Review

Isozymes in macroalgae (seaweeds): genetic differentiation, genetic variability and applications in systematics

PEDRO A. SOSA[1] AND SANDRA C. LINDSTROM[2]

[1] *Department of Biology, University of Las Palmas, Campus Universitario de Tafira, 35017 Las Palmas, Canary Islands, Spain*
[2] *Department of Botany, #3529–6270 University Blvd., University of British Columbia, Vancouver, B.C., Canada V6T 1Z4*

(*Received 10 May 1999; accepted 15 August 1999*)

Use of isozymes (including allozymes) in studies of population genetics and systematics of seaweeds has increased sufficiently in the last decade to allow some generalization. Only a single locus has been observed for about half the enzymes analysed in seaweeds, compared with 29% in vascular plants. Compared with higher plants, macroalgal species generally have low amounts of electrophoretically detectable genetic variation; the lowest levels of genetic variation found in natural populations are those reported for seaweeds. Nonetheless, seaweeds show an association between levels of genetic diversity as revealed by isozymes and species-specific attributes, such as mating system and predominance of asexual versus sexual reproduction. In systematic studies, isozymes have revealed cryptic species and identified pairs of sibling taxa. The quaternary structure of enzymes appears to be conserved at the phylum level. With the current availability of improved techniques for enzyme electrophoresis and for data interpretation, we expect future studies utilizing isozyme electrophoresis to provide further insight into population and evolutionary processes in seaweeds.

Key words: allozymes, electrophoresis, genetic identity, genetic structure, genetic variability, isozymes, population genetics, seaweeds, systematics

Introduction

Prior to Cheney's (1985) compilation of procedures for isozyme gel electrophoresis in macroalgae, there were few published papers applying this technique to seaweeds (Sosa & Garcia-Reina, 1991*a*). Sufficient electrophoretic data for seaweeds have now accumulated that a meaningful synthesis is possible. Here, we review the current application of isozyme analysis to macroalgae. Practical aspects of isozyme electrophoresis are not included, since electrophoretic techniques for algae usually involve only minor modifications of procedures published for other organisms, and they have been described for macroalgae in detail elsewhere (Cheney, 1985; Lindstrom & South, 1989; Sosa & Garcia-Reina, 1991*b*). However, some general genetic concepts commonly used in isozyme analysis will be reviewed in order to explain or emphasize the utility and importance of these techniques.

Hunter & Markert (1957) were the first to apply specific enzyme histochemical stains to detect bands on a gel. Markert & Möller (1959) called them 'isozymes,' indicating multiple forms of an enzyme with the same catalytic specificity but with different kinetic properties and different migration rates on a gel. Since then, the term isozyme has been used in a variety of ways, to refer both to the total number of bands observed, without any genetic interpretation, and to the products of different loci exclusively (Kephart, 1990). Some authors differentiate the gene locus and the isozyme product more effectively by invoking 'isozyme locus' instead of isozyme.

Nowadays, usage of the term isozyme, or isoenzyme, has shifted in accordance with recommendations of the Nomenclature Committee of the International Union of Biochemistry (Kephart, 1990). In practice, two forms of protein data can be gathered simultaneously using electrophoresis (Murphy *et al.*, 1996). One is derived from isozymes, which are all functionally similar forms of enzymes, produced by different gene loci. The other data set consists of allozymes, a subset of isozymes, which are variants of polypeptides representing different allelic alternatives of the same gene locus. In phycological studies, the term isozyme has often been used, even when the products of only a single locus are considered. We follow this broader usage here, recognizing nevertheless that in many cases the term allozyme would be more appropriate.

Isozymes are employed to investigate a variety of interesting and significant problems. At present, the use of isozymes still represents one of the best techniques for analysing and studying large numbers of genes and alleles of different individuals and populations at one time.

Correspondence to: P. A. Sosa. Fax: +34 928.45.29.22. e-mail: pedro.sosa@biologia.ulpgc.es

Multiple enzyme systems can be surveyed with standard electrophoretic techniques to determine levels of genetic differentiation, to detect distributional patterns of genotypes, and to estimate the degree of outcrossing or inbreeding within populations, or to detect cryptic species (the genotypes of which have diverged but which remain morphologically indistinguishable).

