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Article *in* Hydrobiologia · June 1993

DOI: 10.1007/BF00049005

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An examination of the population genetics of *Laminaria* and other brown algae in the laminariales using starch gel electrophoresis

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Abstract

While some investigators have attempted to use isozyme electrophoresis to gain information on the genetics of brown algae, most have reported unsatisfactory results. Through exhaustive screening and modification of sample preparation techniques, gel and tray buffers systems, plus staining recipes, we have developed procedures that consistently provide scorable bands for over 20 enzyme systems in several laminarian algae. We have used our procedures to examine geographically diverse populations of *Laminaria saccharina* and *L. longicruris*, as well as *L. digitata*, *L. groenlandica*, *Agarum cribrosum*, *Alaria esculenta*, *Chorda tomentosa*, and *Macrocystis pyrifera*. Overall, these kelp species seem to have an extremely low degree of enzyme polymorphism, both within and between populations. While some 'rare alleles' occurred in several enzyme systems, only 3–5 loci were found to be polymorphic. Our results are consistent with the few reported studies that have used molecular genetic techniques to look at the intraspecific variability of laminarian algae. We suggest that at the species level the Laminariales, and perhaps other groups of brown algae, are genetically extremely conservative as compared to other divisions of plants. We further suggest that isozyme electrophoresis provides a quick and useful tool for algal population genetic studies.

Introduction

The Laminariales (Phaeophyta) is an ecologically and commercially important group of marine macrophytes. The large brown algae, collectively known as kelps, are a major source of primary production in temperate coastal waters and they provide substratum and cover for a host of other marine organisms (Mann, 1972; Kain, 1979). Organic matter from kelp enters the food chain through grazing organisms, detrital cycles, and the release of dissolved organic matter (Laycock, 1974; Griffiths & Stenton-Dozey, 1981; Robinson *et al.*, 1982). Kelps are used commercially as an important source of the phycocolloid algin, as liquid seaweed agricultural fertilizers, as livestock feed supplements, and as a direct source of human food (Waaland, 1981).

Until recently the taxonomic and evolutionary relationships of laminarian algae have been poorly understood (Kain, 1979). That is, due to their morphological plasticity and interfertility, taxonomic delineations of several kelp species have been confusing (Chapman, 1974; Mathieson

et al., 1980; Yarish *et al.*, 1990). Recent interfertility studies have demonstrated that interspecific and possibly intergeneric hybridization may occur (Lüning *et al.*, 1978; Mathieson *et al.*, 1981; Bolton *et al.*, 1983; Innes, 1984; Egan *et al.*, 1989; Neushul, 1989). For example, Cosson & Olivari (1982) describe interspecific hybrids of *Laminaria digitata* with *L. saccharina* and *L. ochroleuca* and between each of these and *Saccorhiza polyschides*. Cosson (1987) notes that these hybrids are incapable of producing another generation of normal sporophytes. In discussing such intergeneric kelp hybrids Neushul (1989) suggests that it is possible that seemingly major morphological differences between genera may be due to relatively minor genetic differences.

Several recent molecular investigations of kelps have been conducted. Stam *et al.* (1988) employed DNA-DNA hybridizations between single-copy nuclear DNA from *Laminaria digitata*, as well as total DNA from *L. saccharina*, *L. hyperborea*, *L. rodriguezii*, *L. ochroleuca* and *Chorda filum*. They found that the various *Laminaria* species were closely related genetically, while *C. filum* was only distantly related to *L. digitata*. Druehl (1989) employed restriction fragment length polymorphism (RFLP) of kelp chloroplast DNA to evaluate phylogenetic relationships within the Laminariales. Saunders & Druehl (1991) used analogous RFLP techniques on eight Northeast Pacific kelps and found that they were highly conserved, as only three different restriction-site differences were observed among all eight taxa. Using RFLP Bhattacharya & Druehl (1988, 1989) found only one restriction-site difference between two morphologically distinct populations of *Costaria costata*. In two other molecular DNA studies Lim *et al.* (1986) and Hori & Osawa (1987) compared the ribosomal RNA contents of *Eisenia bicyclis* to other brown algae, plus other divisions and phyla.

Although DNA extraction techniques (cf. Stam *et al.*, 1988; Saunders & Druehl, 1991) may give higher genetic resolution than starch gel electrophoresis, the latter method has many advantages, particularly if large numbers of individuals and enzymes are to analyzed (Kephart, 1990). As a consequence, electrophoresis has been extensively used to measure both intra- and interspecific genetic variability within diverse populations of animals (Meizel & Markert, 1967; Lewontin, 1974; Avise, 1975) and vascular plants (Mitton *et al.*, 1977; Soltis *et al.*, 1983; Tansley & Orton, 1983). Isozyme electrophoresis has been used less frequently to investigate the genetic similarity between algal species (Mathieson *et al.*, 1981). Blair *et al.* (1982), Innes (1984, 1987), and Innes & Yarish (1984) investigated genetic variation between species of green algae, while Cheney and Babbel (1978) and Cheney & Mathieson (1979) examined species of red algae. Marsden *et al.* (1981, 1984) and Rice & Crowden (1987) have described polyacrylamide gel electrophoretic techniques (PAGE) for use with brown macrophytes.

