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The edible brown seaweed *Alaria esculenta* (Phaeophyceae, Laminariales): hybridization, growth and genetic comparisons of six Irish populations

Stefan Kraan*, Adriana Verges Tramullas & Michael D. Guiry
Department of Botany, Martin Ryan Marine Science Institute, National University of Ireland, Galway, Republic of Ireland

(*Author for correspondence; phone +353-91-524411; fax +353-91-750539; e-mail stefan.kraan@seaweed.ie)

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Abstract

Alaria esculenta populations from six different geographical locations on the Irish coast were examined for hybridization abilities, growth rates and genetic make-up with a view towards identifying a fast-growing strain suitable for aquaculture. Hybridization experiments under laboratory conditions with the three most geographically dispersed populations showed that all cross combinations were interfertile, although differences were found in survival, and in blade and hapteron morphology. A comparison of relative growth rates showed significant differences amongst the self-crosses and hybrids. The data of the hybridization experiments and growth rates under laboratory conditions show that the best population for the purpose of seaweed aquaculture are the Slea Head and Corbet Head self-crosses and their hybrids. Genetic fingerprinting of the internal transcribed spacer of the ribosomal DNA of five *A. esculenta* isolates from geographically separated populations in Ireland revealed no restriction length polymorphisms between the tested isolates and show that the *A. esculenta* populations around the Irish coast are clearly genetically homogenous in respect of the DNA region examined. The genetic analysis, interfertility of the populations, morphology and growth rates are discussed with a view to potential cultivation.

Introduction

The brown alga *Alaria esculenta* (L.) Greville is a brown seaweed belonging to the family Alariaceae of the order Laminariales (kelp), plants of which can reach a length of 4 m in Ireland (Guiry, 1997). It is found in the North Atlantic as far north as the winter sea ice and as far south as the 16 °C summer isotherm on the Brittany coast (Lüning, 1990). Its absence in the southern North Sea and English Channel is like to be due to the high water temperatures of over 16 °C in summer, which it cannot survive (Munda & Lüning, 1977). The species is found on wave-exposed rocky shores all around the Irish coast.

Alaria esculenta was used in the past in both Scotland and Ireland for human consumption and fodder and was also gathered and spread on infertile land and used as fertiliser (Newton, 1931; Guiry & Hession, 1996; Guiry, 1997). It is rich in sugars, proteins, vitamins and other trace metals, and contains up to 42% alginic acid (Indergaard & Minsaas, 1991; Lewallen & Lewallen, 1996). The species is used for a cultivar of purposes from value-added sea-vegetables to fodder and bodycare products (Guiry & Blunden, 1991; Guiry, 1997). Recently, it has become of economic interest as a foodstuff in aquaculture for herbivorous molluscs, urchins, shrimp and fish (Yone et al., 1986; Moss, 1994; Stuart & Brown, 1994; Mai et al., 1996; Nakagawa et al., 1997), and strain selection will undoubtedly become important. So far, several cultivation trials with *A. esculenta* have been carried out with success on the Isle of Man (Kain & Dawes, 1987) and Ard Bay, Connamora, Ireland (Kraan & Guiry, unpublished), and harvest of 9 t ha^{-1} within 3 months is

possible, although no attention has been paid to strain selection. A study by Kraan & Guiry (2000) on hybridization in *Alaria* showed that four Atlantic strains of *A. esculenta* are able to hybridize among themselves and with some Pacific *Alaria* species.

Strain selection through hybridization or simply selecting the most suitable cultivars has been practised in a number of macroalgal genera, including *Porphyra*, *Chondrus* and *Gracilaria* (Patwary & van der Meer, 1992), *Laminaria* (Wu, 1998) and *Undaria* (Pang et al., 1997). In China, extensive studies on hybridization and inbreeding have led to genetically stable and improved strains of *Laminaria japonica* Areschoug. Large-scale production experiments showed that the new cultivars yield up to 40% more biomass and an iodine content 20–58% higher than control plants. These new cultivars were introduced to *Laminaria* farms and grown in large quantities in northern China (Wu, 1998).

