological responses depending on the identity of the neighbour (de
125 Kroon 2007, Biedrzycki *et al.* 2010, Wu *et al.* 2013). However, as the Hamilton's (1964)
126 rule predicts, fusions may be an adaptive trait only if the benefits overpass the costs, and
127 close contact among individuals within a holdfast is likely to induce strong competition
128 among them (Novoplansky 2009). This cost can probably be reduced if fusion occurs
129 among relatives, so that benefits are expressed in terms of inclusive fitness (i.e. the direct
130 contribution of each genotype to the next generation's gene pool plus their indirect
131 contribution through the progeny of their relatives). Therefore, if fusion between relatives
132 enhances survival rate, then positive kin selection is a likely explanation (File *et al.* 2013).
133 In this context fusions between relatives in *L. berteroana* would be more frequent in low
134 environmental qualities (i.e. low density) in were isolated individuals (unigenotypic
135 organisms) may not persist.

136 The goal of this study was to shed light onto the factors that regulate the fusion of

137 *genets* into a single organism, by assessing genetic diversity of holdfasts at low and high
138 plant density and the genotypic relatedness within plants in the kelp *L. berteroana*.

139

140 **Material and Methods**

141

142 Sampling, field measurements, and DNA extraction

143

144 Tissues of *L. berteroana* were sampled in an extensive intertidal kelp bed in Lagunillas,
145 Chile (30°06'S, 71°22'W) on areas showing no evidence of recent kelp harvest. In order to
146 test for density dependence, twelve plants of different sizes were sampled in two sites of
147 low holdfast density (LD: 0.5 to 1 holdfasts/m²) and two of high holdfast density (HD: 5 to
148 8 holdfasts/m²). We use the term plant to refer to the organism identifiable macroscopically
149 on the shore, and which may be made of either a single or multiple *genets*.

150 From each of the 48 sampled plants, six tissue samples were collected each from a
151 different stipe selected at random, except for some small plants (i.e. less than six stipes). In
152 these cases, the samples were taken from all the available stipes (at least 5). Fresh tissue
153 samples of 2 cm in diameter were collected from the base of fronds avoiding reproductive
154 tissue and epiphytes. Collected pieces were dried using paper towel, placed in sealed
155 individual bags with silica gel, and stored at room temperature until DNA extraction. DNA
156 was extracted from 30-40 mg of dried powdered tissue using a slightly modified version of
157 the Cetyltrimethyl Ammonium Bromide (CTAB) DNA extraction that adds Polyvinyl
158 Pyrolidone to discard polyphenols (Martínez *et al.* 2003). Extracted DNA was quantified in
159 a spectrophotometer (NanoDrop Technologies) and kept at -20°C.

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

166

167 Informative microsatellite loci determination

168

169 To evaluate which loci were informative for the study, ten DNA samples from different
170 plants were randomly picked to explore eight microsatellite loci available for *L. berteroana*
171 (Faugeron *et al.* 2009). PCR's were performed in a final volume of 20 µl with: 1.5 ng of
172 DNA, 2 mM of 10X Buffer, 0.6 mM of dNTP mix, 1.8 mM of MgCl₂, 0.2 mM of each
173 primer, 0.075 mg/mL of Bovine Serum Albumin, and 0.2 U of *Taq* DNA polymerase
174 (Fermentas). Temperature cycling consisted of an initial soak of three min at 95 °C, then 10
175 touchdown cycles at 95 °C for 45 sec, 60 °C- 63 °C for 45 sec, and an extension of 45 sec
176 at 72 °C. Following, 30 cycles at 95 °C for 45 sec, 50 °C for 45 sec, 72 °C for 45 sec and a
177 final extension of 72 °C for 7 min. Amplicons were genotyped in an ABI Prism 3700
178 (Applied Biosystems) and the electrophenograms were visualized using GeneMarker v.
179 1.81 (SoftGenetics PA, USA).