In macroalgae, isozyme studies are still uncommon. Technical difficulties associated with polysaccharides, especially in some red and brown algae, the tendency of phycologists to prefer more traditional approaches in systematics and, conversely, the current tendency of phycologists to utilize DNA-based molecular techniques employing the polymerase chain reaction (PCR) for population or phylogenetic purposes, are some of the reasons so few studies of seaweeds have examined isozymes. The application and advantages of electrophoresis of isozymes and the limitations of the technique have been discussed in detail elsewhere (Gottlieb, 1981; Tanksley & Orton, 1983; Soltis & Soltis, 1989a; May, 1992; Murphy *et al*., 1996), and although direct DNA studies are increasing, there has not been a commensurate reduction in investigations using isozymes. In fact, DNA techniques and enzyme electrophoresis can provide complementary information (van Oppen *et al*., 1995). It is clear that isozymes deserve a wider application than they have thus far seen (van der Meer, 1986).

Genetic interpretation

The genetic interpretation of banding patterns constitutes one of the most important aspects of isozyme electrophoresis. Electrophoretic data can contain significantly more information than simply relative mobility or presence and absence of bands. Moreover, the possibility of non-homologous or even artefactual bands can lead to erroneous conclusions if genetic interpretation is foregone (Gottlieb, 1977; Wendel & Weeden, 1989; Murphy *et al*., 1996).

Although genetic control of banding differences can be established by formal genetic analysis using breeding studies, such tests may be impossible or impractical due to difficulties in culturing seaweeds or in inducing sexuality. As allozymes are usually co-dominantly inherited, crosses between individuals with different electromorphs should result in F1 progeny that display both parental bands. To date, however, isozyme analysis and breeding studies have not been carried out together. Thus, the genetic basis and inheritance of isozymes in seaweeds is an area of research yet to be broached experimentally. Until such studies are made, assumptions regarding Mendelian inheritance and conclusions based on these assumptions are tentative and warrant caution.

In the absence of breeding studies, other important factors for appropriate interpretation of enzyme banding patterns are: (1) the number of coding genes, (2) the ploidy of the individual, (3) the quaternary structure of the enzyme, (4) the subcellular localization of the enzyme, and (5) artefacts. These factors are discussed below.

Number of genes

The number of loci coding an enzyme system affects the interpretation of bands. Obviously, the more genes, the more bands, and the more complex the banding pattern. In seaweeds, the number of genes observed depends on both the enzyme and the species analysed. To date, only a single locus has been identified for about half of the enzyme systems analysed in seaweeds (Table 1). In vascular plants, 29 % of enzymes are coded by a single gene; most are coded by two or more loci (Weeden & Wendel, 1989). Although the low percentage of multilocus genes found in seaweeds has facilitated genetic interpretation, it has also limited the utility of this technique in analysing genetic variation in natural populations (Neefus *et al*., 1993). The relatively high proportion of single-locus enzymes in algae is especially striking for enzymes assayed with artificial substrates, such as phosphatases, esterases, peroxidases and superoxide dismutases. In vascular plants and animals, these multiple-substrate enzymes almost always display many bands for an individual. Because these bands are usually derived from heterogeneous enzymes, coded by more than one locus, their interpretation can be difficult (Innes, 1984). In land plants, peroxidase is often represented by many loci, whereas in seaweeds only one gene has been encountered (Table 1). For esterase, the maximum number of loci is three in macroalgae (Table 1), whereas more than 10 loci have been observed for many land plant species (Weeden & Wendel, 1989).

In macroalgae, four or more loci have been recognized for only three enzyme systems, all of them enzymes detected by non-natural substrates: acid phosphatase, superoxide dismutase and diaphorase (Table 1). Superoxide dismutase (SOD) activity has been described for some peroxidase enzymes (Kiang & Gorman, 1983), which could indicate that some SOD loci correspond to peroxidase enzymes. Also, a diverse group of flavoproteins is capable of using reduced pyridine co-factors to reduce acceptor substrates, and these stains may have little specificity; therefore, diaphorase stains may also reveal a heterogeneous group of enzymes. In the same way, seaweeds can contain a variety of enzymes with phosphatase activity, some of which may have broad and overlapping substrate specificity *in vitro*.

In contrast to vascular plants, where conservation is particularly striking with respect to enzymes of glycolysis and the pentose phosphate pathway, a variable number of loci has been described for these pathways in seaweeds. Thus, a maximum of two loci has been described for triose phosphate isomerase (TPI) and phosphoglucomutase (PGM), whereas between one and three loci have been observed for malate dehydrogenase (MDH) and glucose phosphate isomerase (GPI), depending on the species or genus (Table 1).

