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

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Abstract

Here we investigate the density-dependent and genetic relatedness that regulate the occurrence of inter-individual (*genet*) fusion forming plurigenotypic organisms in the brown alga *Lessonia berteroana*. Recruitment generally occurs at high densities in the intertidal, allowing contact of neighbouring holdfasts as they grow and expand on the substrate. Algal density, on the other hand, 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 intertidal kelp *L. berteroana*. 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 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 while 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 on 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. Because 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 into 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. Nachtomy *et al.* 2002; Santelices 1999; 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 (Santelices *et al.* 1996, 1999; Paine 1990). 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: (1) fusions are correlated with density of recruits which, during their development, grow and eventually get in such a close contact with their neighbours, that they fuse together (i.e. they integrate their cell lines into a single tissue), or (2) 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 rate of aggregates compared to isolated individuals of red and brown algae suggest a selective advantage of clumping (Santelices & Aedo 2006, Santelices & Alvarado 2008, Wernberg 2005). For instance, holdfast aggregations of the kelp *Ecklonia radiata* have been observed in higher frequency 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 berteroana* Montagne (formerly *L. nigrescens*, González *et al.* 2012). This species dominates the low intertidal 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 grown and their tissues mix forming a single unit that externally mimicks 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. berteroana* under two contrasting density of holdfasts in one natural population.

In *L. berteroana*, density variability, spatial distribution, establishment of new recruits, mortality rates, and growth patterns are regulated mainly by herbivory, wave impact and intraspecific competition for hard substrate (Ojeda & Santelices 1984; Santelices & Ojeda 1984). The strength of these three selective agents differs in association with distance between holdfasts. Net recruitment of *L. berteroana* is reduced in the presence of herbivores (e.g. chitons and urchins) (Camus 1994; Ojeda & Santelices 1984), while distance between holdfasts enhances herbivory (Vásquez & Santelices 1990). This 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. berteroana*. Wave impact is another major cause of mortality in benthic algae (Dayton *et al.* 1984; 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 produce 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 produce a wave movement known as the Whiplash Effect (WE, *sensu* Dayton 1994). Because of the WE, high holdfast density is beneficial for the kelp populations, allowing the persistence of new recruits (Ojeda & Santelices 1984). Vásquez (1995) showed that when distance between holdfasts exceeds 2 m, the WE is reduced (i.e. environment is less

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

High holdfast density implies intraspecific 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 intraspecific 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 the Hamilton's (1964) rule predicts, fusions may be an adaptive trait only if the benefits overpass the costs, and close contact among individuals within a holdfast is likely to induce strong competition among them (Novoplansky 2009). This cost can probably be reduced if fusion occurs among 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. berteroana* would be more frequent in low environmental qualities (i.e. low density) in were 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 density and the genotypic relatedness within plants in the kelp *L. berteroana*.

Material and Methods

Sampling, field measurements, and DNA extraction

Tissues of *L. berteroana* were sampled in an extensive intertidal 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, twelve plants of different sizes were sampled in two sites of low holdfast density (LD: 0.5 to 1 holdfasts/m²) and two of high holdfast density (HD: 5 to 8 holdfasts/m²). 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. less than six stipes). In these cases, the samples were taken from all the available stipes (at least 5). 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 (CTAB) DNA extraction that adds Polyvinyl Pyrolidone to discard polyphenols (Martínez *et al.* 2003). Extracted DNA was quantified in a spectrophotometer (NanoDrop Technologies) 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, ten DNA samples from different plants were randomly picked to explore eight microsatellite loci available for *L. berteroana* (Faugeron *et al.* 2009). PCR's were performed in a final volume of 20 µl with: 1.5 ng of DNA, 2 mM of 10X Buffer, 0.6 mM of dNTP mix, 1.8 mM of MgCl₂, 0.2 mM of each primer, 0.075 mg/mL of Bovine Serum Albumin, and 0.2 U of *Taq* DNA polymerase (Fermentas). Temperature cycling consisted of an initial soak of three min at 95 °C, then 10 touchdown cycles at 95 °C for 45 sec, 60 °C- 63 °C for 45 sec, and an extension of 45 sec at 72 °C. Following, 30 cycles at 95 °C for 45 sec, 50 °C for 45 sec, 72 °C for 45 sec and a final extension of 72 °C for 7 min. Amplicons were genotyped in an ABI Prism 3700 (Applied Biosystems) and the electrophenograms were visualized using GeneMarker v. 1.81 (SoftGenetics 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 twice the same multilocus genotype by chance in each site of each density was calculated with Genealex v. 6 (Peakall & Smouse 2006). It was considered a sufficient number of informative loci when each of the ten analysed DNA samples had different multilocus genotypes.