The morphology of lamina and stipe in *A. esculenta* can vary widely between specimens mainly due to wave exposure, with broader lamina and longer stipes in more sheltered areas (Sundene, 1962; Widdowson, 1971). Phenotypic plasticity is a common feature of members of the Laminariales (Norton et al., 1982). Chapman (1974) found significant genetic differentiation between populations of *Laminaria* for stipe morphology. By contrast, Kraan & Guiry (2000) found negligible genetic variation after comparing Rubisco spacer sequences of six dispersed Atlantic *A. esculenta* populations. No genetic differences were found after a comparison of two morphologically distinct populations of the intertidal Pacific kelp *Costaria costata* (C. Agardh) Saunders using restriction fragment length polymormhisms (RFLPs; Bhattacharya & Druehl, 1989).

RFLPs are a common molecular tool to distinguish morphologically similar populations (Hillis et al., 1996). They have also been used in numerous algal studies (e.g. Bhattacharya & Druehl, 1989; Rice & Bird, 1990; Bhattacharya et al., 1991; Lehman & Manhart, 1997). The nuclear ribosomal DNA internal transcribed spacer regions (ITS1 and ITS2) are less conserved then the Rubisco spacer region and have been used to examine relationships among populations, isolates and species in red algae (e.g. Destombe & Douglas, 1991; Maggs et al., 1992; Goff et al., 1994), and in brown algae (e.g. Stache-Crain et al., 1997; Siemer et al., 1998).

The aims of this study were: (1) to isolate and grow strains of *Alaria esculenta* gametophytes from the Irish coast under laboratory conditions; (2) to hybridize and culture hybrid sporophytes and self crosses and screen for cultivars with optimal characteristics in order to identify a suitable strain for seaweed aquaculture in Ireland; (3) to detect genetic variation in the ITS1 and ITS2 spacers amongst geographical dispersed populations of the Irish coast.

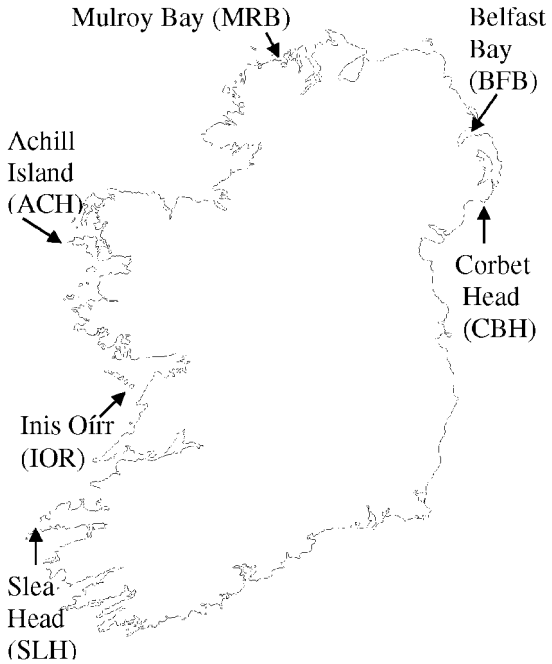

Figure 1. Ireland, showing the collection sites of *Alaria esculenta* indicated with an arrow.

Materials and methods

Species and gametophyte cultures

All *A. esculenta* plants were sampled in the low intertidal from exposed relatively isolated headlands or islands in April and May 1996. Sample locations are shown in Figure 1 and plants used for DNA extraction and hybridization studies are listed in Table 1. Male and female gametophyte cultures, isolated from zoospores collected from five healthy and large sporophytes, were obtained from all collected *A. esculenta* plants in accordance with South (1970) and Nakahara & Nakamura (1973). The *A. esculenta* male and female gametophyte cultures were kept in a vegetative state at 10 °C, 16:8 h L:D, 15 μmol m^{-2} s^{-1} in glass dishes containing sterile enriched seawater, as

Table 1. Origin of *Alaria esculenta* strains from Ireland used for DNA extractions and gametophyte cultures.

Species	Code	Origin
Alaria esculenta[a]	IOR	Trácht Each, Inis Oírr, Co. Galway
Alaria esculenta[a,b]	SLH	Slea Head, Co. Kerry
Alaria esculenta[a,b]	CBH	Corbet Head, Co. Down
Alaria esculenta[b]	BFB	Belfast Bay, Co. Down
Alaria esculenta[b]	ACH	Keem Beach, Achill Island, Co. Mayo.
Alaria esculenta[b]	MRB	Mulroy Bay, Co. Donegal

[a] Plants used for hybridization studies.
[b] Plants used for DNA extraction.

modified by Guiry & Cunningham (1984), which was changed monthly.