180 The main criteria for considering loci as informative were the degree of
181 polymorphism in the local population and PCR amplification success. After checking for
182 polymorphism, genotypes were analysed in Microchecker 2.2 (Van Oosterhout *et al.* 2004)

183 to determine the probability of null alleles. The probability of finding twice the same
184 multilocus genotype by chance in each site of each density was calculated with Genealex v.
185 6 (Peakall & Smouse 2006). It was considered a sufficient number of informative loci when
186 each of the ten analysed DNA samples had different multilocus genotypes.

187

188 Multilocus genotype data analyses

189

190 Any holdfast with more than one multilocus genotype was considered a plurigenotypic
191 organism (PO). The effect of size and density was analysed using Generalized Linear
192 Mixed Models (GLMMs) with Poisson error implemented in the lme4 package in the
193 software R (R Core Team 2013). GLMMs are appropriate for non-normal data influenced
194 by fixed and random effects (Bolker *et al.* 2008; Crawley 2005; Grueber *et al.* 2011). In
195 each model, effects and interaction of plant-to plant morphometric values (MV) (holdfast
196 diameter, total length, and total number of stipes) and density (high and low denoted as LD
197 and HD, respectively) in the occurrence of POs was evaluated using each of the three MV
198 as covariables with the Site (St) nested into Density. The correlation between plant length
199 and holdfast diameter is well known in *Lessonia* (Santelices 1982; Vásquez & Santelices
200 1984; Vásquez 1991), however, the correlation of these variables and the number of stipes
201 is not consistent and only occurs when reproductive stipes are considered (Vásquez 1991).
202 Finally, differences in the number of genotypes between densities were evaluated in a
203 single GLMM using density (D) as categorical variable following the same structure than
204 previous models.

205 Strictly, site has a random effect because all levels of that factor are selected as a

206 random sample from all possible levels (sites) that could be included in the study area
207 (spatial pseudoreplication). So additionally, as an exploratory analysis of the relative
208 importance of the factors in the models, according to the evidence weight of the AIC, the
209 site effect was evaluated as explanatory variable with fixed effects, and in interaction with
210 variables that GLMMs showed to have a significant effect on number of genotypes.

211 Lastly, Principal Component Analyses (PCA) and Lineal Discriminant Analysis
212 (LDA) were carried out with the log-transformed data of the three genet-to-genet variables
213 (length, diameter of the stipe and number of dichotomies) and the three plant-to-plant MV
214 variables (holdfast diameter, total length, number of stipes). The objective of these analyses
215 was to observe possible differences in both densities considering genet-to-genet variance as
216 a way to include the morphological differences within the plant and plant-to-plant
217 differences between densities. Both analyses were carried out using MASS package, and
218 the 95% confident ellipses where calculated with ELLIPSE package, both available in
219 CRAN R project (R Core Team 2013).

220

221 Relatedness among fused genets

222 To investigate the occurrence of kin aggregations within a PO, we tested whether
223 genotypes sharing a holdfast were genetically closer than on average in the population. This
224 was achieved by comparing the average pairwise coefficient of relatedness R (Ritland
225 1996) of each plant with the average inter-plant pairwise R . The upper limit of the one-
226 tailed 95% confidence interval for inter-plant R -values for each site was determined by
227 bootstrapping in R (R Core Team 2013) 10,000 values of mean R among ten randomly

228 sampled (with replacement) pairwise R -values obtained from pairs of genotypes from
229 different plants. Observed intra-plant mean R -values that were higher than the 95 % limit
230 were considered representing a significantly higher genetic relatedness than the background
231 population.

232

233 **Results**

234

235 Informative microsatellite loci identification

236

237 Of the eight explored microsatellite loci, four (Less1T11, Less2D22, Less2D25 and
238 Less2D26) were polymorphic enough to unequivocally identify each of the 10 preliminary
239 sampled individuals, and thus were selected to characterize all samples. The probability of
240 finding twice the same multilocus genotype by chance using these four loci was 9.07×10^{-5}
241 ± 0.0004 and 0.001 ± 0.020 for LD and HD, respectively.