Multilocus genotype data analyses

Any holdfast with more than one multilocus genotype was considered a plurigenotypic organism (PO). The effect of size and density was analysed using Generalized Linear Mixed Models (GLMMs) with Poisson error 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 (Bolker *et al.* 2008; Crawley 2005; Grueber *et al.* 2011). In each model, effects and interaction of plant-to plant morphometric values (MV) (holdfast diameter, total length, and total number of stipes) and density (high and low denoted as LD and HD, respectively) in the occurrence of POs was evaluated using each of the three MV as covariables with the Site (St) nested into Density. The correlation between plant length and holdfast diameter is well known in *Lessonia* (Santelices 1982; Vásquez & Santelices 1984; Vásquez 1991), however, the correlation of these variables and the number of stipes is not consistent and only occurs when reproductive stipes are considered (Vásquez 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 than previous models.

Strictly, site has 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 evidence weight of the AIC, the site effect was evaluated as explanatory variable with fixed effects, and in interaction with variables that GLMMs showed to have a significant effect on number of genotypes.

Lastly, Principal Component Analyses (PCA) 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 MASS package, and the 95% confident ellipses were calculated with ELLIPSE package, both available in CRAN R project (R Core Team 2013).

Relatedness among 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 among ten 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 representing 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 twice the same multilocus genotype by chance using these four loci was $9.07 \times 10^{-5} \pm 0.0004$ and 0.001 ± 0.020 for LD and HD, respectively.

Fusion of individuals in *Lessonia berteroana*

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. On the opposite, 22.9 % showed at least five different multilocus genotypes, including small kelps

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

The evaluation of the effects of plant-to-plant MV using GLMM's revealed that neither total length nor holdfast diameter influenced on 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 response variable was significant only in HD, while in LD there was no relationship (Fig. 2). Moreover, holdfast density had a significant effect in the average number of genotypes per holdfast (df : 41, p = 0.0036) (Table 1). In LD 100% of the holdfasts were plurigenotypic, whereas only 62.5 % at HD. The average number of genotypes was also greater in LD, with 3.54 genotypes/holdfast, versus 2.79 genotypes/holdfast in HD. This suggests that plant density explains the observed data better than MV; a marginal association between number of stipes and number of genotypes can be observed only in HD (Fig. 2).

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), 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 by the three variables that had similar loadings, and the second axis was mainly of stipe diameter, suggesting that the three genet-to-genet variables are associated to morphological variation between densities. The PCA showed two groups with an overlap of few genets that were significantly differentiated (one-way ANOVA; $F_{1,258}=52.59$, $p<10^{-12}$) (Fig. 3). In this analysis, results showed that genets in LD are in general smaller than in HD. The trend is more evident for stipe diameter which tends to be thinner in LD than HD. The LDA approach, with one canonical discriminant function (i.e. two classes HD-LD), showed that the stipe diameter was the variable with the highest discriminant coefficient. This discriminant function was capable to correctly assign 89.7% of the samples to their density. Wrong predictions were in almost all the cases (except for one) for HD samples assigned as LD. On the other hand, 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 the cases.

Relatedness analyses

From a total of 39 POs, 15 in HD and 24 in 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 in HD and 79.16% in LD. There was strong heterogeneity in intra-plant relatedness: site A in HD (SA-HD) had the lowest number of plants (three out of seven) with significantly higher mean relatedness than population level, whereas every PO in the site B (SB-HD) displayed significantly

higher intra-plant mean R (Table 3). In LD, where relatedness is more common, most intra-holdfast pairwise R -values are 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 plurigenotypic organisms (POs) in *L. berteroana* is a highly frequent phenomenon. Thirty-nine of the forty-eight analysed plants bared 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 (Rodriguez *et al.* 2014). The average number of genotypes per holdfasts was surprisingly high, with 11 out of 35 POs bearing at least 5 genotypes. Together with the observation that fusions were more frequent in low-density (LD) than in high-density (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. Rodriguez *et al.* (2014), shows that the coalescence in *L. berteroana* is a continuous process in which recruits may fuse with other isolated recruits, groups or even in and between adults and senescent plants (Rodriguez *et al.* 2014).