Table 1. Number of isozyme loci described or inferred for macroalgae using electrophoresis

Enzyme	Abbreviation	No. of loci	Reference
Acid phosphatase[a]	ACP	2	O
		3–6	B
Aconitase	ACO	1	R
Alanine peptidase	ALAP	1	L
Alanine aminotransferase	ALT	1	T
Aldolase	ALD	1	J, K, L, M, R, T
Alkaline phosphatase[a]	ALP	1	O, U, V, W
Amylase	AMY	1	H
Aspartate aminotransferase	AAT/GOT	1	A, C, E, F, D, G, L, M, R, X
		2	H, J, K, L, M
Catalase[a]	CAT	1	O, P, Q, C, D, E, F, G
Diaphorase[a]	DIA	1	A, C, D, G, E
		2	O, W
		4	U, V
Esterase[a]	EST	1	L
		2	W
		3	U, V
Formate dehydrogenase	FDH	1	R
Fructose biphosphatase	FBF	1	T
Glucose dehydrogenase	GD	1	I
Glucose-6-phosphate dehydrogenase	G6PDH	1	C, D, E, F, I, M, J, K, L, O, R
Glutamate dehydrogenase[b]	GDH	1	D, E, F, G, L, M, J, K, S, P, Q, W
6-Phosphogluconic dehydrogenase	6PGD	1	A,C, D, G, E, F, L, J, K, M, I, R
		2	A
Glyceraldehyde-3-phosphate dehydrogenase	G3PDH	1	I, R
Isocitrate dehydrogenase	IDH	1	C, D, G, J, K, M, N, Q, Y
		2	T
		3	R
Hexokinase	HEX	2	T
Lactate dehydrogenase	LDH	1	I, J, K
Leucine aminopeptidase	LAP	1	P, C
L-Leucylglycine peptidase	PEP	1	O
Leucyltyrosine peptidase	LT	1–2	A
Malate dehydrogenase	MDH	1	A, D, E, F, G, I, N, P, Q, R, Y
		2	A, B, C, O, U, V
		3	A, B, W
Malic enzyme	ME	1	A, J, K, M, Y
		2	A
		3	R
Mannose phosphate isomerase	MPI	1	C, D, E, F, G, I, R
Peptidase	PEP	2	I
Peroxidase[a]	PER	1	J, K, P
		2	R
Phosphoglucoisomerase	PGI/GPI	1	A, B, D, E, F, G, J, K, L, N, Q, R, S, X
		2	C, I, O, P, Y
		3	U, V, W
Phosphoglucomutase	PGM	1	B, C, D, E, F, G, H, I, J, L, M, N, Q, S, U, V, W, X, Y
		2	J, K, M
Ribulose-biphosphate carboxylase	RBC	2	T
Shikimic dehydrogenase	SKDH	1	J, K, M
		2	J, K, R
Superoxide dismutase[a]	SOD/IPO	1	B, C, D, E, F, G, H, I, K, L, O, P
		2	K, L
		3	W
		5	V
		6	U
Triosephosphate isomerase	TPI	1	N, R, Y
		2	X, T

A, Benzie *et al.* (1997), B, Cheney & Babbel (1978), C, Fujio *et al.* (1985); D, Fujio *et al.* (1987); E, Gil-Kodaka *et al.* (1988); F, Gil-Kodaka *et al.* (1990); G, Hara *et al.* (1986); H, Innes & Yarish (1984); I, Intasuwan *et al.* (1993); J, Lindstrom & Cole (1990*a*); K, Lindstrom & Cole (1992*a–c*); L, Lindstrom & South (1989); M, Lindstrom (1993*a*); N, Lu & Williams (1994); O, Malinowski (1974); P, Miura *et al.* (1978*a*); Q, Miura *et al.* (1979); R, Neefus *et al.* (1993); S, Okumura & Fujino (1986); T, Pearson & Murray (1997); U, Sosa & Garcia-Reina (1992); V, Sosa & Garcia-Reina (1993); W, Sosa *et al.* (1996); X, van Oppen *et al.* (1995); Y, Williams & Di Fiori (1996).

[a] Multiple substrate enzymes.

[b] NAD- and NADP-dependent.

These results appear to indicate that the number of loci coding proteins is lower in macroalgae compared with vascular plants, where new loci have been incorporated by duplication and mutation during evolution. One of us believes this difference is real, and one of us believes it is artefactual due to technical difficulties associated with electrophoresis of proteins in seaweeds. The capacity of polysaccharides found in most seaweeds to bind to proteins (especially in brown algae) can influence directly the number of bands and, therefore, the number of loci observed. Most authors have described bands with uninterpretable variation or with inconsistent staining, which they omitted from their analyses; such bands may represent the missing loci.