Hybridization studies

Hybridization experiments were carried out with gametophytes from Corbet Head (Co. Down), Slea Head (Co. Kerry), and Inis Oírr (Aran Islands, Co. Galway), by gently grinding the gametophytes of the relevant cross in a mortar to produce a suspension of male and female filaments 1–10 cells long (Lüning et al., 1978). Each suspension was poured into a Repli-dish (Sterilin, U.K.) consisting of 25 small squares, and incubated at 10 °C, 16:8 h L:D, 20 μmol m^{-2} s^{-1}. In addition, controls of 25 self-crosses and 25 isolated male and female gametophytes were incubated in parallel with each crossing experiment to identify possible parthenogenesis or apogamy (see Nakahara & Nakamura, 1973). After three weeks, the largest sporophyte in each of the 25 squares in the Repli-dish of each cross was selected and placed individually in 20-mL Petri dishes under the same conditions. The medium was changed fortnightly. The length of the sporophytes, 25 in total per cross combination, was measured weekly under a dissecting microscope. After 6–8 weeks the sporophytes, about 5 cm long, were transferred into 20-mL sterile Universal containers containing the same culture medium and placed on a rotary shaker at 45 rev. min^{-1}. After 10–12 weeks sporophytes with a length of 10 cm were transferred to small aquaria with an aeration system. Daylength conditions were changed to 14:10 h L:D, 30 μmol m^{-2} s^{-1} mimicking spring or autumn conditions to initiate fast growth of the developing sporophytes. After five months general appearance, survival, hapteron development and lamina length and width were recorded and measured with an image analyser or a clear ruler. To provide an indication of the shape of the hybrid sporophytes the ratio length:width was calculated (a high value indicating a long and thin sporophyte and a low value a broad and small sporophyte). The hybrid and self-cross specimens are preserved in the phycological herbarium, National University of Ireland, Galway (GALW).

Growth experiments

Relative growth rates (RGR) were determined at 10 °C for length and width of the 25 self-crosses and hybrid sporophytes of the hybridization experiments. Lengths were measured weekly using a clear ruler. RGR (% d^{-1}) was calculated for each plant during the exponential growth phase using the following equation: $RGR = \ln(l_2) - \ln(l_1) / t_2 - t_1 100\%$, where l_2 and l_1 are the lengths of the plant (mm) at days t_2 and t_1, respectively. The resulting data set was statistically examined by a one-way ANOVA to test the null hypothesis that all the relative growth rates in all crosses were not significantly different. If the null hypothesis was rejected multiple comparisons were made *a posteriori* using the Tukey HSD test for unequal sample sizes -Spjotvoll-Stoline test (Zar, 1996).

DNA extraction, PCR, and genetic fingerprinting

DNA was extracted from fresh sporophytic material of the *A. esculenta* isolates listed in Table 1, as described in Kraan & Guiry (2000). The intertranscribed spacers ITS1 and ITS2 of the ribosomal DNA were PCR amplified in an Omn-E thermal cycler (Hybaid Ltd, UK). Primer pair P1 and G4 were used to amplify the region in the ribosomal cistron (Figure 3) from position 1542 in the 18S across the ITS1, the 5.8S, and ITS2 to position 42 in the 26S (Saunders & Druehl, 1993), using the following temperature profile: a denaturation step of 95 °C for 3 min followed by 30