242

243 Fusion of individuals in *Lessonia berteroa*

244

245 Of the 260 tissue samples analysed, from 48 plants, 150 corresponded to different
246 individuals based on multilocus genotypes, which correspond to a total of 39 plurigenotypic
247 organisms (81.3% of the sampled holdfasts). There was an average of 3.17 multilocus
248 genotypes per plant, of a maximum of six that could be detected with the sampling scheme.
249 Only 18.5 % of the sampled holdfasts showed only one multilocus genotype. On the
250 opposite, 22.9 % showed at least five different multilocus genotypes, including small kelps

251 (e.g., all five stipes with a different multilocus genotype) (Fig. 1).

252 The evaluation of the effects of plant-to-plant MV using GLMM's revealed that
253 neither total length nor holdfast diameter influenced on the number of genotypes per
254 holdfast ($df: 41, p= 0.1130$ and 0.1891 , respectively), even when interacting with density
255 ($df: 41, p= 0.0970$ and 0.0879 , respectively) (Table 1). Interestingly, the number of
256 genotypes varied significantly with the number of stipes per holdfast ($df: 41, p = 0.00721$)
257 with a significant effect of density in this model ($df: 41, p = 0.00261$), although the
258 interaction of density with the MV was not significant ($df: 41, p= 0.15932$) (Table 1). The
259 effect of the size, particularly with the number of stipes as response variable was significant
260 only in HD, while in LD there was no relationship (Fig. 2). Moreover, holdfast density had
261 a significant effect in the average number of genotypes per holdfast ($df: 41, p = 0.0036$)
262 (Table 1). In LD 100% of the holdfasts were plurigenotypic, whereas only 62.5 % at HD.
263 The average number of genotypes was also greater in LD, with 3.54 genotypes/holdfast,
264 versus 2.79 genotypes/holdfast in HD. This suggests that plant density explains the
265 observed data better than MV; a marginal association between number of stipes and
266 number of genotypes can be observed only in HD (Fig. 2).

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

273 In the PCA analyses, using genet-to-genet morphological variables, the first two axes

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

288

289 Relatedness analyses

290

291 From a total of 39 POs, 15 in HD and 24 in LD, 30 (i.e. 77%) displayed an intra-plant mean
292 relatedness significantly higher than expected given the inter-plant pairwise relatedness in
293 the population (Table 3). From these 30 holdfasts, 66.6% occurred in HD and 79.16% in
294 LD. There was strong heterogeneity in intra-plant relatedness: site A in HD (SA-HD) had
295 the lowest number of plants (three out of seven) with significantly higher mean relatedness
296 than population level, whereas every PO in the site B (SB-HD) displayed significantly

297 higher intra-plant mean R (Table 3). In LD, where relatedness is more common, most intra-
298 holdfast pairwise R -values are significant with only two and three POs from SA-LD and
299 SB-LD, respectively, with values within the expected range of inter-plant values.

300

301 **Discussion**

302

303 Occurrence of plurigenotypic holdfasts

304

305 Our results indicate that the formation of plurigenotypic organisms (POs) in *L. berteroana*
306 is a highly frequent phenomenon. Thirty-nine of the forty-eight analysed plants bared at
307 least two different genets. As for a number of red and green coalescing algae (Santelices *et*
308 *al.* 1999; 2003), fusion of *L. berteroana* at the holdfast level results in a single macroscopic
309 organism in which the different genets are indistinguishable (Rodriguez *et al.* 2014). The
310 average number of genotypes per holdfasts was surprisingly high, with 11 out of 35 POs
311 bearing at least 5 genotypes. Together with the observation that fusions were more frequent
312 in low-density (LD) than in high-density (HD) areas, the results indicate that the occurrence
313 of fusions is not a simple consequence of holdfast proximity. Therefore, fusions do not
314 occur during the plants' ontogeny, but instead as a consequence of reduced space when
315 plants grow and the increased distance between plants. Rodriguez *et al.* (2014), shows that
316 the coalescence in *L. berteroana* is a continuous process in which recruits may fuse with
317 other isolated recruits, groups or even in and between adults and senescent plants
318 (Rodriguez *et al.* 2014).