Like DNA extraction techniques, sample preparation and extraction procedures for PAGE makes it difficult to use for large scale population assessments. The primary advantages of starch versus polyacrylamide gel electrophoresis are five-fold: 1) simplicity of starch gel preparation; 2) use of less toxic chemicals (the acrylamide in PAGE is a neurotoxin); 3) reduced costs of equipment and supplies; 4) ease of sample loading; and 5) a greater amount of data obtainable per gel. That is, each gel can be cut into 5 horizontal slices, allowing a gel to be stained for 5 enzymes. As a result of these advantages, starch gel isozyme electrophoretic techniques have recently been perfected with macroalgae (Penniman *et al.*, 1985; Penniman, 1987). Typically extraction of enzymes from brown seaweeds is difficult due to the presence of high levels of polyanionic polysaccharides (*i.e.* algin and fucoidan) and polyphenolics that freely bind proteins. A protective extraction buffer used for conifer studies (Gagnon *et al.*, 1988; Mitton *et al.*, 1977, 1979) has been successfully modified for use with brown macrophytes (Penniman *et al.*, 1985), including kelp tissues.

The overall objectives of the present study were to further refine starch gel electrophoresis techniques for kelp, increase the number of enzyme systems that can be scored, and to use these pro-

cedures to examine the population genetics of *Laminaria longicruris*, *L. saccharina* and several other kelp species.

Materials and methods

Collections of *Agarum cribrosum* (Mertens) Bory, *Alaria esculenta* (L.) Greville, *Chorda tomentosa* Lyngbye, *Laminaria digitata* (Hudson) Lamouroux, L. *groenlandica* Rosenvinge, *L. longicruris* De la Pylaie, *L. saccharina* (L.) Lamouroux, and *Macrocystis pyrifera* (L.) C. Agardh were obtained from a variety of northwest Atlantic and northeast Pacific sites in order to compare interspecific variation and geographical differences (intraspecific) of several taxa (Table 1). *Laminaria* collections were made from shallow subtidal and deep water coastal locations and from estuarine sites. Typically 30–60 individuals were collected from each sampling location. Samples were kept refrigerated or on ice until protein extraction was completed. Proteins were extracted from each sample by grinding 3.0 cm^2 pieces of meristematic tissues (0.5 g each) in a mortar and pestle with liquid nitrogen. 1–2 ml of modified Mitton extraction buffer (Mitton *et al.*, 1979; also see Table 2) was added to the mortar and mixed with the macerated sample before it thawed. The extract was then absorbed onto paper wicks (Whatman 3MM). Wicks that were not electrophoresed immediately were placed in an ultralow freezer (−80 °C).

The wicks were electrophoresed in a 12% (w/v)

Table 1. Source and sample size of Kelp collections

Taxa	*n*	Location
Agarum cribrosum	50	Isles of Shoals, New Hamphire, USA
	30	Halifax Harbor, Nova Scotia, Canada
	20	Cape Neddick, Maine, USA
Alaria esculenta	60	Isles of Shoals, New Hampshire, USA
	36	Fink (Sandy) & Morris Pt, Nova Scotia, Canada
Chorda tomentosa	30	Portsmouth Harbor, New Hampshire, USA
	30	Fink (Sandy) Cove, Nova Scotia, Canada
Laminaria digitata	20	Fink (Sandy) Cove, Nova Scotia, Canada
	74	Dover Point, Little Bay, New Hampshire, USA (estuarine)
	62	Jaffrey Point, Newcastle Island, New Hampshire, USA
	20	Smuttynose Island, Isles of Shoals, Maine, USA
	10	Black Ledge, Connecticut, USA
Laminaria groenlandica	60	Friday Harbor, San Juan I., Washington, USA
Laminaria longicruris	116	Yarmouth, Nova Scotia, Canada
	64	Portsmouth Harbor, New Hampshire, USA
	14	Dover Point, Little Bay, New Hampshire, USA (estuarine)
	73	Black Ledge, Connecticut, USA
Laminaria saccharina	47	Yarmouth, Nova Scotia, Canada
	20	Isles of Shoals, New Hampshire, USA
	42	Rye, New Hampshire, USA
	84	Jaffrey Point, Newcastle Island, New Hampshire, USA
	44	Portsmouth Harbor, New Hampshire, USA
	91	Dover Point, Little Bay, New Hampshire, USA (estuarine)
	108	Black Ledge, Connecticut, USA
Macrocystis pyrifera	50	Leo Carillo Beach, Malibu, California, USA
	50	San Clement Island, Channel Islands, California, USA

Table 2. Extraction buffer (after Mitton *et al.*, 1979)

Substance	Amount
Geramium dioxide	0.93 g
0.16 M phosphate buffer, pH 7.0	88 ml
Cysteine	0.025 g
Diethyldithiocarbamic acid	3.0 g
Sodium ascorbate	44.0 g
Sodium metabisulfite	3.3 g
Sodium borate	12.1 g
Polyvinyl pyrrolidone (PVP 40)	44.0 g
Bovine serum albumin	1.0 g
EDTA	1.5 g
Glutathione, reduced	9.0 g
Glycerol	120 ml
NAD	0.01 g
NADP	0.01 g
Dimethyl sulfoxide (DMSO)	4.4 ml

