Unraveling the complexity of salt marsh "*Fucus cottonii*" forms (Phaeophyceae, Fucales)

Kjersti Sjøtun[1*], Svenja Heesch[2#], Jordi Rull Lluch[3], Rafael Martín Martín[3], Amelia Gómez Garreta[3], Anne K. Brysting[4], James A. Coyer[5]

[1] University of Bergen, Department of Biology, PO Box 7803, 5020 Bergen, Norway

[2]Irish Seaweed Research Group, Ryan Institute, National University of Ireland Galway, University Road, Galway, Ireland

[3] University of Barcelona, Laboratory of Botany, Faculty of Pharmacy & Biodiversity Research institute (IrBIO), Av. Joan XXIII s/n, 08028, Barcelona, Spain

[4] University of Oslo, Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, PO Box 1066 Blindern, 0316 Oslo, Norway

[5] Shoals Marine Laboratory, University of New Hampshire, Morse Hall 113, 8 College Road, Durham, NH 03824, USA

[#]current address: Station Biologique de Roscoff, UMR 8227, Place Georges Teissier, 29680 Roscoff, France

* Corresponding author

Running title: Salt marsh "*Fucus cottonii*" forms

Abstract

Genetic affiliation, nuclear DNA content, and gamete functioning were examined in small salt marsh *Fucus* from three localities in western Ireland. Individuals with small and dioecious receptacles were found at all localities, but production of germlings was only evident from those at Locality 1. Here, the *Fucus* vegetation formed a morphological cline from *F. vesiculosus* with bladders in the mid-intertidal to small *Fucus* individuals lacking bladders in the salt marsh of the upper intertidal. Measurements of nuclear DNA content ranged from 1-1.8 pg at this locality, with the *F. vesiculosus* individuals in the lower range. At the two other localities, the small salt marsh *Fucus* formed distinct morphological entities. Microsatellite analyses revealed that the small salt marsh *Fucus* individuals from Locality 2 were derived mainly from *F. vesiculosus*, whereas those from Locality 3 were hybrids between *F. vesiculosus* and *F. spiralis* with greatest affiliation to *F. spiralis*. While the small salt marsh *Fucus* forms from Locality 2 had high nuclear DNA content (ca. 4 pg) and were probably octoploids, the small salt marsh *Fucus* from Locality 3 formed two groups: one with high (3.9-4.6 pg) and one with low (1.5-1.9 pg) nuclear DNA content. Nuclear DNA content measured in individuals from Locality 3 varied between 1.1-2.8 pg in *F. vesiculosus* and 2-3.5 pg in *F. spiralis*, and showed a more or less stepwise increase in both species, consistent with polyploidy. We hypothesize that the small salt marsh *Fucus* forms originate from genome size changes in the parental taxa.

Key words: *Fucus cottonii*, *Fucus spiralis*, *Fucus vesiculosus*, hybridization, nuclear DNA content, polyploidy

INTRODUCTION

The brown macroalgal genus *Fucus* is remarkably adaptive and consequently, has evolved into several species in the North Atlantic since the migration of ancestral forms from the North Pacific across the Bering Strait (Cánovas *et al*., 2011). Two main lineages are apparent within the genus, one consisting of *F. distichus* L. and *F. serratus* L. and the other one comprising several species, including *F. spiralis* L. and *F. vesiculosus* L., all of which are widespread on shores of the North Atlantic. (Cánovas *et al*., 2011). *Fucus vesiculosus* and *F. spiralis* are closely related and frequently hybridize (Engel *et al*., 2005), and two new European species within the *F. vesiculosus* - *F. spiralis* lineage have been recognized: *F. radicans* L.Bergstrøm & L.Kautsky from the Baltic Sea (Bergström *et al*., 2005) and *F. guiryi* G.I.Zardi, K.R.Nicastro, E.S.Serrão & G.A.Pearson, distributed from Ireland and Britain to the Canary Islands (Zardi *et al*., 2011). *Fucus radicans* has evolved in sympatry with *F. vesiculosus* after the Last Glacial Maximum in Europe (Pereyra *et al*., 2009), whereas *F. guiryi* (*F. spiralis*-south in Coyer *et al*., 2011) is a sister species of *F. spiralis* (Cánovas *et al*., 2011).

A miniaturized *Fucus* form without holdfasts and occurring only in high intertidal salt marshes has been described as *F. cottonii* M.C.Wynne & Magne (Wynne & Magne, 1991). Another small *Fucus* species, *F. chalonii* Feldmann, shares a number of similarities with *F. cottonii* but is not associated with salt marsh habitats and only occurs in a few localities on rocky shores in north Spain (Feldmann, 1941). The small (1-5 cm tall) *F. chalonii* is distinguished from the small salt marsh forms by always being attached and by forming verrucose receptacles.

The small salt marsh form of *F. cottonii*, however, does not represent one genetic entity. Molecular studies have demonstrated that it can be a hybrid between *F. vesiculosus* and *F. spiralis* in the USA and Iceland (Wallace *et al*., 2004, Coyer *et al*., 2006), and either a polyploid

form of *F. vesiculosus* (Coyer *et al*., 2006) or derived from *F. spiralis* (Neiva *et al*., 2012) in Ireland. Miniaturized salt marsh forms have also been observed in *F. distichus* from the *F. distichus – F. serratus* lineage, suggesting that small salt marsh fucoids represent convergent growth forms (Neiva *et al*., 2012). However, while growth and morphology of small salt marsh *F. cottonii* was shown to vary with tidal level in a transplant study in a salt marsh locality in Maine (USA) (Mathieson *et al*., 2006), high stress does not fully explain the habit of these small salt marsh fucoids. For example, *Fucus* individuals with normal morphology are often found growing side by side with the miniaturized forms (Fig. 1), and the latter retain their miniaturized morphology when incubated several weeks in culture with normal salt water (K. Sjøtun, pers. obs.).

A characteristic feature of small salt marsh *F. cottonii* (hereafter abbreviated ssm*F*) is their vegetative reproduction, by which side branches eventually form new, but genetically identical individuals (Cotton, 1912). However, ssm*F* from Ireland occasionally develop receptacles, and the original description of *F. cottonii* (as *F. vesiculosus* var. *muscoides* Cotton) included individuals with small and dioecious receptacles (Cotton, 1912). On the other hand, receptacles have not been observed in ssm*F* in France (Wynne & Magne, 1991), the USA (Mathieson *et al*., 2006) or in Norway (K. Sjøtun, pers. obs.).

The findings of Coyer *et al*. (2006) and Neiva *et al*. (2012) that the ssm*F* forms can have multiple independent origins, suggest some common processes leading to a miniaturized growth form. Coyer *et al*. (2006) showed that ssm*F* could display at least partial genome duplication, suggesting that processes involved in genome size changes can be an underlying cause for miniaturization of *Fucus*. To address this possibility, we collected ssm*F* forms from three sites in western Ireland including Achill Sound, which is close to the type locality of *F. cottonii*.

Microsatellite analysis was combined with analysis of nuclear DNA content (from DAPI analysis of nuclear DNA) and tests of gamete functionality.

MATERIALS AND METHODS

Sampling sites and collections

Small salt marsh forms of *Fucus* were collected from three localities on the western coast of Ireland (Fig. 2) during June 2012 and June 2014. Locality 1 was situated in an enclosed estuary northwest of Galway (Illaunnginga, 53°23'02 N, 09°33'50 W) and Locality 2 was situated in the bay outside Clifden (53°29'04 N, 10°01'49 W). At Locality 3, most individuals were collected on the shore below Achill Sound village (53°55'52.78''N, 09°56'47.81''W), with additional specimens collected at a similar site around 2.5 km further south within Achill Sound (53°54'36.23''N, 09°57'26.87''W).