319 This has been proved to explain the early benefits of coalescence in microscopic stages of

320 red algae (Santelices *et al.* 1996; Santelices & Aedo 2006), which show a positive
321 relationship between the number of coalescing spores and the probability of survivorship
322 (Santelices *et al.* 1999; Santelices 2001; 2004). Small plants (i.e. less than 5 stipes) of *L.*
323 *berteroana* were found at both densities, but in LD every small plant is formed of up to five
324 multilocus genotypes, giving further support for the early selection of PO's against
325 unigenotypic organisms in LD. On the other hand, high densities seem to result from the
326 survival of both uni- and pluri-genotypic organisms, at least during early stages.

327 There are three main ways of coalescing at the microscopic stages of kelps: (1)
328 aggregated settlement of spores within a few mm² producing a single PO when observable
329 by naked eye; (2) recruitment of spores on top of established holdfasts; and (3) vegetative
330 ramification of the female gametophyte that then produces multiple eggs (Avila *et al.*
331 1985) that could be fertilized by different males. This study was not designed to
332 discriminate among these possibilities. However, two major findings of this study allow
333 inferring on the causes of the fusions. On one hand, aggregated settlement should vary
334 among sites and be negatively correlated to population density in order to explain the
335 higher occurrence of PO in LD than in HD. On the other hand, multiple paternities in a
336 single ramified female gametophyte should create half-sibs relatedness, increasing the
337 degree of genetic relatedness among genotypes within a plant, compared to the population.
338 Alternatively, both aggregated and non-aggregated settlements occur everywhere, but only
339 closely settled gametophytes survive and reproduce. These points are discussed in the
340 following sections.

341

342 Fusions of individuals as an environmentally mediated process

343

344 The occurrence of intraspecific fusion in *L. berteroana* seems strongly correlated with
345 environmental quality, as well as in other kelps (Wernberg 2005, Malm & Kausky
346 2004). Indeed, differences in population density of *L. berteroana* result from environmental
347 quality, which is heterogeneous, and optimal conditions favour high densities. However,
348 the negative correlation between holdfast density and occurrence of POs strongly suggests
349 that POs have certain advantages over non-PO in sub-optimal conditions, i.e. in low-density
350 areas. Unigenotypic organisms only occur at HD accounting for 75% of the small plants
351 with less than five stipes, suggesting that the optimal environmental conditions allow
352 recruitment and survival of the different kinds of organisms (uni- and plurigenotypic), as
353 opposed to LD. Similar results were obtained by Malm & Kausky (2004) in *Fucus*
354 *vesiculosus* in which the proportion of fused individuals was greater in wave exposed areas.
355 In addition to that, our ordination analyses interestingly showed that plant density could be
356 effectively differentiated according to the morphology of the stipes. As is suggested by
357 Novoplasnky (2009), plant morphology can be modified as a response to competition in
358 contrasting densities.

359 Our results suggest that the quality of the environment on *L. berteroana* influences
360 both the occurrence and composition of the POs and the stipe-to-stipe intra-plant
361 morphology. High density implies intraspecific competition for substrate and light.
362 Concordantly, a positive correlation between mortality of recruits and plant density has
363 been reported for other algae (e.g. of the genus *Fucus*, Steen & Scrosati 2004). As the
364 presence of adult conspecific inhibit recruitments (Santelices & Ojeda 1984), fusion poses
365 an additional challenge to each individual genotype, by potentially adding intra-plant

366 competition on top of intra-population competition. A precise evaluation of intra-specific
367 competition between genets within and between plants is necessary to further understand
368 the balance between both the environmental quality and the intraspecific competition.