(= 950 ml and pH 7.2)

starch gel made from Sigma starch (Sigma Chemical Co., St. Louis, Mo.). Thirty samples were run on each gel in groups of five separated by spacer wicks for ease of scoring. Electrophoresis procedures were essentially those described by Gagnon *et al.* (1988) for conifer foliage. Electrophoresis was carried out under refrigeration at 4 °C on the ten buffer systems outlined in Table 3. Wicks were inserted into a slit (*i.e.* the origin) made across the gel approximately 3 cm from the cathodal edge and were removed from the gels 4 minutes after the voltage was applied. Gels were run until the front migrated approximately 8 cm (Conkle B2, A and Ryu #3) (4–5 hours at 35–65 mA) or until the brilliant blue G/amaranth marker dye migrated a similar distance (B, D, E, Shaw I & V, S10 and HC7). See Table 3 for abbreviations of different buffer systems. After electrophoresis, five horizontal slices per gel were stained (Table 4) and incubated in the dark at 37 °C.

In an effort to find a sufficient number of scorable enzymes for the study, we screened staining recipes for a total of 44 different enzymes on each of the 10 buffer systems for each of the 8 species.

Results

After exhaustive screening and modification of sample preparation techniques, gel and tray buffer systems, and staining recipes we have developed procedures (Tables 2–4) that can provide scorable bands for 20 enzymes within these different taxa. Of these 20 enzymes, 14 produce single, monomorphic bands within all or nearly all species with scorable activity. The remaining 6 enzymes produced polymorphic bands from at least some of the species examined. Because many of our buffer systems do not produce a well defined 'front', the 'RF' values in the results presented below are calculated relative to the migration distance of bands resolved for 'standard' *Laminaria longicruris* plants that were run on all gels.

Table 3. Electrophoretic buffer systems.

Symbol	Buffer	Gel pH	Tray pH	Source
A	Lithium borate	8.5	8.1	Ridgeway *et al.*, 1970
B	Histidine citrate	5.7	5.7	Stuber *et al.*, 1977
D	Morpholine citrate	6.1	6.1	Clayton & Tretiak, 1972
E	Morpholine citrate	8.1	8.1	Clayton & Tretiak, 1972
Conkle B2	Sodium borate/citric acid	8.8	8.1	Conkle *et al.*, 1982
HC7	Histidine citrate	7.0	7.0	Fildes & Harris, 1966
S10	Tris EDTA borate	8.6	8.6	Soltis *et al.*, 1983
Shaw I	Tris citrate III	7.0	7.0	Meizel & Markert, 1967
Shaw V	Tris citrate II	8.0	8.0	Shaw & Prasad, 1970
Ryu #3	Sodium borate/citric acid	7.7	7.5	Mitton *et al.*, 1977

Table 4. Electrophoresis stains and abbreviations.

E.C. #	Abbrev.	Enzyme	Source
2.6.1.1.	ATT	Aspartate Aminotransferase	Marty *et al.*, 1984
4.2.1.3.	ACO	Aconitase	Marty *et al.*, 1984
4.1.2.13.	ALD	Aldolase	Marty *et al.*, 1984
1.2.1.2.	FDH	Formate Dehydrogenase	Soltis & Soltis, 1989
1.1.1.8.	aGPD	a-Glycerophosphate Dehydrogenase	Buth, 1984
1.2.1.12.	G3PDH	Glyceraldlehyde-3-Phosphate Dehydrogenase	Soltis & Soltis, 1989
1.1.1.49.	G6PDH	Glucose-6-Phosphate Dehydrogenase	Marty *et al.*, 1984
1.1.1.42.	IDH	Isocitrate Dehydrogenase	Marty *et al.*, 1984
1.1.1.37.	MDH	Malate Dehydrogenase	Marty *et al.*, 1984
1.1.1.40.	ME	Malic Enzyme	Marty *et al.*, 1984
5.3.1.8.	MPI	Mannose Phosphate Isomerase	Buth, 1984
1.1.1.44.	6PGD	6-Phosphogluconic Dehydrogenase	Marty *et al.*, 1984
5.3.1.9.	PGI	Phosphoglucose Isomerase	Marty *et al.*, 1984
5.4.2.2.	PGM	Phosphoglucomutase	Marty *et al.*, 1984
1.11.1.7.	PER	Peroxidase	Shaw & Prasad, 1970
1.3.99.1.	SDH	Succinate Dehydrogenase	Buth, 1984
1.1.1.24.	SKD	Shikimic Dehydrogenase	Marty *et al.*, 1984
5.3.1.1.	TPI	Triosephosphate Isomerase	Soltis & Soltis, 1989
2.7.7.9.	UGPP	Uridine Diphosphologlucose Pyrophosphorylase	Marty *et al.*, 1984
1.1.1.204.	XHD	Xanthine Dehydrogenase	Buth, 1984

Monomorphic enzymes

6-Phosphogluconic dehydrogenase (6PGD) E.C. 1.1.1.44

On buffer system E, 6-PGD staining produced a single band for all samples of all 8 species. *Laminaria longicruris* and *L. saccharina* had identical RFs (1.0). Some of the samples of *L. groenlandica* (about 10%) also had an RF of 1.0, although a second, more common, electromorph was found with a single band RF = 0.95 rather than 1.0. The band for *L. digitata* had an RF of 0.93. The remaining species had RFs faster than *L. longicruris*; *Alaria esculenta*'s band had an RF = 1.10, *Chorda tomentosa* had an RF = 1.17, and *Macrocystis pyrifera* produced a single band with an RF of 1.07.