Ploidy and life-history phase

Seaweeds exhibit a diversity of life histories ; these may be monophasic, biphasic or even triphasic (DeWreede & Klinger, 1988). Different phases may exist simultaneously as free-living haploid and diploid thalli, and these phases may be isomorphic or heteromorphic. Seaweeds are therefore good biological models to analyse isozyme variation among individuals with different ploidy levels. Data obtained by pooling thalli with unknown ploidy levels should be avoided because differences observed in such pooled data may simply reflect different proportions of the haploid and diploid stages in the sample rather than true genetic differences (Innes, 1984).

An assumption regarding banding patterns is that they are simpler in haploid thalli. In haploid individuals only one allele is present for each locus, so consequently each band corresponds to just one locus. Thus, hybrid bands between products of different alleles of the same locus cannot occur as they can for diploid individuals. For example, in species of *Porphyra*, in which only the haploid macroscopic gametophyte has been sampled, each band has been interpreted as the product of a single gene, and multiple bands would necessarily have to be the products of different loci (however, see Fujio *et al*., 1988, for an exception). In contrast, in other genera, species may be polyploid in origin, and this multigenicity complicates the interpretation of the zymograms. Complex banding patterns for several enzyme systems in individuals of *Caulerpa peltata* suggest that this species is polyploid (Benzie *et al*., 1997). This hypothesis could be verified by inheritance studies.

In very few species have both haploid and diploid life-cycle stages been analysed. In the two phases of *Gelidium arbuscula* and *G. canariensis*, which are isomorphic, no differential expression of alleles was detected (Sosa & Garcia-Reina, 1992, 1993). Thus, the haploid gametophyte banding pattern was used as a tool in interpreting the zymograms genetically. No consistent differences in banding patterns between thalli identified as male (haploid) and those known to be tetrasporophytes (diploid) were seen in other red algae with isomorphic life histories (Cheney & Babbel, 1978; Lindstrom & South, 1989; Pearson & Murray, 1997). Because species with a heteromorphic life cycle have two phases with very different morphologies, seasonalities, and even habitats, it would be very interesting to compare and to analyse the enzyme loci of the two phases. Only Abo *et al*. (1996) have observed isozymes in both phases of heteromorphic species (*Porphyra tenera* and *P. yezoensis*). These authors did not report using controls or replicates nor did they interpret their data genetically. Thus, further studies on the two phases of heteromorphic species are warranted.

Quaternary structure

Many proteins contain more than one polypeptide chain (subunit) bound together by hydrogen bonds, van der Waals forces, ionic bonds, disulphide bridges and/or hydrophobic interactions. Proteins having more than one polypeptide are said to have a quaternary structure. Proteins with only one polypeptide chain are called *monomers*. A *dimer* is formed by two subunits, each consisting of a single polypeptide chain; an enzyme is a *tetramer* if four subunits are required to form the active protein. The identification of the quaternary structure of an enzyme by electrophoresis can be determined from the number of bands obtained from heterozygous diploid individuals (Wendel & Weeden, 1989; Kephart, 1990; May, 1992; Murphy *et al*., 1996): two bands for a monomer, three for a dimer, and five for a tetramer.

For most enzymes in vascular plants, genetic inferences can be made from gel banding patterns, because the quaternary structure of most enzymes is highly conserved in biochemical evolution at the level of class, especially for enzymes with known natural substrates. Thus, for vascular plants, enzymes such as PGM and ACO are monomers, whereas ADH, MDH, PGI and TPI are dimers (Weeden & Wendel, 1989; Kephart, 1990).

In seaweeds, inferences regarding quaternary structure have been made directly from electrophoretic banding patterns (Table 2). From results to date, it seems that quaternary structure is also evolutionarily conserved in seaweeds. For example, PGI (described as a dimer in vascular plants) has been interpreted as a monomer in *Silvetia compressa* (as *Pelvetia fastigiata*) and *Macrocystis pyrifera* (Phaeophyceae), but as a dimer in *Gelidium canariensis*, *G. arbuscula*, *Gracilaria cervicornis* and *Porphyra yezoensis* (Rhodophyta) (Table 2). Similar results have been observed for the enzymes MDH, PGM, GOT and SOD. For example, MDH is considered dimeric in all red and brown seaweeds analysed so far but is monomeric in *Codium fragile* (Chlorophyta) (Table 2). However, more studies, especially formal crossing analyses, are required to determine with certainty the quaternary structure of isozymes in seaweeds and to confirm evolutionary conservation within phyla.