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. less than 5 stipes) of *L. berteriana* were found at both densities, but in LD every small plant is formed of up to five multilocus genotypes, giving further support for the early selection of PO's against unigenotypic organisms in LD. On the other hand, high densities seem to result from the survival of both uni- and pluri-genotypic organisms, at least during early stages.

There are three main ways of coalescing at the microscopic stages of kelps: (1) aggregated settlement of spores within a few mm² producing a single PO when observable by naked eye; (2) recruitment of spores on top of established holdfasts; and (3) vegetative ramification of the female gametophyte that then produces multiple eggs (Avila *et al.* 1985) that could be fertilized by different males. This study was not designed to discriminate among these possibilities. However, two major findings of this study allow inferring on the causes of the fusions. On one hand, aggregated settlement should vary among sites and be negatively correlated to population density in order to explain the higher occurrence of PO in LD than in HD. On the other hand, multiple paternities in a single ramified female gametophyte should create half-sibs relatedness, increasing the degree of genetic relatedness among genotypes within a plant, compared to 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 intraspecific fusion in *L. berteroana* seems strongly correlated with environmental quality, as well as in other kelps (Wernberg 2005, Malm & Kausky 2004). Indeed, differences in population density of *L. berteroana* 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-PO in sub-optimal conditions, i.e. in low-density areas. Unigenotypic organisms only occur at HD accounting for 75% of the small plants with less than five stipes, suggesting that the 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 & Kausky (2004) in *Fucus vesiculosus* in which the proportion of fused individuals was greater in wave exposed areas. In addition to that, our ordination analyses interestingly showed that plant density could be effectively differentiated according to the morphology of the stipes. As is suggested by Novoplasnky (2009), plant morphology can be modified as a response to competition in contrasting densities.

Our results suggest that the quality of the environment on *L. berteroana* influences both the occurrence and composition of the POs and the stipe-to-stipe intra-plant morphology. High density implies intraspecific 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 conspecific inhibit recruitments (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 both the environmental quality and the intraspecific competition.

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

Kin aggregation

An important result of this study was 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 the 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), evidencing 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 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 a same family or a 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 were 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 the 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 to 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 between interacting individuals is not by itself sufficient evidence that kin selection is the driving force (Griffin & West 2002). In

the case of *L. berteroana*, 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 PO in low-density 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 PO should be selected for, including traits at the group level (File *et al.* 2013). Such multilevel 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, *L. berteroana* has been considered as a unitary, aclonal species (*sensu* Santelices 1982), wherein each plant corresponds to one individual (e.g. Ojeda & Santelices 1984; Santelices & Ojeda 1984). Here, we showed that one organism, made 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 intertidal species of red or 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 intertidal 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 characterizing traits of the PO that influence the individual *genet*, as well as the relative contribution of PO's traits and individual genets traits to the fitness of the *genet*. It requires also measuring 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 in LD stipes tend to be thinner than HD. The number of stipes is a good indicator of the reproductive potential in *L. berteriana* (Santelices & Ojeda 1984), and therefore the observed morphological differences could mean fitness differences in terms of reproductive success.

At the individual level, the higher number of stipes in PO may indicate that the trade-off between sharing the holdfast and the opportunity to produce external structure (the stipes) is reduced, as opposed to a situation in which the number of stipes would be fixed by external factors. The predominance of POs in low density areas suggests that benefits largely exceed the eventual costs of sharing a holdfast, despite that plants tends to be smaller in low- than in high-density 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 to 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 and approaches that focus on 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). While an important debate still holds around universal definitions of individual and organism (Nachtomy *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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References