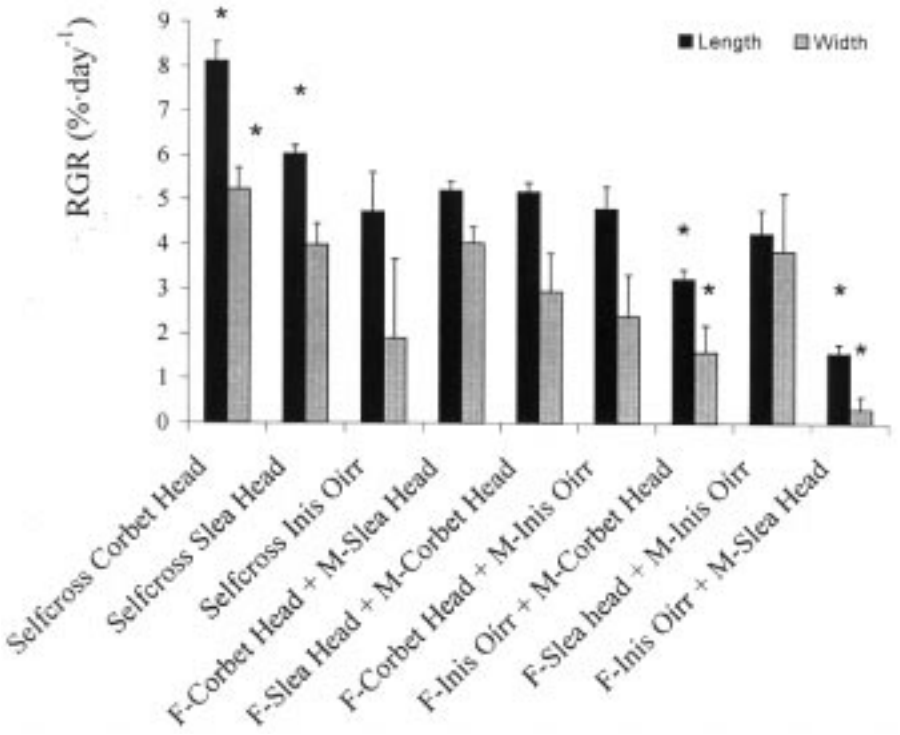

Figure 2. Mean relative growth rates (% day $^{-1}$) for length and width (with 95% confidence limits; n = 25) of the cross combinations between the three *Alaria esculenta* strains from Inis Oírr, Aran Islands, Co. Galway, Corbet Head, Co. Down and Slea Head, Co. Kerry, Ireland, grown at 10 °C, 14:10 h L:D, 30 μmol m^{-2} s^{-1}. F = female gametophyte, M = male gametophyte, * = significant difference.

cycles of 1 min 95 °C, 2 min 50 °C, and 2 min 72 °C, and ending with one extension step of 72 °C for 5 min. The reaction volume was 100 μL and comprised of 10–100 ng genomic DNA, 50 μM of each dATP, dNTP, dCTP and dGTP, 0.5 μM of each primer, 10 μL of 10x reaction buffer, 6 μL 25 mM mg$Cl_2$and 2.5 units of Taq (Sigma). Amplifications were checked for correct length, purity and yield on 1.5% agarose TAE gels stained with EtBr in accordance with the methods of Sambrook et al. (1989). Isolation of PCR products from amplification reactions was carried out using the High Pure PCR Product Purification Kit of Boehringer Mannheim according to the manufacturer's protocol.

Genetic fingerprinting of the *A. esculenta* isolates was performed with the RFLP technique using restriction endonucleases according to the manufacturer's protocol (Boeringer Mannheim). The following restriction endonucleases were applied: PST I, RSA I, MSP I, HAE III, CFO I, CLA I, SCA I, BAM HI, ECO RV, DRA I, TAQ I, XBA I, ALU I, ECO RI, BCL I.

Restriction fragments were run on 3% TAE agarose gels at 20 V for 20 h and stained afterwards with EtBr. The stained restriction fragments were visualised by UV-fluorescence, captured with a video camera and printed with a thermal printer. Fragment sizes were determined according to standard methods (Hillis et al., 1996). All digestions were performed twice to assure accurate calculation of size of the fragments. Presence and absence of restriction fragments were scored manually from the thermal prints and a presence/absence matrix was constructed for all individuals.

Results

Hybridization experiments

The results of the hybridization experiments, shown as survival, hapteron development, ratio length:width and lamina length and width between gametophytes from Corbet Head (Co. Down), Slea Head (Co. Kerry), and Inis Oírr (Aran Islands, Co. Galway), are given in Table 2; parental codes are listed in Table 1. All plants crossed successfully only at 10 °C. The most successful crossings in terms of survival, growth, and hapteron development are the male Slea Head with

Table 2. Hybridization matrix of three Irish *Alaria esculenta* populations. Success of the crossings in percentages after a 5-month cultivation period is indicated as morphology, maximum length (in mm) and mean length:width ratio that was achieved during cultivation (n = 25) L = length, W = width + = over 3 roots as haptera, +/– = 1–3 roots as haptera, – = no haptera. For origin codes see Table 1.