Aspartate aminotransferase (AAT) E.C. 2.6.1.1

Scorable bands were found for AAT on buffer system E for 4 of the 8 species. The RF for the single band produced by *Laminaria saccharina* samples was identical to that for *L. longicruris*. *Macrocystis pyrifera* had a single band that was faster than *Laminaria*'s with an RF = 1.07. *Alaria esculenta* had a single band with an RF of 1.18.

Aconotase (ACO) E.C. 4.2.1.3

Aconotase produced a very sharp single band with low to moderate activity on gels using the E buffer system. All 4 of the *Laminaria* species as well as *Agarum cribrosum* had identical RFs (1.0). The other three species have not been scored.

Aldolase (ALD) E.C. 4.1.2.13

Alaria esculenta, *Laminaria longicruris* and *L. saccharina* had single ALD bands with the same RF (1.0) on buffer system E. The RF for *Macrocystis pyrifera* was 0.94 and for *Agarum cribrosum* it was 0.96. *L. groenlandica* and *L. digitata* each had RF's faster than *L. longicruris*, the values were 1.07 and 1.11, respectively.

Glyceraldlehyde-3-phosphate dehydrogenase (G3PDH) E.C. 1.2.1.12

On the Shaw V buffer system, *Laminaria longicruris*, *L. saccharina*, *L. digitata*, and *L. groenlandica* produced single G3PDH bands with RF = 1.0;

Agarum cribrosum had the slowest migration rate RF = 0.77, and *Macrocystis* had an RF of 0.93. We have no results for the remaining two species.

Glucose-6-phosphate dehydrogenase (G6PDH) E.C. 1.1.1.49

On the SHAW V buffer system, G6PDH produces a single, well defined band with good activity for all 8 species, but only three species *Chorda tomentosa*, *Laminaria saccharina* and *L. longicruris* had identical RFs (Fig. 1). The band for *Agarum cribrosum* had the slowest RF, 0.76; *L. digitata* was at RF = 0.83, *Alaria esculenta* RF = 0.86, *L. groenlandica* RF = 0.96, and *Macrocystis pyrifera* RF = 0.97.

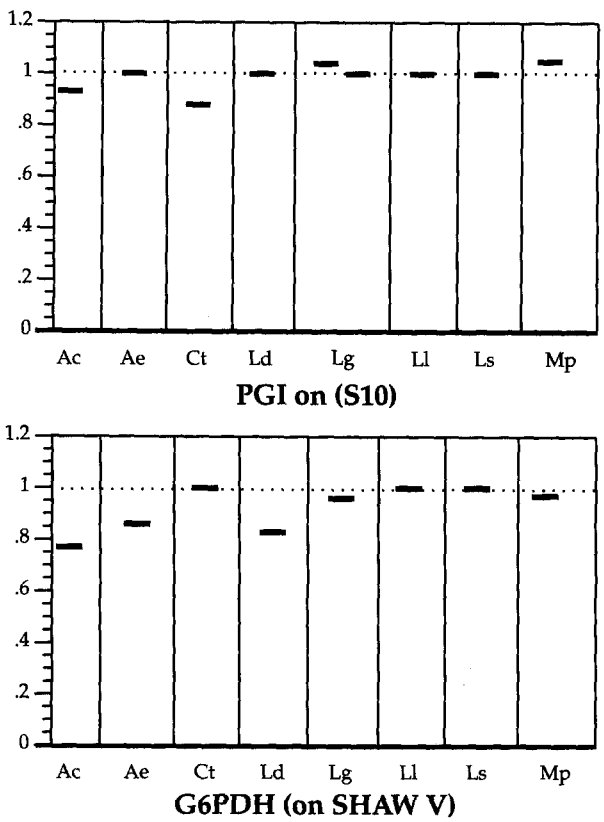

Fig. 1. Band patterns for PGI and G6PDH from all eight species in the study. The vertical axis indicates electrophoretic migration distance of bands for each species relative to *Laminaria longicruris*. Ac = *Agarum cribrosum*, Ae = *Alaria esculenta*, Ct = *Chorda tomentosa*, Ld = *Laminaria digitata*, Lg = *Laminaria groenlandica*, Ll = *Laminaria longicruris*, Ls = *Laminaria saccharina*.

Phosphoglucose Isomerase (PGI) E.C. 5.3.1.9

PGI stains extremely well on nearly all of our gel buffer systems, and produces a single monomorphic band with good resolution for seven of the eight species (Fig. 1), *Laminaria groenlandica* being the exception. The single bands for *L. saccharina*, *L. digitata*, and *Alaria esculenta* had RFs identical to *L. longicruris*. Two electromorphs were found for *L. groenlandica*, each had a single band, one was identical to *L. longicruris*, but the second had a faster RF (1.04); 71% of the samples had the faster band. *Agarum cribrosum* and *Chorda tomentosa* both had slower bands than *L. longicruris*, with RFs of 0.93 and 0.88, respectively. *Macrocystis pyrifera* had a single band with an RF of 1.05 for all samples.