At Locality 1 (Illaunnginga), the *Fucus* vegetation consisted of *F. vesiculosus* attached to rock in the lower zone, gradually transforming into small salt marsh-like individuals in mud and turf substrata of the middle and upper zones. In the middle zone, *Fucus* individuals possessed small and evesiculose thalli (up to around 10 cm) with rounded receptacles (Fig. 3), whereas in the upper zone, the individuals decreased further in size and all were vegetative. At both Locality 2 (Clifden) and Locality 3 (Achill Sound), the *Fucus* vegetation consisted of *F. vesiculosus*, *F. spiralis* and ssm*F*, with ssm*F* growing in the upper part of the *Fucus* zone at both localities. Some ssm*F* individuals bearing receptacles were found at both Localities 2 and 3 (Figs 4, 5), but less abundantly than what was observed at Locality 1. *Fucus spiralis* formed a broad band above *F. vesiculosus* at Locality 3, but was less abundant at Locality 2. Individuals of all taxa were collected haphazardly but normally separated by at least one metre. An overview of sampling

dates, collection data and numbers of individuals analyzed is given in Table 1. The sampled material was transported back to the laboratory in a cool bag.

Gamete functionality test

Individuals of ssm*F* bearing receptacles were collected at all three localities during June 2012, and receptacles from 11-27 individuals per site were examined, i.e. one receptacle per individual (Table 1). The examination of ssm*F* individuals and receptacles was done using a microscope. Separate receptacles with male and female gametes were found at Locality 1 and 3, and a test of gamete functionality was conducted with material from these localities. For each locality, three receptacles of each sex that appeared to be mature were dried at air temperature for 2.5 hours to initiate gamete release. Thereafter, pairs of male and female receptacles were added to small beakers with sterile unenriched sea water, incubated in a culture chamber (SanyoMLR-351, Sanyo Electric Co., Osaka, Japan) at 10 °C with a photon fluence rate of 20-50 μmol photons m^{-2} s^{-1} (18:6 h light:dark), and examined for released eggs or developing germlings after one, four, and six days.

Microsatellite analyses

In order to determine the genetic origins of ssm*F*, samples of *F. vesiculosus* (all localities), *F. spiralis* (Locality 2 and 3) and ssm*F* (all localities) were collected for microsatellite analyses during June 2012. After returning to the laboratory, one clean (no epiphytes) branch tip per individual was excised and dried in silica crystals. Tissue from 10-29 individuals per taxon per locality was collected (Table 1). Individuals other than those examined for gamete functionality were selected for microsatellite analyses to ensure extraction of high quality DNA. Vouchers of 10-20 ssm*F* were preserved from each locality (photos of pressed voucher specimens are added

as supplementary material, Figs S1, S2, S3-1, S3-2). DNA was extracted from 8-16 individuals per taxon per locality using a modification of the CTAB method (Coyer *et al*., 2009) and purified with Zymo One-Step-96 PCR™ Inhibitor Removal Kit (Zymo Research) following the manufacturer's protocol. From 8 to 16 individuals of each taxon per locality (Table 1) were genotyped using four microsatellite loci (L20, L38, L58 and L94) and the protocols by Engel *et al*. (2003). Due to methodological problems, no individuals from Locality 1 were successfully amplified, thus, this locality was not included in the microsatellite analyses. Genotypes were visualized on an ABI 3730 gene analyzer (Applied Biosystems) and analysed using GENOTYPER (Applied Biosystems) software.

Genetic diversity measured as Nei's non-biased H_{exp} (Nei, 1978) and estimators of F_{IS} and F_{ST} (Wright 1969) (as f and θ, Weir & Cockerham, 1984) were calculated using GENETIX 4.02 (Belkhir *et al*., 2001). Standardized allelic richness was determined for each population with GENCLONE 2.0 (http://wwz.ifremer.fr/clonix/Logiciels/GenClone-2.0). Clustering of microsatellite genotypes was done with STRUCTURE (Pritchard *et al*., 2000) using the Lifeportal computing service at the University of Oslo. STRUCTURE uses a Bayesian algorithm to identify clusters or groups of genetically homogeneous individuals (*K*), and has earlier been used to study hybridization in *Fucus* (e.g. Engel *et al*., 2005, Coyer *et al*., 2006). Analyses were run with *K*=1-6, and each run was replicated 10 times to ensure proper convergence of the MCMC with the parameters: ancestry model = admixture (to account for recent divergence and shared ancestral polymorphisms); frequency model = independent; burn-in = 1 000 000; MCMC length = 2 000 000 after burn-in. Since ssm*F* show vegetative reproduction and potentially form clones and *F. spiralis* has a high degree of selfing (Engel *et al*., 2005), clustering of the microsatellite genotypes was also examined with INSTRUCT (Gao *et al*., 2007), which eliminates the assumption of Hardy–Weinberg equilibrium within clusters and calculates expected genotype

frequencies on the basis of inbreeding or selfing rates. In both cases the most likely *K* value was inferred from the rate of change in estimated Ln probability of data between successive *K* values (delta K) (Evanno *et al*., 2005), using STRUCTURE HARVESTER web version 0.6.94 (Earl & vonHoldt, 2012) and CLUMPAK beta version (Kopelman *et al*., 2015). The results were visualized with DISTRUCT 1.1 (Rosenberg, 2004). The genetic affinities of ssm*F* from Localities 2 and 3 were further analysed with principal component analysis (PCA) in R 3.2.5 using the R package ADEGENET (Jombart, 2008). The genotype spreading was very large in *F. vesiculosus* from Locality 3, and two extreme outliers were removed before the final PCA. Multivariate statistical methods such as PCA are free from assumptions of underlying population genetic models and represent an independent method to measure population differentiation (Jombart *et al*., 2010).

Nuclear DNA content analyses

To detect variation in genome size and possible polyploidy in the ssm*F* individuals, nuclear DNA content was estimated with microspectrofluorometry analyses of DAPI stained tissue. Individuals of ssm*F* collected from all three localities during June 2014 were included, but *Fucus vesiculosus* and *F. spiralis* were only collected from Locality 1 and 3, and Locality 3, respectively (Table 1), since we initially did not expect a large variation in nuclear DNA content of these species on a small spatial scale (Gómez Garreta *et al*., 2010). A small tissue section was excised from each individual and fixed in Carnoy's solution (3:1 100 % ethanol:glacial acetic acid, mixed just before use) for 24 hrs, then stored in 70% ethanol at 4 ˚C. Fixation in Carnoy's solution prevents staining inhibition due to phlorotannins. Nuclear DNA content was estimated by image analysis using METAMORPH software (Molecular Devices, Toronto, ON, Canada) and comparing the If (Intensity of fluorescence) of *Fucus* nuclei with those of chicken erythrocytes, which have a DNA content of 2.4 pg (Clowes *et al*., 1983). Analyses of nuclei DNA content were carried out

using an AxioCam HRm Zeiss digital camera on an Axioplan Zeiss fluorescence microscope (see Gómez Garreta *et al*., 2010). Estimates of nuclei contents were obtained from 3-7 ssm*F* from each locality (Table 1). Additionally, nuclei contents were estimated for three *F. vesiculosus* individuals from Locality 1 and 3, and five *F. spiralis* individuals from Locality 3. Mean nuclear DNA contents per individual were based on 68-247 nuclei (Table 4).