Table 2. Quaternary structure of enzymes described or inferred for macroalgae using isozyme gel electrophoresis

Enzyme	Structure	Species	Reference
ACP	Monomer	*Eucheuma isiforme* var. *denudatum*[a]; *E. isiforme* var. *isiforme*; *Meristiella gelidium*[b]	Cheney & Babbel (1978)
ALP	Monomer	*Gelidium arbuscula*	Sosa & Garcia-Reina (1992)
		Gelidium canariensis	Sosa & Garcia-Reina (1993)
AMY	Monomer	*Enteromorpha linza*	Innes & Yarish (1984)
CAT	Monomer	*Codium fragile*	Malinowski (1974)
DIA	Monomer	*Gelidium arbuscula*	Sosa & Garcia-Reina (1992)
		Gelidium canariensis	Sosa & Garcia-Reina (1993)
EST	Monomer	*Gelidium arbuscula*	Sosa & Garcia-Reina (1992)
		Gelidium canariensis	Sosa & Garcia-Reina (1993)
		Gracilaria cervicornis	Sosa *et al.* (1996)
GDH	Monomer	*Gelidium arbuscula*	Sosa & Garcia-Reina (1992)
GOT	Dimer	*Enteromorpha linza*	Innes & Yarish (1984)
MDH	Monomer	*Codium fragile*	Malinowski (1974)
	Dimer	*Halidrys dioica*	Lu & Williams (1994)
		Gelidium arbuscula	Sosa & Garcia-Reina (1992)
		Gelidium canariensis	Sosa & Garcia-Reina (1993)
		Porphyra yezoensis	Fujio *et al.* (1985)
		Porphyra sp.	Fujio *et al.* (1988)
MPI	Monomer	*Porphyra* sp.	Fujio *et al.* (1988)
PEP	Monomer	*Codium fragile*	Malinowski (1974)
PGI/GPI	Monomer	*Silvetia compressa*[c], *Macrocystis pyrifera*	Williams & Di Fiori (1996)
	Dimer	*Gelidium arbuscula*	Sosa & Garcia-Reina (1992)
		Gelidium canariensis	Sosa & Garcia-Reina (1993)
		Gracilaria cervicornis	Sosa *et al.* (1996)
		Porphyra yezoensis	Fujio *et al.* (1985)
PGM	Monomer	*Halidrys dioica*	Lu & Williams (1994)
	Dimer	*Enteromorpha linza*	Innes & Yarish (1984)
SOD	Monomer	*Eucheuma isiforme* var. *isiforme*, *E. isiforme* var. *denudatum*	Cheney & Babbel (1978)
	Dimer	*Enteromorpha linza*	Innes & Yarish (1984)
TPI	Dimer	*Halidrys dioica*	Lu & Williams (1994)

[a] As *Eucheuma nudum*.
[b] As *Eucheuma gelidium* and *E. acanthocladum*.
[c] As *Pelvetia fastigiata*.

Subcellular distribution

In vascular plants, many routinely examined isozymes have chloroplast, mitochondrial, microbody and cytosolic forms (Weeden & Wendel, 1989). Generally, such isozymes, coded by different loci, are unable to associate into active enzymes, and therefore they form no intergenic heteromultimers (there are exceptions). Thus, band phenotypes may be simplified by subcellular compartmentalization of isozymes but no research on compartmentalization of electrophoretically interesting enzymes in seaweeds has been published, although its existence has been suggested. Malinowski (1974), comparing patterns obtained for chloroplast and whole plant extracts, established that the GOT-1 locus was localized in the chloroplasts of *Codium fragile*. Lindstrom (1993*a*) reported occasional faint secondary bands for several enzymes in species of *Porphyra*, which she thought could represent isozymes from different subcellular locations.

Artefacts and null alleles

Artefacts from extraction, electrophoresis and staining of seaweed enzymes have also been observed on gels. Most of these can be dismissed by proper experimental controls or testing. Description and discussion of artefacts and their control can be found elsewhere (Oelshlegel & Stahman, 1973; Cheney; 1985; Kephart, 1990; May, 1992; Murphy *et al.*, 1996). Finally, alleles that are no longer transcribed or that code for defective polypeptides lacking enzymatic activity are generally referred to as 'null' or 'silent' alleles. These variants often remain undetected because their presence is masked in the heterozygous condition. As yet, no null alleles have been described in seaweeds.