- Aedo D. (2007) Esporas de algas rojas coalescentes y no coalescentes: diferencias funcionales y factores ambientales afectado la coalescencia. *Doctoral Thesis, Departamento de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.*
- Andrew N.L., Viejo R.M. (1998) Effects of wave exposure and intraspecific density on the growth and survivorship of *Sargassum muticum* (Sargassaceae: Phaeophyta). *European Journal of Phycology*, **33**, 251-258.
- Avila M., Hoffmann A.J., Santelices B. (1985) Interacciones de temperatura, densidad de flujo fotónico y fotoperiodo sobre el desarrollo de etapas microscópicas de *Lessonia*

481 *nigrescens* (Phaeophyta, Laminariales). *Revista Chilena de Historia Natural*, **58**, 71-
482 82.

483 Biedrzycki M., Jilany T., Dudley S., Bais H. (2010) Root exudates mediate kin recognition
484 in plants. *Communicative & Integrated Biology*, **3**, 1–8.

485 Blanchette C.A. (1997) Size and survival of intertidal plants in response to wave action: A
486 case study with *Fucus gardneri*. *Ecology*, **78**, 1563-1578.

487 Bolker B.M., Brooks M.E., Clarck C.J., Geange S.W., Poulsen J.R., Henry M., White J.S.S.
488 (2008) Generalized linear mixed models: a practical guide for ecology and evolution.
489 *Trends in Ecology and Evolution*, **24**, 127-135.

490 Broitman B.R., Navarrete S.A., Smith F., Gaines S.D. (2001) Geographic variation of
491 southeastern Pacific intertidal communities. *Marine Ecology Progress Series*, **224**,
492 21-34.

493 Camus P. (1994) Recruitment of intertidal kelp *Lessonia nigrescens* Bory in northern Chile:
494 successional constraints and opportunities. *Journal of Experimental Marine Ecology*,
495 **184**, 171-181.

496 Crawley M.J. (2005) Statistics: an introduction using R. Wiley, New York

497 Dayton P.K. (1974) Experimental evaluation of ecological dominance in a rocky intertidal
498 algal community. *Ecological Monographs*, **45**, 137-159.

499 Dayton P.K., Currie V., Gerrodette T., Keller B., Ronsenthal R., Tresca D.V. (1984) Patch
500 dynamics and stability of some southern California kelp communities. *Ecological*
501 *Monographs*, **54**, 253-289.

502 de Kroon H. (2007) Ecology: how do roots interact? *Science*, **318**, 1562–1563.

503 Faugeron S., Véliz D., Peralta G., Tapia J., Tellier F., Billot C., Martínez E. (2009)
 504 Development and characterization of nine polymorphic microsatellite markers in the
 505 Chilean kelp *Lessonia nigrescens*. *Molecular Ecology Resources*, **9**, 937-939.
 506 Gerlach G., Hodgins-Davis A., MacDonald B., Hannah R. (2007) Benefits of kin
 507 association: related and familiar zebrafish larvae (*Danio rerio*) show improved
 508 growth. *Behavioral Ecology and Sociobiology*, **61**, 1765–1770.
 509 González A.V., Santelices B. (2008) Coalescence and chimerism in *Codium* (Chlorophyta)
 510 from central Chile. *Phycologia*, **47**, 468–476.
 511 González A., Beltrán J., Hiriart-Bertrand L., Flores V., de Reviers B., Correa J.A.,
 512 Santelices B. (2012) Identification of cryptic species in the *Lessonia nigrescens*
 513 complex (Phaeophyceae, Laminariales). *Journal of Phycology*, **48**, 1153–1165.
 514 Griffin A.S., West S.A. (2002) Kin selection: fact and fiction. *Trends in Ecology and*
 515 *Evolution*, **17**, 15-21.
 516 Grueber C.E., Nakagawa S., Laws R.J., Jamieson I.G. (2011) Multimodel inference in
 517 ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology*, **24**,
 518 699–711.
 519 Hamilton W.D. (1964) The genetical evolution of social behaviour. I. *Journal of*
 520 *Theoretical Biology*, **7**, 1–16.
 521 Hoffmann A.J., Santelices B. (1997) Flora Marina de Chile Central. *Ediciones Pontificia*
 522 *Universidad Católica de Chile*, Santiago.
 523 Johnson M.S., Black R. (1982) Chaotic genetic patchiness in an intertidal limpet,
 524 *Siphonaria* sp. *Marine Biology*, **70**, 157-164.
 525 Lizé A., Khidr S.K., Hardy I.C.W. (2012) Two components of kin recognition influence

526 parasitoid aggression in resource com- petition. *Animal Behaviour*, **83**, 793–799.