RESULTS

Gamete functionality test

Since *Fucus* species can be dioecious (male antheridia and female oogonia on separate individuals, e.g. *F. vesiculosus*) or hermaphroditic (male antheridia and female oogonia within a single receptacle, e.g. *F. spiralis*), ssm*F* receptacles were examined in order to sex the individuals. Only female receptacles (n=11) were found at Locality 2 (Fig. 9), while eight female and six male receptacles were found at Locality 1 (Figs 6, 7), and 11 female and six male receptacles at Locality 3 (Figs 10, 11). The male receptacles from Locality 3 had relatively few conceptacles. No hermaphroditic receptacles of the ssm*F* individuals were found, except for four of 25 receptacles from Locality 3, which had oogonia and structures that may have been immature antheridia. A few sterile receptacles were found at both Locality 2 and 3. Receptacles of ssm*F* from Locality 1 were normal in appearance with viable and functioning gametes, and after six days numerous and normal-looking germlings were observed in the incubated beakers (Fig. 8). The ssm*F* individuals from Locality 1 differed further from those of the other localities by having a more conspicuous mid-rib (Fig. 3). Only a few oogonia with division lines were observed in receptacles from Locality 2 and 3, and oogonia from these localities frequently were

abnormal with cell contents concentrated in the middle (Figs 9, 10). None of the incubations from Locality 3 produced germlings.

Microsatellite analyses

The microsatellite analyses gave results for 5-6 individuals of each taxon from Locality 2, and for 6-15 individuals per taxon from Locality 3. Significant F_{IS}-values were found for all three taxa from Locality 3 and for *F. spiralis* from Locality 2, which suggests more inbreeding in these populations than in the others (Table 2). A relatively high expected heterozygosity (H_{exp}=0.813) was found for *F. vesiculosus* at Locality 3 (Table 2), probably reflecting the larger sampling area. Pairwise F_{ST}-comparisons revealed an overall greater genetic distance among populations at Locality 3 than among those at Locality 2, expect for the comparison between *F. spiralis* and *F. vesiculosus*, which showed a lower F_{ST}-value at Locality 3 than at Locality 2 (Table 3). *Fucus spiralis* showed the highest inbreeding coefficients (F_{IS}) at Locality 2 and 3. At Locality 3 also ssm*F* and *F. vesiculosus* had significant inbreeding coefficients (Table 2). The microsatellite analyses did not reveal clones of ssm*F*. Allelic richness for *F. vesiculosus* was higher than for ssm*F* and *F. spiralis* at Locality 3, while at Locality 2 *F. vesiculosus* and ssm*F* had similar and higher allelic richness than *F. spiralis* (Table 2). Information about allele proportions is included in Table S4.

According to the delta *K* plots from the STRUCTURE and INSTRUCT analyses, the most likely distribution of the microsatellite genotypes at Localities 2 and 3 (not available for Locality 1) was *K*=2, followed by *K*=3. The two clusters obtained by *K*=2 corresponded largely to the individuals sampled as *F. spiralis* and *F. vesiculosus*. Genotyped ssm*F* individuals from Locality 2 showed highest similarity to *F. vesiculosus* as shown by both STRUCTURE and INSTRUCT (Fig.12). Genotyped ssm*F* individuals from Locality 3 showed admixture between *F. vesiculosus*

and *F. spiralis* according to the STRUCTURE results, but were similar to *F. spiralis* according to the INSTRUCT results (Fig. 12).

With *K*=3, the STRUCTURE and INSTRUCT analyses showed identical patterns (only the result of the INSTRUCT analysis is shown in Fig. 12). In this case, the ssm*F* from Locality 3 formed a separate group, but shared this haplotype combination with some *F. spiralis* and *F. vesiculosus* individuals from the same locality. At Locality 2 the analyses indicated some admixture with *F. spiralis* in some of the ssm*F* individuals.

The results of the PCA analysis largely confirmed the results from the STRUCTURE and INSTRUCT analyses (Fig. 13), except that the ssm*F* individuals clustered in a more intermediate position relative to *F. spiralis* and *F. vesiculosus* at both localities. The high genetic diversity of *F. vesiculosus* from Locality 3 was also evident in the PCA, with these individuals spread throughout the plot, overlapping totally with individuals of *F. vesiculosus* and ssm*F* from Locality 2, and partly with ssm*F* from Locality 3.

Nuclear DNA content analyses

Nuclear DNA content was reported as C-value units (Table 4), with C representing the estimated weight (pg) of the nuclear DNA content of non-replicated gametes. Very few direct C values (from sperm) were obtained from the examined individuals, and none from the receptacle-bearing ssm*F* (Table 4). Members of *Fucus* are characterized by a diplontic life cycle with diploid 2C nuclei in non-replicated vegetative cells (G1-phase) and 4C in replicated cells (G2-phase) (Gómez Garreta *et al*., 2010). Most recordings of nuclear DNA content were 2C- or 4C-values (Table 4). The 2C-values of three ssm*F* individuals from Locality 1 ranged from 1 to 1.8 pg, whereas the values of the three individuals from Locality 2 ranged from 4 to 4.3 pg (Fig. 14). The ssm*F* individuals from Locality 3 formed two groups, with three individuals at the lower (1.5-1.9

pg) and four individuals at the upper (3.9-4.6 pg) range (Fig. 14). The three *F. vesiculosus* individuals from Locality 1 had an overall lower, but overlapping range of nuclear DNA content with the ssm*F* individuals from the same locality (Fig. 14). At Locality 3, the three *F. vesiculosus* individuals showed a variable nuclear DNA content, ranging from 1.1 to 2.8 pg. The *F. spiralis* individuals from Locality 3 had 2C-estimates of 2-3.5 pg (with four out of the five estimates between 3 and 3.5 pg). Unfortunately, we have no analyses of nuclear DNA content for *F. vesiculosus* and *F. spiralis* from Locality 2.

DISCUSSION

The ssm*F* showed surprisingly high variation between the three localities in western Ireland with respect to reproduction mode, genetic affiliation, and nuclear DNA content. Previous studies have indicated that ssm*F* can originate from different *Fucus* taxa, even between localities in Ireland (Coyer *et al*., 2006, Neiva *et al*., 2012). This is supported by our microsatellite results, which showed that ssm*F* from Locality 2 were derived mainly from *F. vesiculosus*, whereas the ssm*F* individuals from Locality 3 revealed a higher degree of hybridization or introgression, but with greatest affiliation to *F. spiralis*. The fact that ssm*F* had a significant inbreeding coefficient at Locality 3 but not at Locality 2 also suggest a connection of ssm*F* to *F. spiralis* at Locality 3, since high degree of inbreeding is found in *F. spiralis*. At Locality 3 the sampling was done at two sites about 2.5 km apart, but the microsatellite clustering did not reveal genetic separation between the sites.

Only ssm*F* from Locality 1 displayed successful sexual reproduction as shown by a test of gamete functionality. Unfortunately, no individuals from Locality 1 were included in the microsatellite analyses (due to unsuccessful amplification). However, since the *Fucus* vegetation

formed a cline from *F. vesiculosus*-like individuals in the lower intertidal to ssm*F*-like and dioecious individuals in the upper intertidal, and no *F. spiralis* were observed at or in the vicinity of Locality 1, we assume that ssm*F* from Locality 1 were derived from *F. vesiculosus*. Both *F. vesiculosus* and ssm*F* from Locality 1 had mature receptacles during June, and are likely to be interfertile.

Coyer *et al*. (2006) examined ssm*F* from one locality in Ireland and showed that ssm*F* were polyploids derived from *F. vesiculosus*. To investigate this further, we estimated the nuclear DNA contents of ssm*F*, *F. vesiculosus* and *F. spiralis.* Generally, we found both much higher estimates of, and variability in, nuclear DNA content than has previously been measured for these *Fucus* taxa, even within a single locality (Fig. 14). Previously published estimates of nuclear DNA content (2C) of *F. vesiculosus* from different geographical areas (Norway, Spain, USA and France; Fig. 14) varied between 1 and 2.3 pg (Kapraun, 2005; Gómez Garreta *et al*., 2010; Phillips *et al*., 2011). We obtained estimates between 1 and 2.8 pg for this taxon from two localities less than 100 km apart. The highest nuclear DNA content (up to 4.6 pg) was found in a group of ssm*F* individuals from Locality 3. Variable genome sizes have been reported for some brown algae (Kapraun, 2005; Gómez Garreta *et al*., 2010; Phillips *et al*., 2011), but little is known about the mechanisms causing such variation.