Female gametophyte	Male gametophyte		
	CBH	SLH	IOR
CBH	56%, +/–	100%, +	67%, +/–
	L: 590, W: 11	L: 610, W: 35	L: 440, W: 35
	Ratio: 60.4	Ratio: 33.2	Ratio: 21
SLH	69%, +	88%, +	20%, +/–
	L: 520, W: 22	L: 660, W: 26	L: 75, W: 3.5
	Ratio: 28.9	Ratio 37.8	Ratio: 22.3
IOR	37%, +	13%, +/–, –	23%, –
	L: 660, W: 32	L: 210, W: 13	L: 235, W: 18
	Ratio: 25.2	Ratio 20	Ratio: 20.4

female Corbet Head cross, the Slea Head self-cross, and the Corbet Head self-cross. The least successful crossings are the Inis Oírr self-cross and the female Inis Oírr with male Slea Head cross. Sporophytes derived parthenogenically in the female parallel cultures were easily identified because of their small, round, clump-like appearance and the lack of a hapteron.

Relative growth rates

The RGR for length and width with 95% confidence intervals of the sporophytes of the cross combinations between Corbet Head (Co. Down), Slea Head (Co. Kerry) and Inis Oírr (Aran Islands, Co. Galway) are shown in Figure 2. The null hypothesis for similarity of RGR for length and width amongst the cross combinations was rejected (length: H = 62.25, F(0.01)(8)(61) = 2.82, $p < 0.001$; width: H = 10.53, F(0.01)(8)(61) = 2.82, $p < 0.001$). The Corbet Head and Slea Head self-cross showed a significantly faster RGR for length than all other cross combinations. The female Inis Oírr with male Corbet Head and female Inis Oírr with male Slea Head hybrids showed a significantly slower RGR for length compared with the other cross combinations, which did not differ significantly from each other. The RGR for width was significantly faster in the Corbet Head self-cross and significantly slower in the female Inis Oírr with male Corbet Head and female Inis Oírr with male Slea Head hybrids.

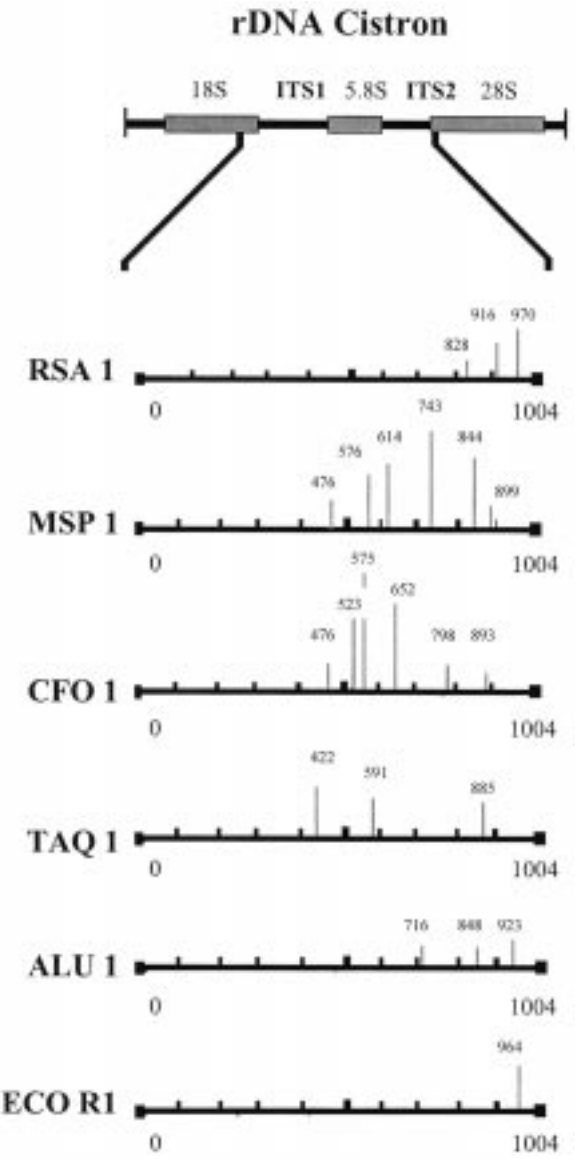

Figure 3. Schematic view of the rDNA cistron and the regions amplified. The restriction fragments produced by the six informative enzymes are plotted in the spacer region. The patterns were the same for all isolates of the five tested *Alaria esculenta* strains.