Triosephosphate isomerase (TPI) E.C. 5.3.1.1

Laminaria longicruris and *L. saccharina* produced single identical TPI bands. *L. digitata* and *Agarum cribrosum* both had slightly lower migration distances with RFs of 0.98 and 0.97, respectively. The RF for the *Alaria esculenta* band was 1.02. Data for this enzyme have not been collected for the remaining 3 species.

Formate dehydrogenase (FDH) E.C. 1.2.1.2

Laminaria longicruris, *L. saccharina*, *L. digitata*, *L. groenlandica* and *Alaria esculenta* all produced a single band with the same RF (1.0). Data have not been collected for the remaining 3 species.

Mannose phosphate isomerase (MPI) E.C. 5.3.1.8

Identical single bands were found for *Laminaria longicruris*, *L. saccharina*, and *L. digitata*. The band for *Alaria esculenta* had an RF of 1.13. Once again, two different electromorphs of *L. groenlandica* were found, each had a single band, but with different RFs: 0.88 and 0.94; the slower band was more common and appeared in 80% of the samples.

Polymorphic Enzymes

Malate dehydrogenase (MDH) E.C. 1.1.1.37

Staining for MDH on buffer system Shaw V produced polymorphic banding patterns for *Alaria*

esculenta, *Laminaria digitata*, *L. longicruris* and *L. saccharina* (Fig. 2). *Chorda tomentosa* was monomorphic for MDH, exhibiting a single band with an RF = 1.0. The remaining species have not been scored. Three electromorphs were found for *A. esculenta*, one with a single band at RF = 0.72, one with two bands RF = 0.72 and 0.92, and a third type with only the faster (0.92) band. The frequencies of the three types were 0.05, 0.85, and 0.10 respectively. Four banding patterns were found for *L. digitata*, 3 of the electromorphs had only single bands with different RF's: 1.0, 0.94, and 0.60. The fourth type had 2 bands, RF's 0.74 and 1.0. *Laminaria longicruris* and *L. saccharina* had 3 different banding patterns, the most common type for both species being a single band at RF = 1.0. The second type, with a single band with RF = 0.95, occurred in 26% of *L. longicruris*

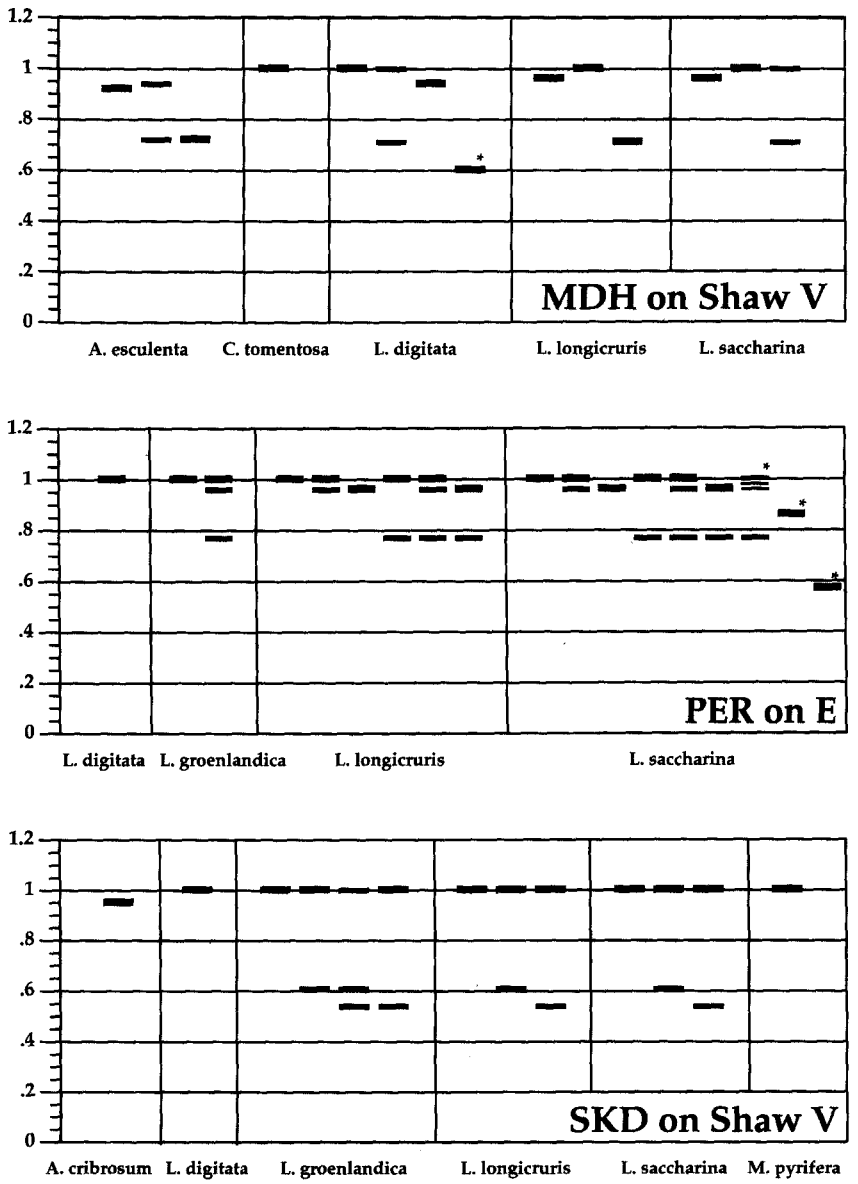

Fig. 2. Band patterns for MDH, PER, and SKD. The vertical axis indicates electrophoretic migration distance of bands for each species relative to *Laminaria longicruris*. (*) indicates a rare electromorph (< 1% occurrence).

samples and 9% of *L. saccharina* samples. The third variant in *L. longicruris* was a single band with an RF of 0.71; it represented <3% of the population sample. The same slow band (0.71) was also found in *L. saccharina*, but was always accompanied by the RF = 1.0 band; it was found in 7% of *L. saccharina* samples.