Variation in genome size has long been studied in land plants, and several mechanisms have been demonstrated (Šmarda & Bureš, 2010; Leitch & Leitch, 2013). Even though it is likely that processes influencing genome size are different in land plants compared to brown algae, which are evolutionarily independent from Archaeplastida (Yoon *et al*., 2004), there are probably also similarities. For example, a commonly occurring process in land plants is polyploidy, a rapid and dramatic doubling of the genome size sometimes from one generation to the next (Bennetzen *et al*., 2005), which can, if repeated, result in a range of ploidal levels. Newly evolved polyploids

are expected to increase the genome size in direct proportion with ploidy. However, many polyploids also show a reduction over time in DNA amount relative to diploids as a result of genome downsizing through various processes (Leitch & Bennett, 2004; Leitch & Leitch, 2013).

Autopolyploids arise within or among populations of a single species by the doubling of homologous chromosome sets. Allopolyploids, on the other hand, combine two or more genomes from different species as a result of interspecific hybridization. In nature, both autopolyploids and allopolyploids often result from the formation of unreduced gametes (Tayalé & Parisod, 2013; De Strome & Mason, 2014) and usually through a triploid intermediate formed by the union of unreduced and reduced gametes. The triploid may in turn produce tetraploids either through selfing, or by crossing with other triploids or diploids. Triploids may produce viable gametes of varying ploidy (n = x, 2x, 3x), leading to individuals with variable genome sizes (Mason & Pires, 2015). Diploids and tetraploids in a population may further cross and form triploids, which in turn may produce viable gametes (Birchler, 2012). As more and more population-scale studies on genome size variation are published, it is clear that odd-numbered ploidies exist within species and populations, suggesting that successful intercytotype crossings may be more widespread than previously thought (Husband *et al*., 2013).

Production of unreduced gametes in land plants occurs more frequently under environmental stress such as low and fluctuating temperatures, water stress and disease (Tayalé & Parisod, 2013; De Strome & Mason, 2014). The estuarine conditions of the localities in the present study provide similar stressful habitats with regard to salinity and temperature variation in the intertidal, which consequently may favour formation of unreduced gametes and further facilitate polyploidization. Polyploidization, followed by repeated crossings between individuals with different ploidal levels, may explain the variable genome sizes observed in *Fucus* at Locality 3. The lowest 2C-value found in *F. vesiculosus* is 1 pg (Kapraun, 2005; this study). Under the

assumption that this value represents a diploid genome size, the more or less stepwise increase in genome size suggests that also tetraploids and hexaploids were found at Locality 3. If the diploid genome size of *F. spiralis* is ca. 1.5 pg (as reported from Spain, Fig. 14), most of *F. spiralis* from Locality 3 could be considered tetraploids with genome sizes around 3 pg. However, with the small number of analysed individuals and the relatively high variation in nuclear DNA estimates, we cannot exclude the possibility that a smaller diploid genome size exists for *F. spiralis* at Locality 3 (e.g. 1 pg). In this case the individual at Locality 3 with a genome size of ca. 2 pg may be a tetraploid, and those with higher ploidy could be hexaploids.

Assuming polyploid origins, the ssm*F* individuals at Locality 2 with large genome sizes (ca. 4 pg) can be considered octoploids that have originated mainly from *F. vesiculosus.* The ssm*F* individuals at Locality 3, however, can be separated in two groups; one with genome sizes ≥ 4 pg (which can be interpreted as hexa- or octoploid), and one with genome sizes from 1.5 to 2 pg. No sexual recruitment was observed for ssm*F* from Locality 3, suggesting that both groups originated from the reproducing *Fucus* populations at the site. Microsatellite analyses showed that ssm*F* from Locality 3 most likely were hybrids and/or introgressed *F. spiralis*. *Fucus vesiculosus* and *F. spiralis* are closely related and may easily hybridize, but a 'normal' F1 hybrid has an intermediate morphology between the two species (Burrows & Lodge, 1951). We propose that inter- or intraploidy crossings between individuals of *F. vesiculosus* and *F. spiralis* may be responsible for the varying ploidy, deviating morphology, and loss of functions among ssm*F* from Localities 2 and 3.

The ssm*F* at Locality 1 seem to represent a special case, since they had functional gametes and sexual reproduction. They also deviated from the ssm*F* at Localities 2 and 3 by having a more conspicuous mid-rib. Compared to estimates from the other localities, the genome size estimates from Locality 1 were at the lower end of the range. However, ssm*F* here also

possessed the highest genome sizes, though overlapping with those of *F. vesiculosus*. The processes leading to variable genome sizes at Locality 1 are unknown, but likely due to processes other than polyploidization (e.g. Piegu *et al*., 2006).

It is not clear what causes the miniaturization of ssm*F* that was observed in all three localities, but it seems to be unrelated to genetic affiliation or genome size *per se* (see also Wallace *et al*., 2004; Coyer *et al*., 2006: Neiva *et al*., 2012). We hypothesize that occurrence of ssm*F* is connected to processes causing variable genome sizes in *Fucus* populations, and that formation of ssm*F* occurs most frequently at localities where one or more members of *Fucus* have an unstable genome. Our hypothesis may explain why ssm*F* are not present in all salt marshes with apparently suitable habitat. For example, Sheehy Skeffington & Curtis (2000) found that the distribution of ssm*F* was limited to the west coast of Ireland, and connected to high precipitation and low salinity in the local salt marshes. A stressful environment may cause genome size instability and variation in plants in various ways (e.g. Mirouze & Paszkowski, 2011, De Strome & Mason, 2014), and estuaries with very low or strongly variable salinity may in a similar way represent stressful biotopes for fucoids, resulting in genome size variations.

Furthermore, in plants it has been demonstrated that high variation in genome size, as a result of polyploidization and subsequent genome downsizing, or activation of epigenetics and transposable elements, can influence gene functioning and expression, through chromosome rearrangements and epigenetic mobilizations (Song & Chen, 2015). Interestingly, a recent study of speciation in angiosperms showed that high rates of phenotypic changes and speciation are positively and strongly correlated to genome size changes, but not to absolute genome size (Puttick *et al*., 2015). Processes that change genome sizes, therefore, may influence both growth and functioning of organisms. In our case, variation in genome size could have resulted in

malfunction of genes necessary for normal development of antheridia (male gametangia) in ssm*F* from Locality 2.

Our study shows that members of the genus *Fucus* display a surprisingly large variation in genome size, even within populations. Although processes regulating speciation in angiosperms and brown algae may be quite different, it is possible that the high genome size variation observed in *Fucus* may partially explain the observed high capacity of morphological plasticity. While we cannot determine the underlying processes causing the patterns reported in the present study, our results clearly show that these processes need to be investigated further in *Fucus*. The genus, therefore, may be an excellent model for testing the connection between variable genome sizes, population adaptation to different environmental conditions, and speciation.

Acknowledgements

We thank Sarah Helyar and Steinunn Magnúsdottir at MATIS, Iceland, for all their help with microsatellite analyses, Kerstin Johannessen, Richardo Pereyra, Mike Guiry, Ester Serrão and an anonymous reviewer for helpful comments to the manuscript, and Ilia J. Leitch for information about processes of genome size variation in land plants. Financial support has been obtained from the Olaf Grolle Olsen's legacy and the University of Bergen (KS). SH acknowledges financial support under the Irish Sea Change Strategy and SSTI (2006-2013) from the Marine Institute, funded under the Irish National Development Plan 2007-2013.