527 Lynch M., Ritland K. (1999) Estimation of pairwise relatedness with molecular markers.

528 *Genetics*, **152**, 1753–1766.

529 Malm T., Kautsky L. (2004) Are Bladderwrack (*Fucus vesiculosus* L.) holdfasts that

530 support several fronds composed of one or several genetic individuals? *Aquatic*

531 *Botany*, **80**, 221–226.

532 Martínez E., Santelices B. (1998) Selective mortality on haploid and diploid microscopic

533 stages of *Lessonia nigrescens* Bory (Phaeophyta, Laminariales. *Journal of*

534 *Experimental Marine Biology and Ecology*, **229**, 219–239.

535 Martínez E., Cárdenas L., Pinto R. (2003) Recovery and genetic diversity of the intertidal

536 kelp *Lessonia nigrescens* (Phaeophyceae) 20 years after EL NIÑO 1982/83. *Journal*

537 *of Phycology*, **39**, 504–508.

538 Muñoz V., Hernandez-Gonzalez M.C., Buschmann A., Graham M.H., Vásquez J.A. (2004)

539 Variability in per capita oogonia and sporophyte production from giant kelp

540 gametophytes (*Macrocystis pyrifera*, Phaeophyceae). *Revista Chilena de Historia*

541 *Natural*, **77**, 639–647.

542 Nachtomy O., Shavit A., Smith J. (2002) Leibnizian organisms, nested individuals, and

543 units of selection. *Theory in Biosciences*, **121**, 205–230.

544 Nelson W.A. (2004) Life history and growth in culture of the endemic New Zealand kelp

545 *Lessonia variegata* J. Agardh in response to differing regimes of temperature,

546 photoperiod and light. *Journal of Applied Phycology*, **17**, 23–28.

547 Novoplansky A. (1998) Picking battles wisely: plant behaviour under competition. *Plant,*

548 *Cell and Environment*, **32**, 726–741.

549 Ojeda F.P., Santelices B. (1984) Ecological dominance of *Lessonia nigrescens*
550 (Phaeophyta) in central Chile. *Marine Ecology Progress Series*, **16**, 83-91.

551 Paine R. (1990) Benthic macroalgal competition: Complications and consequences. *Journal*
552 *of Phycology*, **26**, 12-17.

553 Peakall R., Smouse P. (2006) GENEALEX 6: genetic statistic analysis in Excel. Population
554 genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288-295.

555 Pepper J.W., Herron M.D. (2008) Does Biology Need an Organism Concept? *Biological*
556 *Reviews*, **83**, 621-627.

557 R Core Team. (2013) R: a language and environment for statistical computing. *R*
558 *Foundation for Statistical Computing*. Vienna, Austria. <http://www.R-project.org/>.

559 Ritland K. (1996) Estimators for pairwise relatedness and individual inbreeding
560 coefficients. *Genetics Research*, **67**, 175-185.

561 Rodriguez D, Oróstica M.H, Vásquez J.A. (2014) Coalescence in wild organisms of the
562 intertidal population of *Lessonia berteroana* in northern Chile: management and
563 sustainability effects. *Journal of Applied Phycology*, **26**, 1115–1122

564 Santelices B. (1982) Bases biológicas para el manejo de *Lessonia nigrescens*
565 (Phaeophyta Laminariales) en Chile Central. *Monografías Biológicas*, **2**, 135 –150.

566 Santelices B. (1999) How many kind of individuals are there? *Trends in Ecology and*
567 *Evolution*, **14**, 152-155.

568 Santelices B. (2001) Implications of clonal and chimeric-type thallus organization on
569 seaweed farming and harvesting. *Journal of Applied Phycology*, **13**, 153-160.

570 Santelices B. (2004) A comparison of ecological responses among aclonal (unitary), clonal
571 and coalescing macroalgae. *Journal of Experimental Marine Biology and Ecology*,

572 **300**, 31-64.