369 Finally, our results add complexity to demographic studies in *L. berteroana* that
370 thus far have considered the whole plant as the individual entity (e.g. Santelices & Ojeda
371 1984, Ojeda & Santelices 1984). Our finding suggests that rather than the whole plant, it is
372 important to consider the stipes, which take into account possible differences between
373 genets into the clump.

374

375 Kin aggregation

376

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

384 At least three non-exclusive hypotheses could explain this strong trend, each as
385 post-settlement processes. First, higher *R-values* can result from fusions between
386 sporophytes sharing the same mother and multiple fathers (siblings or half-siblings).
387 Vegetative ramification and multiple egg production of the female gametophyte is a
388 common process in Laminariales (Muñoz *et al.* 2004; Nelson 2004). To determine whether

389 genotypes do correspond to full or half-sibs, a higher number of loci would be required in
390 order to reduce the large sampling variance that lowers the precision of estimated
391 relatedness (Lynch & Ritland 1999). Second, dispersal and settlement may be non-random.
392 Kin-structured dispersal (the joint dispersal of seeds or juveniles that come from a same
393 family or a same mother) is an example of process that leads to kin aggregation in the adult
394 stage. It explains some cases of small-scale genetic structure in plants (Torimaru *et al.*
395 2007) and genetic patchiness in marine invertebrates (Johnson & Black 1982). Such
396 dispersal modes have been reported in some seaweeds that bear unitary reproductive organs
397 like the cystocarp in red algae, which can release bunches of spores surrounded by their
398 maternal mucilage that keeps the spores together until settlement (Aedo 2007). This
399 phenomenon seems unlikely to apply to kelps, which release motile spores individually that
400 can hardly maintain proximity with their relatives because of the turbulence of the coastal
401 waters were they are released.

402 The third hypothesis argues that dispersal and settlement are random but survival of
403 the young sporophytes is determined by the genetic relatedness of the surrounding
404 individuals. If fusions between genets are environmentally mediated, it is possible that the
405 aggregation between relatives provides higher benefits to the PO than non-kin aggregates
406 would. This scenario is possible whenever intra-plant competition is reduced by the genetic
407 relatedness of individuals sharing the holdfast. This is particularly noteworthy because it
408 opens to a potential role of selection in determining the occurrence of kin aggregations
409 within plants. It is not necessarily kin selection, as it is known for social animal species,
410 particularly because the existence of high relatedness between interacting individuals is not
411 by itself sufficient evidence that kin selection is the driving force (Griffin & West 2002). In

412 the case of *L. berteroana*, genotypes within a kin aggregate may have a higher survival up
413 to the adult stage than those within non-kin aggregates, as evidenced by the predominance
414 of PO in low-density areas, suggesting that kin aggregation brings fitness advantages over
415 random interaction. In this context, every life history trait that favours the fusion between
416 relatives to form PO should be selected for, including traits at the group level (File *et al.*
417 2013). Such multilevel selection (Wilson 1997) on group-living traits in kelps is an
418 interesting and testable hypothesis that emerges from the pattern observed in this study.

419

420 Perspective: Fusion of individuals as an adaptive strategy

421

422 Traditionally, *L. berteroana* has been considered as a unitary, aclonal species (*sensu*
423 Santelices 1982), wherein each plant corresponds to one individual (e.g. Ojeda & Santelices
424 1984; Santelices & Ojeda 1984). Here, we showed that one organism, made of a single
425 holdfast and a variable number of stipes and fronds can be composed of one or more
426 individuals, and large kelps are most likely colonies of different genets. As several other
427 intertidal species of red or green algae share this fusion capacity, it is possible that the
428 phenomenon of coalescence in algae is an adaptation to highly stressful and heterogeneous
429 environments such as the marine intertidal rocky shore. Direct benefits (i.e. higher survival,
430 protection of the recruits) to the individual *genet* when integrated within a PO lead to the
431 question whether fusion or coalescence is an adaptive strategy. This may be difficult to
432 answer because it requires characterizing traits of the PO that influence the individual
433 *genet*, as well as the relative contribution of PO's traits and individual genets traits to the
434 fitness of the *genet*. It requires also measuring the exact costs and benefits of being part of a