References

Belkhir, K., Borsa, P., Goudet, J., Chikhi, L. & Bonhomme, F. (2001). GENETIX 4.02, *logiciel sous Windows TM pour la génétique des populations*. Laboratoire Génome, Populations, Interactions: CNRS UMR 5000, Université de Montpellier II, Montpellier.

Bennetzen, J.L., Jianxin, M.A. & Devos, K.M. (2005). Mechanisms of recent genome size variation in flowering plants. *Annals of Botany*, **95**: 127-132.

Bergström, L., Tatarenkov, A., Johannesson, K., Jönsson, R.B. & Kautsky, L. (2005). Genetic and morphological identification of *Fucus radicans* sp. nov. (Fucales, Phaeophyceae). *Journal of Phycology*, **41**: 900-905.

Birchler, J.A. (2012). Chapter 2. Genetic consequences of polyploidy in plants. In *Polyploidy and genome evolution* (Soltis, P.S. & Soltis, D.E., editors), 21-32. Springer-Verlag, Berlin-Heidelberg.

Burrows, E.M. & Lodge, S. (1951). Autecology and the species problem in *Fucus*. *Journal of the Marine Biological Association of the United Kingdom,* **30**: 161-176.

Cánovas, F.G., Mota, C.F., Serrão, E.A. & Pearson, G.A. (2011). Driving south: a multi-gene phylogeny of the brown algal family Fucaceae reveals relationships and recent drivers of a marine radiation. *BCM Evolutionary Biology,* **11**: 371.

Clowes, A.W., Reidy, M.A. & Clowes, M.M. (1983). Kinetics of cellular proliferation after arterial injury. I. Smooth muscle growth in absence of endothelium. *Laboratory Investigation*, **49**: 327-333.

Cotton, A.D. (1912). 15. Marine Algae. Proceedings of the Royal Irish Academy. *Section B: Biological, Geological, and Chemical Science, A Biological Survey of Clare Island in the County of Mayo, Ireland and of the Adjoining District (Sections 1-3) (1911 - 1915),* **31**: 1-178.

Coyer, J.A., Hoarau, G., Pearson, G.A., Serrão, E.A., Stam, W.T. & Olsen, J.L. (2006). Convergent adaptation to a marginal habitat by homoploid hybrids and polyploid ecads in the seaweed genus *Fucus*. *Biology Letters*, **2**: 405-408.

Coyer, J.A., Hoarau, G., Beszerti, B., Pearson, G. & Olsen, J.L. (2009). Expressed sequence tag derived polymorphic SSR markers for *Fucus serratus* and amplification in other species of *Fucus*. *Molecular Ecology Resources*, **9**:168-170.

Coyer, J.A., Hoarau, G., Costa, J.F., Hogerdijk, B., Serrão, E.A., Billard, E., Valero, M., Pearson, G. & Olsen, J.L. (2011). Evolution and diversification within the intertidal brown macroalgae *Fucus spiralis/F. vesiculosus* species complex in the North Atlantic. *Molecular Phylogenetics and Evolution*, **58**: 283-296.

De Strome, N. & Mason, A. (2014). Plant speciation through chromosome instability and ploidy change: Cellular mechanisms, molecular factors and evolutionary relevance. *Current Plant Biology,* **1**: 10-33.

Earl, D.A. & vonHoldt, B.M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**: 359-361.

Engel, C.R., Brawley, S., Edwards, K.J. & Serrão, E. (2003). Isolation and cross-species amplification of microsatellite loci from the fucoid seaweeds *Fucus vesiculosus*, *F. serratus*, and *Ascophyllum nodosum* (Heterokontophyta, Fucaceae). *Molecular Ecology Notes*, **3**: 180-182.

Engel, C.R., Daguin, C. & Serrão, E. (2005). Genetic entities and mating system in hermaphroditic *Fucus spiralis* and its close dioecious relative *F. vesiculosus* (Fucaceae, Phaeophyceae). *Molecular Ecology,* **14**: 2033-2046.

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology,***14**: 2611-2620.

Feldmann, J. (1941). Sur une nouvelle espèce de *Fucus* de la côte Basque: *Fucus chalonii* nov. sp. *Bulletin de la Société Botanique de France*, **88**: 143-147.

Gao, H., Williamson, S. & Bustamante, C.D. (2007). A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. *Genetics*, **176**: 1635-1651.

Gómez Garreta, A., Ribera Siguan, M.A., Salvador Soler, N., Rull Lluch, J. & Kapraun, D.F. (2010). Fucales (Phaeophyceae) from Spain characterized by large-scale discontinuous nuclear DNA contents consistent with ancestral cryptopolyploidy. *Phycologia*, **49**: 64-72.

Husband, B.C., Baldwin, S.J. & Suda, J. (2013). The incidence of polyploidy in natural plant populations: major patterns and evolutionary processes. In *Plant genome diversity Vol. 2* (Leitch, I.J., Greilhuber, J., Dolezel, J. & Wendel, J.F., editors), 255-277. Springer-Verlag, Vienna.

Jombart, T. (2008). ADEGENET: an R package for the multivariate analysis of genetic markers. *Bioinformatics,* **24**: 1403-1405.

Jombart, T., Devillard, S. & Balloux. F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, **11**: 94.

Kapraun, D.F. (2005). Nuclear DNA content estimates in multicellular green, red and brown algae: phylogenetic considerations. *Annals of Botany*, **95**: 7-44.

Kopelman, N.M., Mayze, J., Jakobsson, M., Rosenberg, N.A. & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, **15**: 1179-1191.

Leitch, I.J. & Bennett, M.D. (2004). Genome downsizing in polyploid plants. *Biological Journal of the Linnean Society,* **82**: 651-663.

Leitch, I.J. & Leitch, A.R. (2013). Genome size diversity and evolution in land plants. In *Plant genome diversity Vol. 2* (Leitch, I.J., Greilhuber, J., Dolezel, J. & Wendel, J.F., editors), 307-322. Springer-Verlag, Vienna.

Mason, A.S. & Pires, J.C. (2015). Unreduced gametes: meiotic mishap or evolutionary mechanism? *Trends in Genetics*, doi: 10.1016/j.tig.2014.09.011.

Mathieson, A.C., Clinton, J.D., Wallace, A.L. & Klein, A.S. (2006). Distribution, morphology, and genetic affinities of dwarf embedded *Fucus* populations from the Northwest Atlantic Ocean. *Botanica Marina*, **49**: 283-303. Article in press - uncorrected proof2006/67

Mirouze, M. & Paszkowski, J. (2011). Epigenetic contribution to stress adaptation in plants. *Current Opinion in Plant Biology,* **14:** 267–274.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583-590.

Neiva, J., Hansen, G.I., Pearson, G.A., Van De Vliet, M.S., Maggs, C.A. & Serrão, E.A. (2012). *Fucus cottonii* (Fucales, Phaeophyceae) is not a single genetic entity but a convergent salt-marsh morphotype with multiple independent origins. *European Journal of Phycology*, **47**: 461-468.

Pereyra, R.T., Bergström, L., Kautsky, L. & Johannesson, K. (2009). Rapid speciation in a newly opened postglacial marine environment, the Baltic Sea. *BMC Evolutionary Biology*, **9**: 70.

Peters, A.F., Marie, D., Scornet, D., Kloareg, B. & Cock, J.M. (2004). Proposal of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) as a model organism for brown algal genetics and genomics. *Journal of Phycology*, **40**: 1079-1088.

Phillips, N., Kapraun, D.F., Garreta, A.G., Siguan, M.A.R., Rull, J.L., Soler, N.S., Lewis, R. & Kawai, H. (2011). Estimates of nuclear DNA content in 98 species of brown algae (Phaeophyta). *AoB PLANTS*, 2011: plr001, doi:10.1093/aobpla/plr001.