573 Santelices B., Aedo D., Hormazabal M., Flores V. (2003) Field testing of inter- and
574 intraspecific coalescence among mid-intertidal red algae. *Marine Ecology Progress*
575 *Series*, **250**, 91-103.

576 Santelices B., Aedo D. (2006) Group recruitment and early survival of *Mazzaella*
577 *laminarioides*. *Journal of Applied Phycology*, **18**, 583-589.

578 Santelices B., Alvarado L. (2008) Demographic consequences of coalescence in sporeling
579 populations of *Mazzaella laminaroides* (Gigartinales, Rhodophyta). *Journal of*
580 *Applied Phycology*, **44**, 624-636.

581 Santelices B., Correa J., Meneses I., Aedo D., Varela D. (1996) Sporeling coalescence and
582 intraclonal variation in *Gracilaria chilensis* (Gracilariales, Rhodophyta). *Journal of*
583 *Phycology*, **32**, 313-322.

584 Santelices B., Correa J., Aedo D., Flores V., Hormazabal M., Sanchez P. (1999)
585 Convergent biological processes in coalescing Rhodophyta. *Journal of Phycology*,
586 **35**, 1127-1149.

587 Santelices B., Ojeda F.P. (1984) Recruitment, growth and survival of *Lessonia nigrescens*
588 (Phaeophyta) at various tidal levels in exposed habitats of central Chile. *Marine*
589 *Ecology Progress Series*, **19**, 73-82.

590 Selkoe K.A., Gaines S.D., Caselle J.E., Warner R.R. (2006) Current shifts and kin
591 aggregation explain genetic patchiness in fish recruits. *Ecology*, **87**, 3082-3094.

592 Steen H., Scrosati R. (2004) Intraspecific competition in *Fucus serratus* and *F. evanescens*
593 (Phaeophyceae: Fucales) germings: effects of settlement density, nutrient
594 concentration, and temperature. *Marine Biology*, **144**, 61-70.

595 Torimaru T., Tani N., Tsumura Y., Nishimura N., Tomaru N (2007) Effects of kin-
 596 structured seed dispersal on the genetic structure of the clonal dioecious shrub *Ilex*
 597 *leucoclada*. *Evolution*, **61**, 1289-1300.

598 Vadas R.L., Wright A.W., Miller S.L. (1990) Recruitment of *Ascophyllum nodosum*: wave
 599 action as a source of mortality. *Marine Ecology Progress Series*, **61**, 263-272.

600 Vadas R.L., Johnson S., Norton T.A. (1992) Recruitment and mortality of early t-settlement
 601 stages of benthic algae. *British Phycological Journal*, **27**, 331-351.

602 Van Oosterhout C., Hutchinsnson W.F., Willis D.P.M., Shipley P. (2004) Micro-checker:
 603 software for identifying and correcting genotyping errors in microsatellite data.
 604 *Molecular Ecology Notes*, **4**, 535-538.

605 Vásquez J.A. (1991) Variables morfométricas y relaciones morfológicas de *Lessonia*
 606 *trabeculata* Villouta & Santelices 1984, en poblaciones submareales del Norte de
 607 Chile. *Revista Chilena de Historia Natural*, **64**, 271-279.

608 Vásquez J.A. (1992) *Lessonia trabeculata*, a subtidal bottom kelp in northern Chile: a case
 609 of study for a structural and geographical comparison. In Coastal communities of
 610 Latin America. U. Seeliger (Ed) Academic Press Inc., San Diego.

611 Vásquez J.A. (1995) Ecological effect of brown seaweed harvesting. *Botanica Marina*, **38**,
 612 251-257.

613 Vásquez J.A., Buschmann A. (1997) Herbivory-kelp interactions in subtidal Chilean
 614 communities: a review. *Revista Chilena de Historia Natural*, **70**, 41-52.

615 Vásquez J.A., Santelices B. (1984) Comunidades de macroinvertebrados en discos de
 616 adhesion de *Lessonia nigrescens* en Chile central. *Revista Chilena de Historia*
 617 *Natural*, **57**, 131-154.