Genetic variation

The detection of genetic variability in seaweed populations is a function of sample size and number of loci analysed. Sufficient numbers of individuals and enzymes are essential for meaningful results. Surveys of 10 or more loci in several hundred diploid individuals are often reported in animal and higher plant studies. These numbers of loci and individuals can be difficult to achieve in seaweeds, and the optimal sampling design will depend on the specific purpose of the study and the nature of the populations.

Table 3. Measures of genetic variability in natural populations of seaweeds averaged across those populations

Species	S	NP	NL	A	P	H	Reference
CHLOROPHYTA							
Caulerpa cupressoides (d)	12–15	2	13	1·10	7·7	0·013	Benzie *et al.* (1997)
C. lentillifera (d)	14	1	8	1·00	0·0	0·000	Benzie *et al.* (1997)
C. racemosa var. *imbricata* (d)	7	1	10	1·40	30·0	0·129	Benzie *et al.* (1997)
C. racemosa var. *laetevirens* (d)	2–21	7	9	1·30	30·1	0·122	Benzie *et al.* (1997)
C. racemosa var. *racemosa* (d)	16–20	3	9	1·06	7·4	0·023	Benzie *et al.* (1997)
Caulerpa sp. (d)	8	1	12	1·30	25·0	0·091	Benzie *et al.* (1997)
C. serrulata (d)	5–22	3	14	1·10	9·5	0·036	Benzie *et al.* (1997)
C. sertularoides (d)	21	1	8	1·00	0·0	0·000	Benzie *et al.* (1997)
C. taxifolia (d)	7–25	7	11	1·30	14·3	0·067	Benzie *et al.* (1997)
Codium fragile (d)	10–100	14	14	1·36	31·0	0·150	Malinowski (1974)
Enteromorpha linza (d)	47–479	4	5	1·90	100	0·180	Innes & Yarish (1984)
Average		4·0	10·3	1·26	23·2	0·074	
Average (excluding *E. linza*)		4·0	10·8	1·19	15·5	0·063	
PHAEOPHYCEAE							
Halidrys dioica (d)	55–72	5	5	1·83	50·0	0·193	Lu & Williams (1994)
Silvetia compressa[a] (d)	30–60	5	7	1·14	10·0	0·014	Williams & Di Fiori (1996)
Agarum cribrosum (d)	20–50	3	8	1·00	0·00	0·000	Neefus *et al.* (1993)
Halosiphon tomentosus[b] (d)	30	2	5	1·00	0·00	0·000	Neefus *et al.* (1993)
Laminaria digitata (d)	10–74	5	11	1·00	0·00	0·000	Neefus *et al.* (1993)
Laminaria groenlandica (d)	60	1	11	1·36	36·0	0·120	Neefus *et al.* (1993)
Macrocystis pyrifera (d)	50	2	8	1·00	0·00	0·000	Neefus *et al.* (1993)
Average		3·3	7·9	1·19	13·7	0·047	
Average (excluding invariate kelps)		3·7	7·7	1·44	32·0	0·109	
RHODOPHYTA							
Eucheuma isiforme var. *isiforme* (h, d)	24–56	3	11	1·36	36·4	–	Cheney & Babbel (1978)
E. isiforme var. *denudatum*[c] (h, d)	50–58	3	11	1·31	31·3	–	Cheney & Babbel (1978)
Gelidium arbuscula (d, h)	11 – 49	3	22	1·15	13·6	0·044	Sosa & Garcia-Reina (1992)
G. canariensis (d, h)	11–50	3	22	1·14	13·6	0·041	Sosa & Garcia-Reina (1993)
Gracilaria cervicornis (h)	22–63	3	16	1·06	5·9	0·082	Sosa *et al.* (1996)
G. chilensis (d)	Ca 20	17	14	–	2·5	0·007	Intasuwan *et al.* (1993)
Lithothrix aspergillum (d)	53 – 75	5	16	1·38	35·0	0·131	Pearson & Murray (1997)
Meristiella gelidium[d] (h, d)	12–29	2	8	1·27	26·8	–	Cheney & Babbel (1978)
Palmaria palmata (d)	24–31	2	10	1·20	10·0	0·036	Lindstrom & South (1989)
Phycodrys rubens (d)	2–33	18	3	1·00	0·0	0·000	van Oppen *et al.* (1995)
Porphyra abbottae (h)	46	1	15	1·00	0·0	0·000	Lindstrom (1993*a*)
P. fallax (h)	60	1	16	1·06	6·3	0·032	Lindstrom (1993*a*)
P. kanakaensis (h)	45	1	18	1·06	5·6	0·029	Lindstrom (1993*a*)
P. mumfordii (h)	30–99	2	17	1·56	26·5	0·104	Lindstrom (1993*a*)
P. perforata (h)	75	1	17	1·00	0·0	0·000	Lindstrom (1993*a*)
P. pseudolanceolata (h)	55–104	2	16	1·59	25·1	0·087	Lindstrom (1993*a*)
P. pseudolinearis (h)	45	1	11	1·54	54·5	0·068	Gil-Kodaka & Fujio (1990)
Porphyra sp. (h)	60	1	11	1·36	27·3	0·111	Gil-Kodaka & Fujio (1990)
P. torta (h)	32–105	2	16	1·22	6·3	0·029	Lindstrom (1993*a*)
P. yezoensis[e] (h)	12–224	11	8	1·50	39·8	0·152	Miura *et al.* (1979)
P. yezoensis (h)	21–228	3	12	1·64	33·3	0·127	Fujio *et al.* (1985)
P. yezoensis (h)	78–319	1	12	3·08	91·7	0·210	Hara *et al.* (1986)
P. yezoensis (h)	23–111	11	12	2·29	58·3	0·197	Fujio *et al.* (1987)
P. yezoensis[e] (h)	5–125	3	11	1·99	78·8	0·239	Gil-Kodaka *et al.* (1988)
P. yezoensis (h)	91–105	1	11	1·91	63·6	0·160	Gil-Kodaka & Fujio (1990)
Average		4·0	13·4	1·44	27·7	0·086	
Average (excluding *P. yezoensis*)		3·7	14·2	1·24	17·2	0·050	
AVERAGES							
Seaweeds		3·9	11·7	1·35	24·2	0·076	This study
Seaweeds (excluding *E. linza, P. yezoensis* and invariate kelps)		3·8	12·5	1·24	18·1	0·061	
Bryophytes				1·43	28·4	0·098	Wyatt *et al.* (1989)
Pteridophytes				1·60	37·8	0·121	Soltis & Soltis (1989*b*)
Higher plants				2·00	54·4	0·152	Frankel *et al.* (1995)