Piegu, B., Guyot, R., Picault, N., Roulin, A., Saniyal, A., Kim, H., Collura, K., Brar, D.S., Jackson, S., Wing, R.A. & Panaud, O. (2006). Doubling genome size without polyploidization: Dynamics of retrotransposition-driven genomic expansions in *Oryza australiensis*, a wild relative of rice. *Genome Research,* **16**: 1262-1269.

Pritchard, J.K., Stephens, P. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, **155**: 945-959.

Puttick, M.N., Clark, J. & Donoghue, C.J. (2015). Size is not everything: rates of genome size evolution, not C-value, correlate with speciation in angiosperms. *Proceedings of the Royal Society B*, **282**: 20152289. doi:org./10.1098/rspb.2015.2289.

Rosenberg, N.A. (2004). DISTRUCT: a program for the graphical display of population Structure. *Molecular Ecology Notes*, **4**: 137-138.

Sheehy Skeffington, M.J. & Curtis, T.G.E. (2000). The Atlantic element in Irish salt marshes

In *Biodiversity: The Irish dimension* (Rushton, B.S., editor), 179-196. Royal Irish Academy, Dublin.

Šmarda, P. & Bureš, P. (2010). Understanding intraspecific variation in genome size in plants. *Preslia*, **82**: 41-61.

Song, Q. & Chen, Z.J. (2015). Epigenetic and developmental regulation in plant polyploids. *Current Opinion in Plant Biology,* **24**: 101-109.

Tayalé, A. & Parisod, C. (2013). Natural pathways to polyploidy in plants and consequences for genome reorganization. *Cytogenetic and Genome Research*, **140**: 79-96.

Wallace, A.L., Klein, A.S. & Mathieson, A.C. (2004). Determining the affinities of salt marsh fucoids using microsatellite markers: evidence of hybridization and introgression between two species of *Fucus* (Phaeophyta) in a Maine estuary. *Journal of Phycology*, **40**: 1013–1027.

Weir, B.S. & Cockerham, C.C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**: 1358-1370.

Wright, S. (1969). *Evolution and the Genetics of Populations, Vol. 2. The Theory of Gene Frequencies*. Chicago: University of Chicago Press.

Wynne, M.J. & Magne, F. (1991). Concerning the name *Fucus muscoides* (Cotton) J. Feldmann et Magne. *Cryptogamie, Algologie*, **12**: 55–65.

Yoon, H.S., Hackett, J.D., Ciniglia, C., Pinto, G. & Bhattacharya, D. A. (2004). Molecular timeline for the origin of photosynthetic eukaryotes. *Molecular Biology and Evolution*, **21**: 809-818.

Zardi, G.I., Nicastro, K.R., Canovas, F., Costa, J.F., Serrão, E.A. & Pearson, G.A. (2011). Adaptive traits are maintained on steep selective gradients despite gene flow and hybridization in the intertidal zone. *PLoS ONE*, **6**: 1-13.

Table 1. Sampling specifics. Dates of sampling, , taxa sampled, and numbers of individuals per taxa examined or processed are presented for *Fucus spiralis* (Fs), *F. vesiculosus* (Fv) and small salt marsh *Fucus* (ssmF).

Collection information	**Locality 1 (Illaunnginga)**	**Locality 2 (Clifden)**	**Locality 3 (Achill Sound)**
Field sampling 14 and 18 June 2012, taxa:	***Fv, ssmF***	***Fv, Fs, ssmF***	***Fv, Fs, ssmF***
Individuals of ssmF examined under microscope	15	11	17
Individuals of ssmF with receptacles examined	15	11	27
Dried for microsatellite analyses; Taxon (no)	Fv (10), ssmF (15)	Fv (10), Fs (10), ssmF (15)	Fv (20), Fs (23), ssmF (29)
DNA extraction; Taxon (no)	Fv (10), ssmF (15)	Fv (8), Fs (8), ssmF (8)	Fv (16), Fs (16), ssmF (16)
Individuals successfully genotyped by microsatellite analyses; Taxon (no)	-	Fv (6), Fs (6), ssmF (5)	Fv (14), Fs (15), ssmF (6)
Voucher samples	ssmF (10)	ssmF (12)	Fv (1), Fs (1), ssmF (20)
Field sampling 22-23 June 2014, taxa:	***Fv, ssmF***	***ssmF***	***Fv, Fs, ssmF***
Fixated for nuclear DNA content analyses; Taxon (no)	Fv (9), ssmF (16)	ssmF (10)	Fv (5), Fs (10), ssmF (12)
Nuclear DNA content analyses; Taxon (no)	Fv (3), ssmF (3)	ssmF (3)	Fv (3), Fs (5), ssmF (7)

Table 2. Calculated F_{IS}, H_{exp} and allelic richness ($\mathring{A}$) for n individuals from populations of *Fucus spiralis* (Fs), *F. vesiculosus* (Fv) and small salt marsh *Fucus* (ssmF) from Locality 2 (Clifden) and Locality 3 (Achill Sound). Significant F_{IS}-values are marked with an asterisk. Allelic richness ($\mathring{A}$) is standardized to n=5.

Locality-taxon	**n**	$\boldsymbol{F_{IS}}$	$\boldsymbol{H_{exp}}$	$\boldsymbol{\mathring{A}}$
2-Fs	6	0.590*	0.327	1.400
2-ssmF	5	0.271	0.513	2.800
2-Fv	6	0.102	0.570	2.726
3-Fs	15	0.618*	0.295	1.472
3-ssmF	6	0.510*	0.374	2.600
3-Fv	14	0.504*	0.813	4.266

Table 3. Pairwise F_{ST}-values comparing populations of *Fucus spiralis* (Fs), *F. vesiculosus* (Fv) and small salt marsh *Fucus* (ssmF) from Locality 2 (Clifden) and Locality 3 (Achill Sound).

Locality-taxon	**2-ssmF**	**2-Fv**	**3-Fs**	**3-ssmF**	**3-Fv**
2-Fs	0.408	0.447	-0.059	0.469	0.238
2-ssmF		0.073	0.492	0.349	0.064
2-Fv			0.526	0.464	0.078
3-Fs				0.497	0.327
3-ssmF					0.182

Table 4. Overview of results from estimated nuclear DNA content from Locality 1 (Illaunnginga), Locality 2 (Clifden) and Locality 3 (Achill sound) of small salt marsh *Fucus* (ssmF), *F. vesiculosus* (Fv) and *F. spiralis* (Fs). The results are given in pg for 2C, 4C, and for C (sperm) when possible, with standard deviations (Stdv). Nuclei (n) = number of nuclei examined per sample. Presence or absence of receptacles in the analysed individuals is indicated.