618 Vázquez J.A., Santelices B. (1990) Ecological effects of harvesting *Lessonia* (Laminariales,
619 Phaeophyta) in central Chile. *Hydrobiologia*, **204**, 41-47.

620 Vázquez J.A., Tala F., Vega J.M.A., Zuñiga S., Edding M., Piaget N. (2008) Bases
621 ecológicas y evaluación de usos alternativos para el manejo de praderas de algas
622 pardas de la III y IV regiones. *Final Report FIP 2005-22*.

623 Veliz D., Duchesne P., Bourget E., Bernatchez L. (2006) Genetic evidence for kin
624 aggregation in the intertidal acorn barnacle (*Semibalanus balanoides*). *Molecular*
625 *Ecology*, **15**, 4193–4202.

626 Venegas M., Tala F., Fonck E., Vázquez J.A. (1992) Sporangial sori on stipes of *Lessonia*
627 *nigrescens* Bory (Laminareacea, Phaeophyta): a high frequency phenomenon in
628 intertidal populations of northern Chile. *Botanica Marina*, **35**, 573-578.

629 Wernberg T., Kendrick G.A., Toohey B.D. (2005) Modification of the physical
630 environment by an *Ecklonia radiata* (Laminariales) canopy and implications for
631 associated foliose algae. *Aquatic Ecology*, **39**, 419-430.

632 Wernberg T. (2005) Holdfast aggregation in relation to morphology, age, attachment and
633 drag for the kelp *Ecklonia radiata*. *Aquatic Botany*, **82**, 168-180.

634 Wilson D.S. (1997) Introduction: multilevel selection comes of age. *American Naturalist*,
635 **150**, S1-4.

636 Wu C.C., Diggle P.K., Friedman W.E. (2013) Kin recognition within a seed and the effect
637 of genetic relatedness of an endosperm to its compatriot embryo on maize seed
638 development. *Proceedings of the National Academy of Sciences*, **110**, 2217-2222.

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Table captions

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

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

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

Figure Captions

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

Fig. 2. Relationship between the number of multilocus genotypes per plant and the number of stipes in 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 PCA 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 for low density and orange points for high density; the 95% confidence ellipses for each group are shown.

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

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

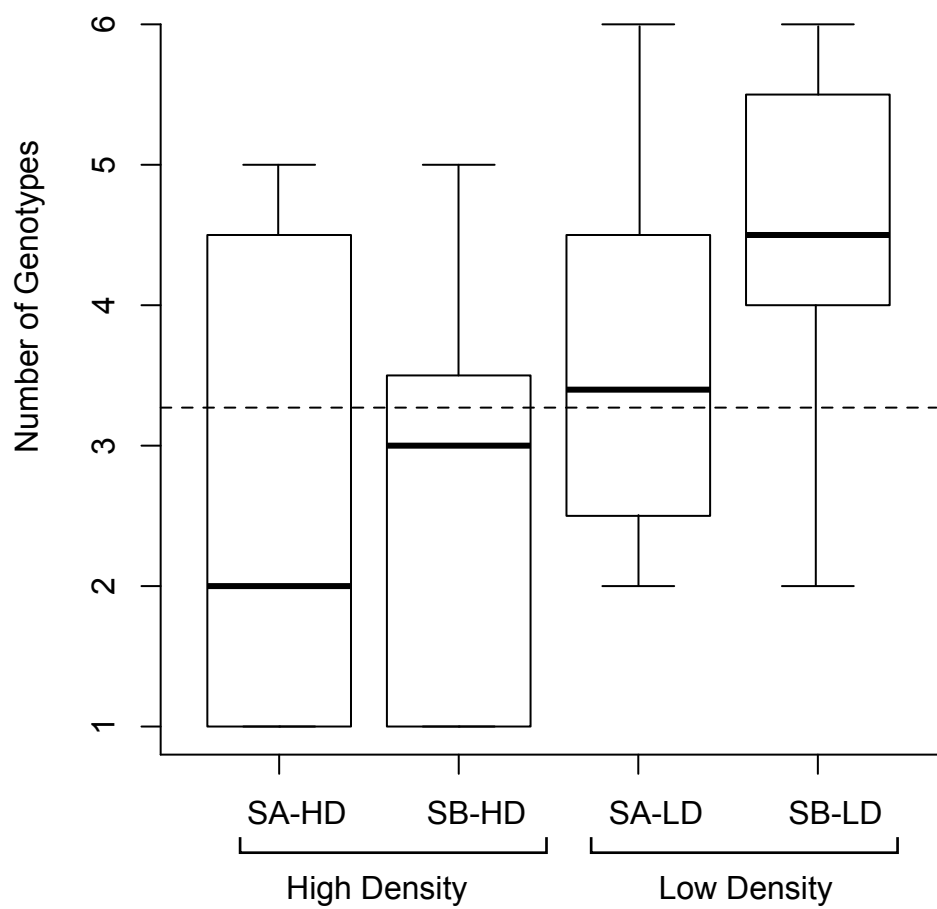
Model	Estimate	Std. Error	Z value	p value ⁶⁹⁸
Ng= D + St(D) (AIC: 22.2,df= 5, k = 3)				
Intercept	0.9328	0.1280	7.286	⁶⁹⁹ < 0.001*
Density	0.4535	0.1637	2.769	0.006*