Genetic fingerprinting

PCR amplification of the ITS 1 and ITS 2 region resulted in a single product of 1004 bp. Of the 15 restriction enzymes used, only Rsa I (4 fragments), Msp I (7 fragments), Cfo I (7 fragments), Taq I (4 fragments), Alu I (4 fragments) and Eco RI (2 fragments) proved informative and produced 28 scorable bands (Figure 3). The total number of base pairs recognised by the

six informative REs was 116 bp, which is 11.6% of the amplified spacer region.

Comparison of the restriction enzyme digestion patterns of the five tested isolates of *A. esculenta* showed no polymorphisms, resulting in similar patterns (data not shown).

Discussion

The results presented in this study for crosses between *A. esculenta* from different geographical locations on the Irish coast show that the three strains are interfertile, which is in agreement with Kraan & Guiry (2000), who showed that *A. esculenta* populations throughout the North Atlantic Ocean were interfertile. The morphologically best and fastest-growing crosses under laboratory conditions were produced by the Slea Head and Corbet Head self-crosses and are probably most suitable for field cultivation. None of the crosses with Inis Oírr gametophytes produced sporophytes with well-developed haptera in the cultivation tanks and therefore are not directly suitable for rope cultivation in Atlantic Irish waters. Slower growing but morphologically healthy hybrids were produced between crosses of Slea Head and Corbet Head gametophytes.

Hybridization and strain selection with *Undaria pinnatifida* (Harvey) Suringar gametophytes from different locations in Japan and China produced F1 sporophytes with substantial size and weight variation (Pang et al., 1997) These authors found that some self-crosses resulted in plants with the largest weight and longest size as well as plants with the lowest weight and smallest size, similarly to what was observed in the *A. esculenta* self-crosses during the present study. It should be noted, however, that these results are laboratory tank cultivation results and do not reflect field conditions.

The restriction fragment analysis of *A. esculenta* isolates from around the Irish coast, showed that they were genetically identical in respect of the DNA region examined. A study on the Rubisco spacer sequences of *A. esculenta* isolates from elsewhere in the North Atlantic confirms the results of this study (Kraan & Guiry, 2000). These authors found a maximum three-base-pair difference amongst *A. esculenta* isolates from Halifax, Canada and Inis Oírr, Ireland, showing negligible genetic variation. By contrast, Coyer et al. (1997) showed distinguishable biogeographic populations along the north-east Pacific coast for the kelp *Postelsia palmaeformis* Ruprecht; however, for ecological reasons, gene flow may be more restricted in this species. These authors showed decreasing genetic relatedness with increasing distance of 25 m or more between populations assessed with M13 Fingerprinting and 16 to 250 km with RAPDs. Bhattacharya et al. (1990) showed with RFLPs 8 distinct populations of *C. costata* over a range of 400 km. They showed that these populations appeared to be discreet breeding groups.

Despite relative isolation of individual *A. esculenta* populations at headlands or islands around the Irish coast, differences observed in the hybridization experiments are not likely to be caused due to genetic variation between the populations considered. The different survival rates and growth rates encountered in the self-crosses are most probably caused due to ecotypic variation such as different temperature tolerance of the gametophyte and/or sporophyte and temperature responses of growth (see Breeman, 1988). The summer and winter isotherm at the sample location Slea Head is consistently 2–3 °C higher than that at the Corbet Head Location (U.S. Navy, 1981). Consequently, it is possible that local and genetically fixed temperature ecotypes have evolved in *A. esculenta*. The performance of field trials may be helpful in assessing strain selection, although these are time consuming and a lot of variation may be encountered (Pang et al., 1997).

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