Sample-id	Locality	Nuclei (n)	2C	Stdv 2C	4C	Stdv 4C	C (sperm)	Stdv C	Receptacles
ssmF-1	2	201	4	0.3	8.4	0.7	-	-	No
ssmF-2	2	184	4.3	0.8	8.2	0.2	-	-	Yes
ssmF-3	2	190	4	0.3	8.4	0.7	-	-	No
ssmF-11	1	172	1.6	0.3	3.3	0.5	-	-	No
ssmF-19	1	208	1	0.1	3.1	0.2	-	-	Yes
ssmF-20	1	164	1.8	0.4	4.3	0.2	-	-	Yes
Fv-27	1	231	1.3	0.3	2.7	0.3	-	-	Yes
Fv-28	1	76	1	0.3	2.3	0.5	-	-	Yes
Fv-30	1	167	1	0.3	-	-	0.7	0.2	Yes
ssmF-36	3	87	1.8	0.5	-	-	-	-	No
ssmF-37	3	153	1.5	0.2	3.4	0.4	-	-	No
ssmF-38	3	69	4.1	0.8	7.3	0.5	-	-	Yes
ssmF-39	3	71	3.9	0.3	6.4	0.1	-	-	Yes
ssmF-40	3	187	4.6	0.6	7.9	0.6	-	-	Yes
ssmF-41	3	173	1.9	0.6	4.2	0.5	-		Yes
ssmF-42	3	154	4	0.3	7.7	0.4	-	-	No

Fs-48a	3	247	3.5	0.3	5.4	0.6	-		-	No
Fs-48b	3	151	2	0.4	3.8	0.4	0.9		0.2	Yes
Fs-50	3	104	3.3	0.4	5.8	0.4	-		-	Yes
Fs-51	3	68	3.1	1.0	5.8	0.8	-		-	Yes
Fs-52	3	144	3	0.3	4.8	0.2	-		-	No
Fv-57	3	123	1.8	0.3	4.4	0.3	-		-	Yes
Fv-58	3	83	2.8	0.5	4.2	0.2	-		-	Yes
Fv-60	3	136	1.1	0.3	2.2	0.3	0.6		0.1	Yes

Legends

Figure 1. Co-occurring morphological forms. Individuals of small salt marsh *Fucus* with narrow thalli adjacent to *F. spiralis* with broader thalli and receptacles (examples shown by arrows) at Locality 3 (Achill Sound) in June 2014.

Figure 2. Sample localities in Ireland. *Fucus* spp. were sampled during 2012 and 2014 at Locality 1 (Illaunnginga), Locality 2 (Clifden) and Locality 3 (Achill Sound).

Figures 3-5. Small salt marsh *Fucus* with receptacles from Locality 1 (Illaunnginga; Fig. 3), Locality 2 (Clifden; Fig. 4) and Locality 3 (Achill Sound; Fig. 5). Scale bar is 1 cm.

Figures 6-11. Conceptacle structure and germling growth. Mature oogonium containing egg cells ready to be released (Fig. 6); antheridia (Fig. 7) and germling after six days in culture (Fig. 8) from Locality 1 (Illaunnginga) (possible damage to the attachment rhizoids due to transfer to microscope slide). Immature oogonia from Locality 2 (Clifden; Fig. 9). Immature oogonia (Fig. 10) and antheridia (Fig. 11) from Locality 3 (Achill Sound). Scale bar is 50 μm.

Figure 12. Results from STRUCTURE (A) and INSTRUCT (B) analyses with K=2; and from INSTRUCT analysis with K=3 (C). Localities 2 and 3 consist of individuals sampled as *Fucus spiralis* (Fs), *F. vesiculosus* (Fv), and small salt marsh *Fucus* (ssmF). Each individual is

represented by a bar and colors represent the proportional assignment to the STRUCTURE/INSTRUCT groups.

Figure 13. The two first principal components (PC) of a Principal Component Analysis showing genetic affiliation of haplotype composition of ssm*F* (blue), *F. vesiculosus* (ves) (green) and *F. spiralis* (spi) (orange-red) from Locality 2 (Loc2) and Locality 3 (Loc3). The circles represent 95% inertia ellipses for the populations, which characterizes the dispersion of each population around its center of gravity. Percentages along axes indicate the proportion of overall variability explained by the principal components.

Figure 14. Average nuclear DNA content (2C; pg) in *Fucus vesiculosus* (Fv, black circles), *F. spiralis* (Fs, grey circles) and small salt marsh *Fucus* (ssmF, white circles). Data from Illaunnginga (Locality 1), Clifden (Locality 2) and Achill Sound (Locality 3) are shown, together with unpublished data from Norway (**) and earlier published data (*) from Spain (Gómez Garreta et al. 2010), USA (Kapraun 2005) and France (Phillips et al. 2011, recalculation from Peters *et al.* 2004).

Figure 1.

Figure 2.

Figure 3-5.

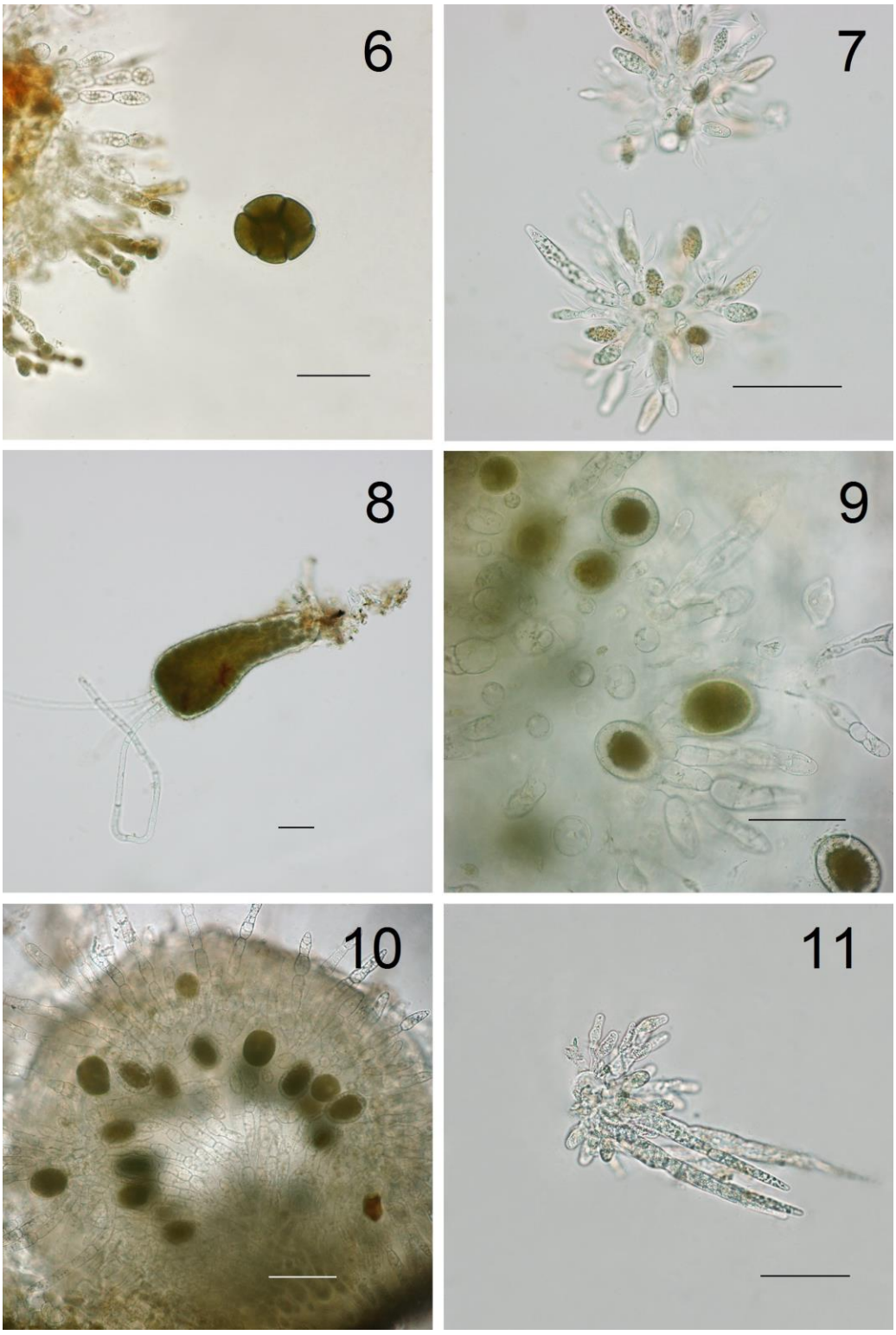

Figure 6-11.