Table 3. Estimates of intra- and inter-plant pairwise relatedness *R*-values. The name of the sampled plant with more than one genotype is between brackets after each *R*-value. * Indicates values significantly higher than expected from one-tailed 95% confidence interval 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) -0.006 (11) 0.023 (24) 0.124 * (27) 0.027 (28) 0.024 (32) 0.211 * (33)	0.215 * (5) 0.094 * (7) 0.624 * (8) 0.233 * (15) 0.026 * (16) 0.300 * (17) 0.088 * (19) 0.360 * (20)	0.058 * (37) 0.030 (38) 0.318 * (39) 0.058 * (40) 0.046 * (41) 0.892 * (42) 0.071 * (43) -0.014 (44) 0.077 * (45) 0.283 * (46) 0.478 * (47) 0.941 * (48)	0.065 * (1) 0.094 * (3) 0.077 * (4) 0.174 * (6) 0.049 * (13) 0.033 (22) 0.041 (29) 0.172 * (31) -0.059 (34) 0.084 * (35) 0.058 * (39) 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

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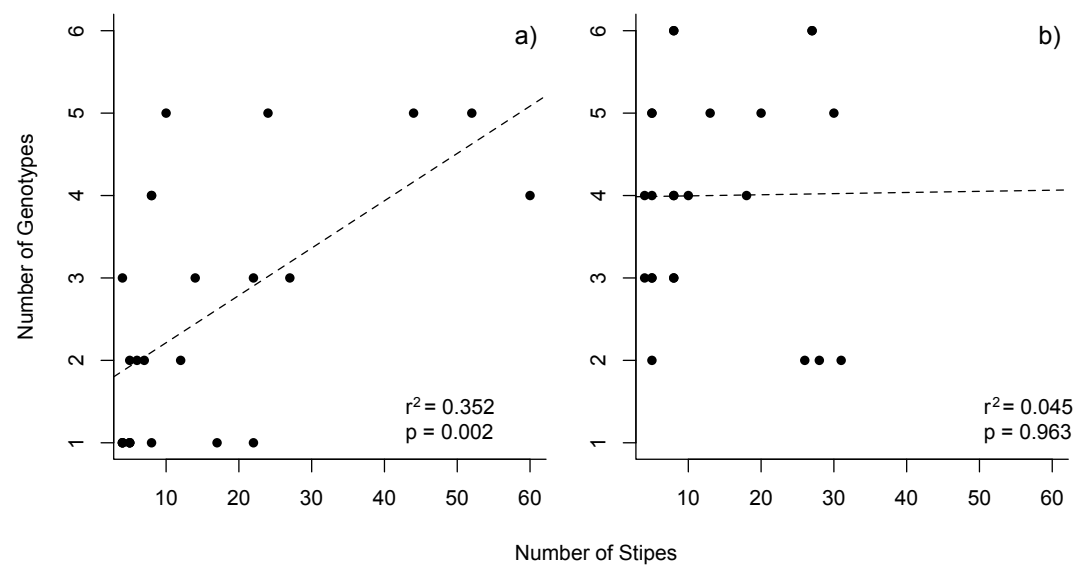
Figure 1



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709 Figure 2



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