S, numbers of individuals sampled per population; NP, numbers of populations or subpopulations sampled; NL, numbers of loci; A, average number of alleles per locus; P, average polymorphism; H, average coefficient of gene diversity (expected heterozygosity); –, not available; (h), haploid individuals; (d), diploid individuals.

[a] As *Pelvetia fastigiata*.

[b] As *Chorda tomentosa*.

[c] As *Eucheuma nudum*.

[d] As *Eucheuma acanthocladum* and *E. gelidium*.

[e] Cultured populations not included.

From 3 to 22 loci have been investigated in seaweed populations (Table 3). In most cases, a low number of loci is a consequence of undetectable enzyme activity and not lack of effort on the part of the researcher. However, conclusions based on few polymorphic loci should be accepted with caution.

Table 3 summarizes several measures of genetic variability for natural populations of 38 macroalgal taxa. Numbers of individuals sampled per population ranged from 2 to 479, with most in the minimally acceptable range of 30–60 for adequate population sampling. The data in Table 3 constitute mean values of all populations.

Table 3 reveals that species of seaweeds differ greatly in their levels and patterns of genetic variation. Some species, such as *Porphyra yezoensis* and *Enteromorpha linza*, seem replete with genetic diversity (however, see caveats below). In contrast, others, including species of *Porphyra* (Lindstrom, 1993*a*) and other red algae such as *Phycodrys rubens* (van Oppen *et al.*, 1995), are genetically depauperate. (Some of these results are a consequence of data for only selected loci being reported; for example, Innes & Yarish (1984) analysed only polymorphic loci in *Enteromorpha linza*.)

Overall, macroalgal species maintain relatively low amounts of electrophoretically detectable genetic variation compared with other plants (Table 3). On average, seaweeds are polymorphic (P) at only about 25% of their loci with an average (A) of 1·39 alleles per locus and a mean expected heterozygosity (H) of 0·074. Since these values are skewed by the high variability reported for *Enteromorpha linza* and *Porphyra yezoensis*, we also calculated averages without these taxa, reducing these values even further (Table 3). Thus, the lowest levels of genetic variation found in any natural populations are those reported for seaweeds.