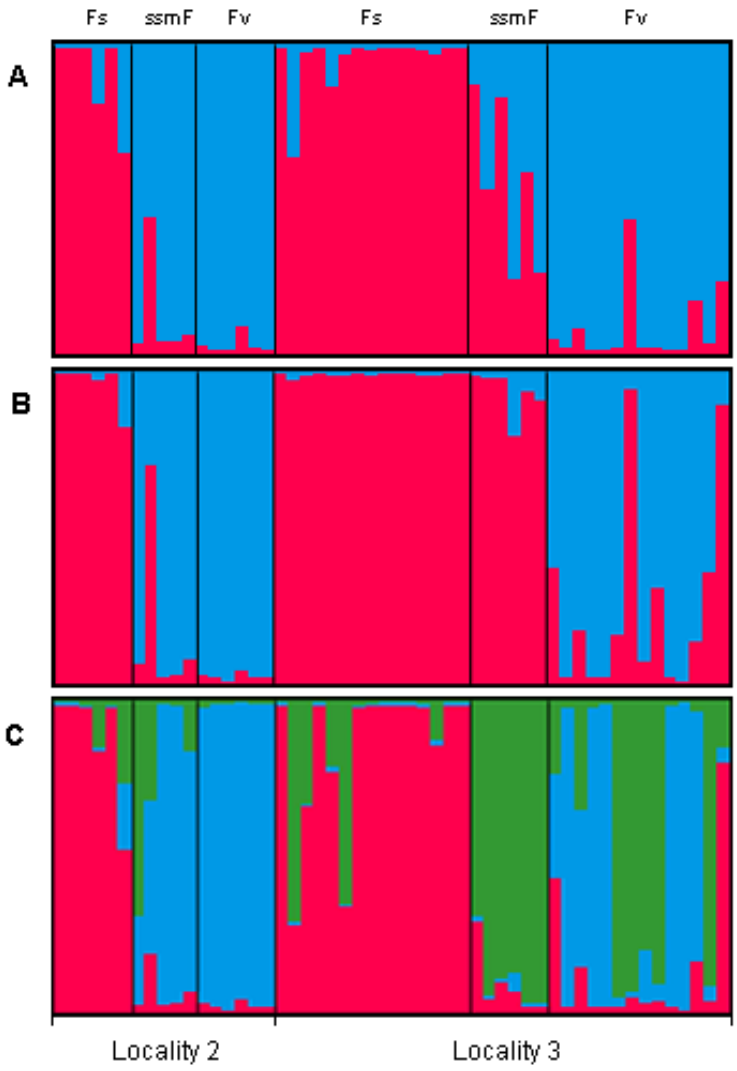

Figure 12.

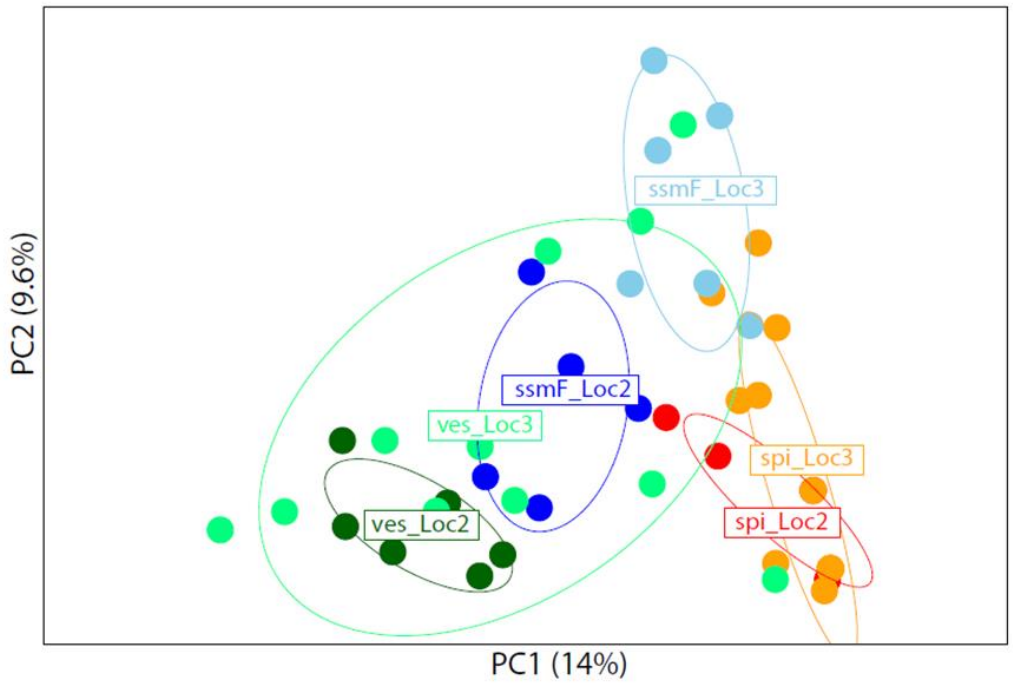
ssmF_Loc3
ssmF_Loc2
ves_Loc3
ves_Loc2
spi_Loc3
spi_Loc2
PC2 (9.6%)
PC1 (14%)

Figure 13.

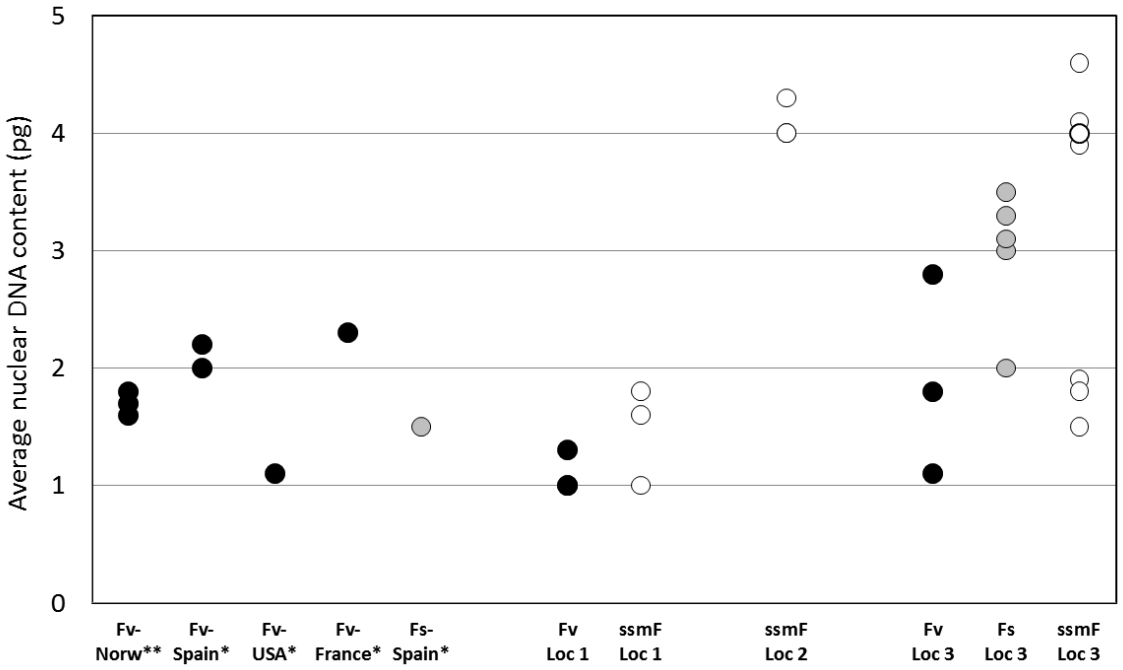
Average nuclear DNA content (pg)
5
4
3
2
1
0
Fv-Norw**
Fv-Spain*
Fv-USA*
Fv-France*
Fs-Spain*
Fv Loc 1
ssmF Loc 1
ssmF Loc 2
Fv Loc 3
Fs Loc 3
ssmF Loc 3

Figure 14.