435 PO. So far, we have shown that the number of stipes of the PO is correlated with the
436 number of genotypes, and in LD stipes tend to be thinner than HD. The number of stipes is
437 a good indicator of the reproductive potential in *L. berteriana* (Santelices & Ojeda 1984),
438 and therefore the observed morphological differences could mean fitness differences in
439 terms of reproductive success.

440 At the individual level, the higher number of stipes in PO may indicate that the trade-off
441 between sharing the holdfast and the opportunity to produce external structure (the stipes)
442 is reduced, as opposed to a situation in which the number of stipes would be fixed by
443 external factors. The predominance of POs in low density areas suggests that benefits
444 largely exceed the eventual costs of sharing a holdfast, despite that plants tends to be
445 smaller in low- than in high-density areas. Better evaluation of these trade-offs (e.g. by
446 quantifying the number of stipes that each genotype is able to produce within a shared
447 holdfast, compared to a solitary holdfast) would allow an exact assessment of these costs
448 and benefits at the individual level.

449 Santelices (1999) showed that genetic homogeneity and uniqueness as well as
450 physiological autonomy of algae, are relevant criteria to define an individual. In this
451 context, the limits of an individual, as a functional basic unit of organization, may vary
452 according to the questions and approaches that focus on the model organism (Pepper &
453 Herron 2008). For example, ramets, clones, colonies and clumps can be defined as
454 individual organisms, although none of these would fit into the more traditional unitary
455 organism concept (Santelices 1999). While an important debate still holds around universal
456 definitions of individual and organism (Nachtomy *et al.* 2002; Santelices 1999, Pepper &
457 Herron 2008 among others), the ecological and evolutionary implications of different ways

458 of organization have received relatively little attention. The propensity of most species to
459 form groups has been considered as an adaptation that may maximize the inclusive fitness
460 of the individual-genotype. The adaptation of these concepts traditionally used in social
461 animals to non-animal models is a most challenging perspective of the study of
462 plurigenotypic organisms.

463

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470

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639

640 **Table captions**

641

642 **Table 1.** Summary of the GLMM models using morphometric values of the plants. Table
643 shows the result of comparing high holdfast density (HD) with the other levels of the
644 factor. Ng: Number of genotypes; D: Density; St: sites; AIC: Akaike Information Criterion.
645 * Significant values ($p < 0.05$).

646

647 **Table 2:** Summary of the GLMM model testing the effect of Density (D) on the Number of
648 Genotypes (Ng). St: sites; AIC: Akaike values; k: number of variables. * Significant values
649 ($p < 0.05$).

650

651 **Table 3.** Estimates of intra- and inter-plant pairwise relatedness R -values. The name of the
652 sampled plant with more than one genotype is between brackets after each R -value. *
653 Indicates values significantly higher than expected from one-tailed 95% confidence interval
654 of mean inter-plant pairwise R -values.

655

656 **Figure Captions**

657

658 **Fig. 1.** Box-plot of number of multilocus genotypes per plant detected at two sites with
659 high (SA-HD and SB-HD) and two with low holdfast densities (SA-LD and SB-LD) in
660 *Lessonia berteriana*. Dashed horizontal line indicates total average of multilocus
661 genotypes in both densities.