Peroxidase (PER) E.C. 1.11.1.7

Using buffer system E, *Laminaria digitata* samples produced a single monomorphic PER band with RF = 1.0 (Fig. 2). *L. groenlandica*, *L. longicruris*, and *L. saccharina* exhibited two zones of activity on the gels. The slower zone in all three species comprised a single band with an RF of 0.77. This band appeared in 50% of the *L. groenlandica* samples, 9% of the *L. longicruris* samples, and 22% of *L. saccharina*. The second zone of activity consisted of two bands, RF = 0.96 and RF = 1.0, which occurred either singly or together. In *L. groenlandica* all samples had either the faster band (RF = 1.0) alone or both bands. In *L. longicruris* 40% had only the faster band, 26% had both bands, and the remaining 34% had only the slower band. Three additional rare (<1.0%) variants were found in *L. saccharina* and are included in Fig. 2.

Shikimic dehydrogenase (SKD) E.C. 1.1.1.24

Using the Shaw V buffer system, SKD bands were scored for 6 of the 8 species. *Agarum cribrosum* had a single fixed band at RF = 0.95. All 4 *Laminaria* species and *Macrocystis pyrifera* had a fixed band at RF = 1.0 in all samples. *Laminaria groenlandica*, *L. longicruris* and *L. saccharina* had a second region of staining activity, with a bands at RF = 0.54 and RF = 0.61. All three possible combinations of the two slower bands were found in *L. groenlandica*, but the faster (RF = 0.61) band alone was the most common type (71%). In *L. longicruris* and *L. saccharina* all samples had only one or the other of the two slower bands, with the RF = 0.54 band being more common in either species.

Malic Enzyme (ME) E.C. 1.1.1.40

Using the S10 buffer system, ME staining produced bands for *Laminaria longicruris* and *L. saccharina* in three zones on the gels (Fig. 3). The fastest zone had a fixed band at RF = 2.11. This band appeared in about 50% of the samples of both species and was absent from the rest. In the middle zone, there was one band (RF = 1.0) that occurred in all *L. longicruris* samples and 93% of the *L. saccharina* samples. A second band, at RF = 1.21, occurred in the zone for about half the samples of either species. Two bands stained in the third zone, one with an RF = 0.37, the other RF = 0.42. The faster of the 2 bands occurred individually in both species. The slower and faster bands occurred together, only in *L. longicruris*. The slower band occurred alone only in *L. saccharina*.

Isocitrate dehydrogenase (IDH) E.C. 1.1.1.42

Using buffer system E, we recorded banding patterns for IDH in all 8 species (Fig. 3). All *Agarum cribrosum* samples produced a band with RF = 0.95, and 50% of those had a second, slower band at RF = 0.47. *Alaria esculenta* samples also produced a band at RF = 0.95 and for a small percentage of the samples, a second band is visible with an RF = 1.0. All *Chorda tomentosa* scored to date have three bands with RFs 0.97, 1.0 and 1.03. *Laminaria digitata* had a band at RF = 1.06 that was sometimes accompanied by an additional band at 1.07 and/or another at 1.0. *Laminaria groenlandica* always has a band at RF = 0.80 or at 0.88, a second band at RF = 1.0 may or may not be present, and a third band RF = 1.40 has been found only in combination with the RF = 0.88 band. All of the *Macrocystis pyrifera* samples had a single band with an RF = 0.90.

IDH banding patterns for *Laminaria longicruris* and *L. saccharina* seemed to vary with collection location. All samples from Nova Scotia had either or both of two bands, with RF = 0.91 and 1.0. In *L. longicruris* from Nova Scotia, the form with both bands is most common (70%), while most Nova Scotian *L. saccharina* had only the faster band (72%). All Connecticut samples of *L. longicruris* scored to date had both of the bands found in the Nova Scotian samples, plus a third band with an RF = 1.08. Connecticut *L. saccharina* had the 2 bands found in the Nova Scotian