662

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

665

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

670

671 **Table 1.** Summary of the GLMM models using morphometric values of the plants. Table
 672 shows the result of comparing high holdfast density (HD) with the other levels of the
 673 factor. Ng: Number of genotypes; D: Density; St: sites; AIC: Akaike Information Criterion.
 674 * Significant values ($p < 0.05$).

Model	Estimate	Std. Error	Z value	p value
Ng = D x Number of Stipes + St(D) (AIC: 35.79)				
Intercept	0.601	0.191	3.137	>0.001
Density (HD-LD)	0.780	0.259	3.010	0.003*
Number of stipes	0.018	0.006	2.687	0.007*
Density High-Low x Number of stipes	-0.018	0.013	-1.407	0.159
Ng = D x Total length + St(D) (AIC: 37.96)				
Intercept	0.527	0.297	1.772	0.076
Density (HD-LD)	0.609	0.367	1.660	0.097
Total length	0.003	0.002	1.585	0.113
Density High-Low x Total length	-0.001	0.002	-0.376	0.706
Ng = D x Holdfast diameter + St(D) (AIC: 38.14)				
Intercept	0.558	0.322	1.730	0.083
Density High-Low	0.622	0.364	1.706	0.087
Holdfast diameter	0.028	0.021	1.313	0.189
Density High-Low x Holdfast diameter	-0.011	0.023	-0.466	0.641

693

694

695 **Table 2:** Summary of the GLMM model testing the effect of Density (D) on the Number of
 696 Genotypes (Ng). St: sites; AIC: Akaike values; k: number of variables. * Significant values
 697 (p < 0.05)

Model	Estimate	Std. Error	Z value	698 p value
Ng= D + St(D) (AIC: 22.2,df= 5, k = 3)				
Intercept	0.9328	0.1280	7.286	699 < 0.001*
Density	0.4535	0.1637	2.769	0.006*

700 **Table 3.** Estimates of intra- and inter-plant pairwise relatedness *R*-values. The name of the
 701 sampled plant with more than one genotype is between brackets after each *R*-value. *
 702 Indicates values significantly higher than expected from one-tailed 95% confidence interval
 703 of mean inter-plant pairwise *R*-values.

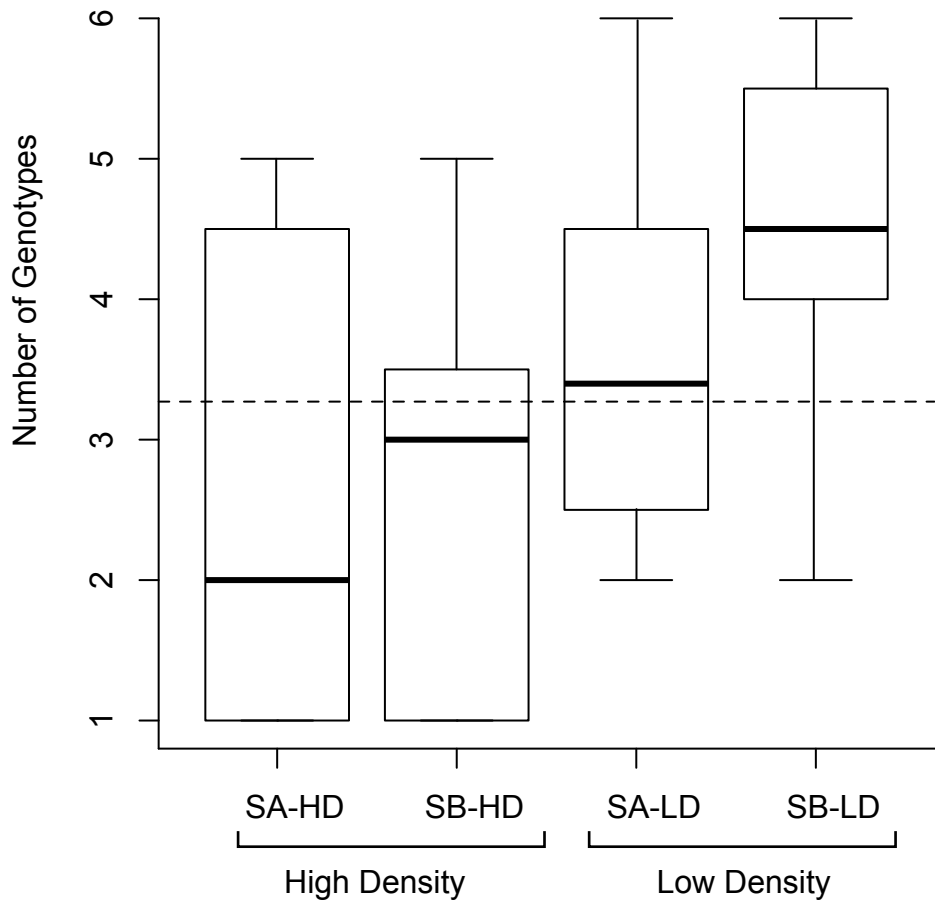
	SA-HD	SB-HD	SA-LD	SB-LD
Intra-plant pairwise <i>R</i> -values	0.046 * (10)	0.215 * (5)	0.058 * (37)	0.065 * (1)
	-0.006 (11)	0.094 * (7)	0.030 (38)	0.094 * (3)
	0.023 (24)	0.624 * (8)	0.318 * (39)	0.077 * (4)
	0.124 * (27)	0.233 * (15)	0.058 * (40)	0.174 * (6)
	0.027 (28)	0.026 * (16)	0.046 * (41)	0.049 * (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 <i>R</i> -values	-0.123 – 0.029	-0.149 – 0.020	-0.169 – 0.046	-0.144 – 0.042