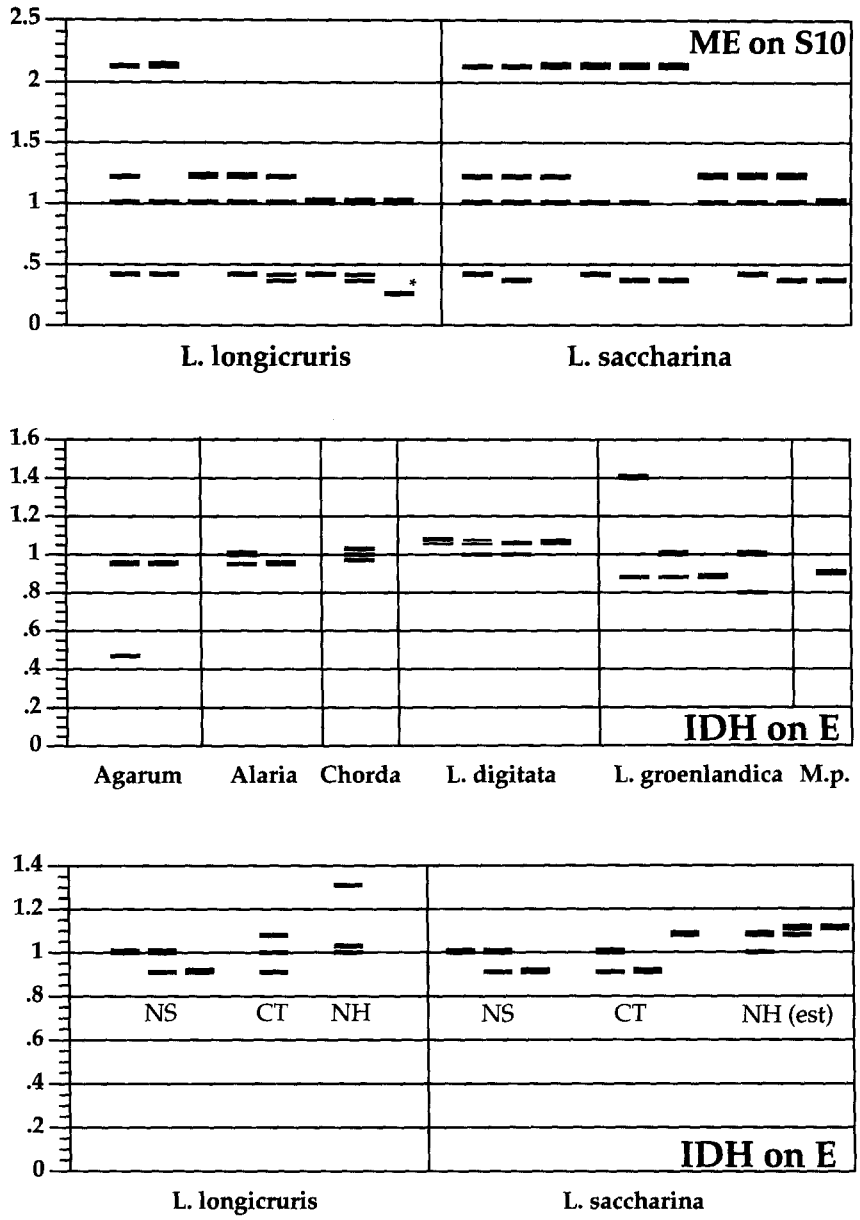

Fig. 3. Band patterns for ME and IDH. The vertical axis indicates electrophoretic migration distance of bands for each species relative to *Laminaria longicruris*. (*) indicates a rare electromorph (< 1% occurrence).

samples or just the slower of the two, or they had only the RF = 1.08 band found in the Connecticut *L. longicruris* samples. Estuarine *L. saccharina* samples from New Hampshire had the band at RF = 1.08 and/or a faster band at RF = 1.11; some estuarine samples from this location also had the RF = 1.0 band.

Discussion

Overall, the number of polymorphic enzymes found for each species was quite low compared to plants from other divisions. This is in agreement with reported population genetics studies of the Laminariales using molecular genetic techniques

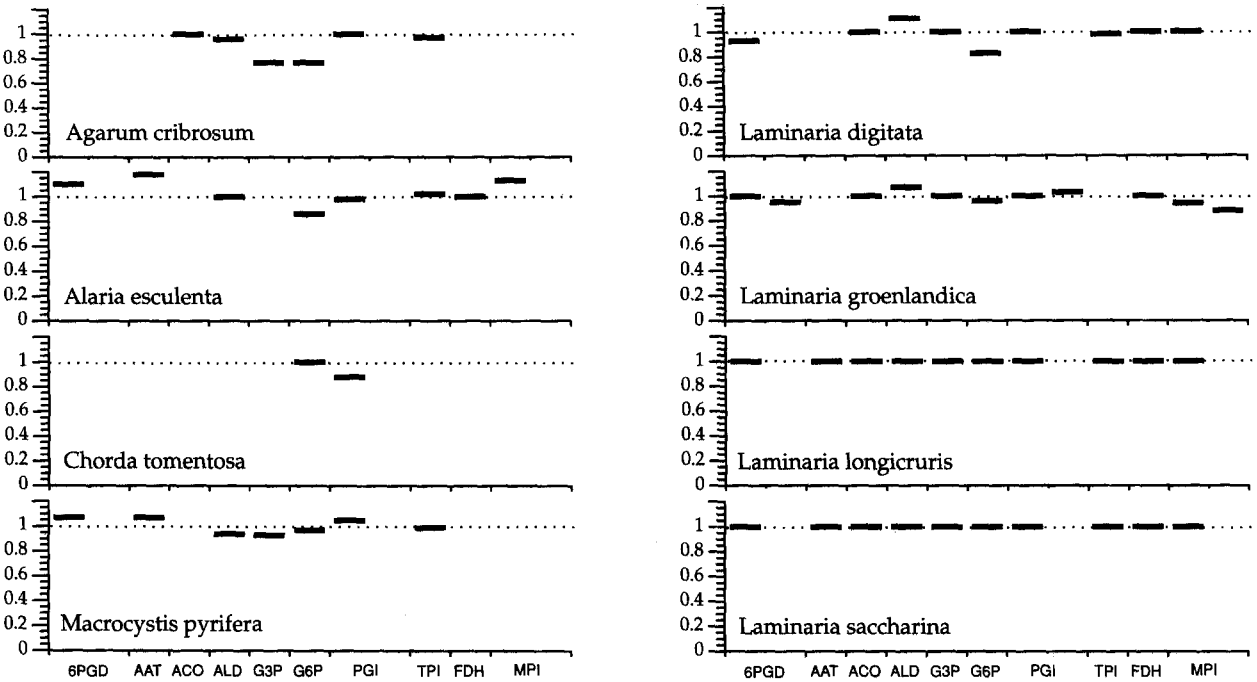

Fig. 4. Comparison of monomorphic bands in relation to *Laminaria longicruris*. The vertical axis indicates electrophoretic migration distance of bands for each species relative to the band for *Laminaria longicruris*.

(Bhattacharya & Druehl, 1988, 1989). While monomorphic enzymes provide little information on the dynamics of population genetics, they can be useful in supporting hypotheses on interspecific genetic relationships. Figure 4 illustrates the migration distances of 10 monomorphic enzymes for all 8 species in relation to *Laminaria longicruris*. On the basis of these results, it is impossible to distinguish between *Laminaria longicruris* and *L. saccharina*. The fact that we have found no phenotypic differences in the isozyme banding patterns for these 2 species does not imply that no differences exist, and caution should be exercised in making taxonomic inferences. On the other hand, if we accept these patterns as indicators of genetic similarity, albeit over a small portion of the genome, then *L. longicruris* and *L. saccharina* are genetically very similar. Both *L. digitata* and *L. groenlandica* have 5 enzyme bands with RF identical to *Laminaria longicruris*, but beyond those 5 bands, *L. digitata* and *L. groenlandica* had no bands in common with each other. *Laminaria groenlandica* is the only species of the eight where variants were found in any of these 10 enzymes; it is interesting to note that in each of the 3 enzymes where *L. groenlandica* variants occurred, one of the variants RF was identical to *L. longicruris* while the other was unique to *L. groenlandica*. If we assume that the two bands for each of these 3 enzymes are allozymes, then it should also be noted that no heterozygous individuals were found; this seems to be an area for further study.

Until crossing experiments have been completed to establish genetic control, the interpretation of banding patterns is tentative at best. While allelic relationships for some of the polymorphic enzymes seem quite evident, others enzyme banding patterns are not so easily interpreted. For example, *Laminaria longicruris*, *L. saccharina* and *L. groenlandica* appear to have two loci for both PER and SKD (Fig. 2); in each case, one locus is monomorphic, while the second locus has a fast and slow allele. For PER, heterozygous individuals, plus homozygous individuals for each of the two alleles have been found for *L. longi-*

cruris and *L. saccharina*; however, in a population sample of 60 plants we have found no individuals of *L. groenlandica* that are homozygous for the slow allele. For SKD in *L. groenlandica* we found heterozygous plants and plants that were homozygous for either the fast or slow allele, yet for what appeared to be the same locus in *L. longicruris* and *L. saccharina*, only homozygous individual were found. Some of the most interesting banding patterns occur in *Laminaria longicruris* and *L. saccharina* for IDH, where there appears to be both geographic and ecotypic differences in populations (Fig. 3).

Our current research efforts are directed at resolving some of the above issues and at establishing evidence for genetic control through laboratory crossing experiments with *Laminaria* cultures. We are also working to determine why some enzymes that should have separate chloroplast and cytoplasmic forms, produce only single bands for kelp. We have tested several additions to the sample extraction buffer including 3 protease inhibitors: phenylmethylsulfonyl fluoride (PMSF), benzamidine, and caproic acid; none of these has revealed additional bands. We will isolate and extract organelle enzymes separately to determine which copies of the enzymes we are seeing.

It has been suggested by Saunders & Druehl (1991) that low levels of genetic variation observed among members of the Laminariales are a reflection of the relatively recent evolution of the group. This could also account for the low degree of enzyme polymorphism found in our study. An alternate hypothesis can be proposed based on van den Hoek & Breeman's (1990) analysis of the biogeographic distributions of kelps during the last ice age (18 000 b.p). They have suggested that the distribution of the cold temperate flora was extremely reduced in the glacial N Atlantic. Range compression was most severe along the NW Atlantic coast and less extreme along NE Atlantic shores. If present day kelp populations within the geographic range of our study arose from very small post glacial populations, they might well be expected to have low genetic variability. This hypothesis can be tested by expanding the geographic range of our study and comparing current results with those from European kelp populations.

Acknowledgements

The present study was supported in part by New Hampshire/Maine and Connecticut Sea Grant College Programs of NOAA. Published as Scientific Contribution no. 265 of the Jackson Estuarine Laboratory and Center of Marine Biology.

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