704

705

706

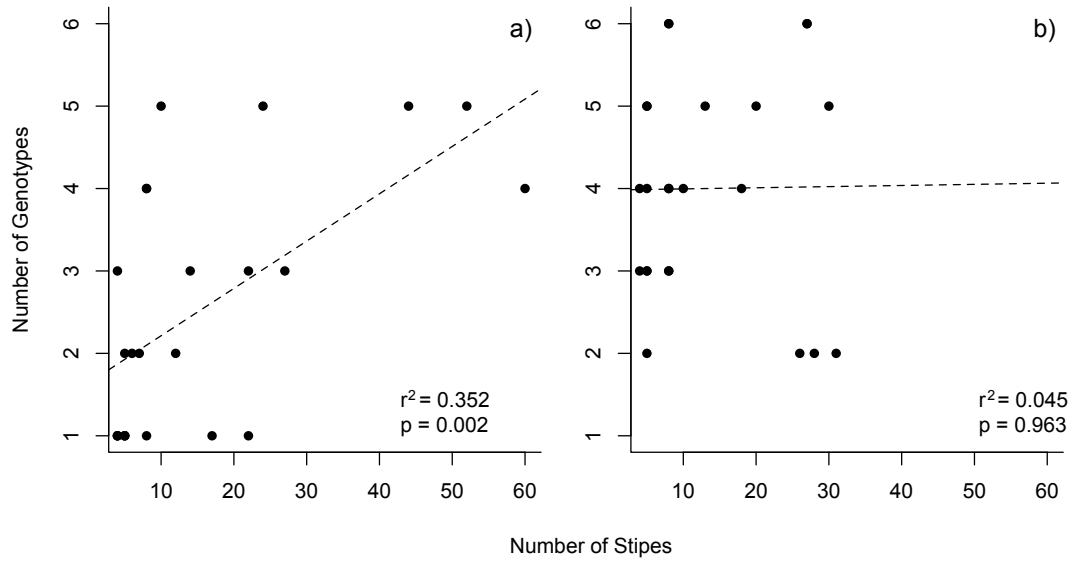
Figure 1



707

708

709 Figure 2



710

711