There is no compelling evidence in Table 3 that isozyme variation is related to phylum. Species in the three phyla (Chlorophyta, Heterokontophyta and Rhodophyta) do not differ significantly in their genetic variation, all phyla showing a similar range and average of genetic variability among their populations (Table 3). However, the scarcity of these kinds of studies means that this conclusion is preliminary.

It is also apparent in Table 3 that species in which the haploid phase is dominant maintain as much genetic variability as species with only a diploid phase or with both haploid and diploid phases. Thus, average genetic variability for haploid individuals is P = 30.0%, A = 1·50 and H = 0·093, and the average for diploid individuals is P = 17·6%, A = 1·22 and H = 0·059 (the values for haploid individuals are skewed by the unusually high levels reported for *Porphyra yezoensis*). Sosa & Garcia-Reina (1992, 1993) described a lower genetic variability for the haploid phase compared with the diploid phase for two *Gelidium* species, but the number of haploid individuals analysed was sufficiently lower than the number of tetrasporophytes to account for the discrepancy (Sosa *et al.*, 1998). The figures given here for the amount of genetic variability in the haploid phase of seaweeds are biased because most of the haploid individuals analysed belong to species of *Porphyra*. The generalization of no difference in diversity in species with different life history patterns requires studies across a broader spectrum of taxa in all seaweed phyla, and interpretation in light of the functionality of haploidy and diploidy in seaweeds (Valero *et al.*, 1992).

Both species-specific attributes (the biological properties of the species) and abiotic ecological factors have a significant influence on the level and distribution of genetic variation in natural populations (Frankel *et al.*, 1995). These factors influence the genetic composition of populations in both a directed and a stochastic fashion through natural selection and random events: in the ways in which new variants arise (mutation) or are sorted into genotypes (recombination), and in the ways in which the gametes are brought together (mating system) or new propagules move to new sites (gene flow/migration). The results of physical factors acting on populations, i.e. genetic differentiation in response to environmental variation, are well established in seaweed ecology. These responses can be recognized in morphological or physiological ecotypes (Russell, 1986). Spatial or temporal organization of genetic variation is another response. Isozyme analysis is especially useful in revealing the latter.

Natural selection

Very little is known about how natural selection affects genetic variation in seaweed populations. Populations that are subjected to selection are expected to show distinct spatial patterns in relation to the environmental variables producing selection pressure. Some researchers have reported a correlation between isozyme loci in seaweeds and environmental or physiological factors (Miura *et al.*, 1978*a*,*b*; Okumura & Fujino, 1986; Innes, 1987, 1988; Intasuwan *et al.*, 1993). However, a positive correlation by itself cannot prove causality, and the possibility of different taxa being sampled cannot be ruled out. Williams & Di Fiori (1996) found a highly structured spatial distribution of PGI alleles in *Silvetia compressa* (as *Pelvetia fastigiata*) on three different reefs. It was highly improbable that the observed pattern was due to chance, suggesting that natural selection was maintaining the distribution of alleles, but no specific abiotic factor was implicated. Thus, microgeographic differentiation may play a crucial role in structuring natural populations of seaweeds. As discussed by Linhart & Grant (1996), genetic differentiation over short distances (a few metres) can be as great as that over several to tens of kilometres.

Genetic drift

In the absence of selection, genetic drift can lead to a loss of genetic variation within populations, exhibited as a loss of heterozygosity and eventual fixation of alleles. The

effects of genetic drift arising from bottlenecks have been estimated to result ultimately in a 75% reduction in heterozygosity (Frankel *et al*., 1995) and a simultaneous reduction in the average number of alleles per locus (Sytsma & Schaal, 1985). Population size is the most important determinant of genetic drift, with small populations being more strongly affected than large ones. The magnitude of random drift can be estimated from the effective size of a population. This is defined as the size of an ideal population whose genetic make-up is affected by random drift to the same degree as is the make-up of the real population under study. A sudden decline in population size can affect the mating process. Fewer individuals are available to participate in mating and, therefore, the next generation will consist of individuals that are more closely related than were individuals in the previous generation. Reduced genetic variability results from such inbreeding.

Neefus *et al*. (1993) suggested that low levels of genetic variation observed among eight species of Laminariales were a reflection of the relatively recent evolution of the group, or due to a bottleneck as populations arose from very small numbers of individuals after the last ice age. Relatively recent colonization following the last glaciation has also been suggested to explain the lack of enzyme diversity in many species of *Porphyra* in British Columbia and adjacent waters (Lindstrom, 1993*a*). The effect of genetic drift following a bottleneck can be so dramatic that genetic variation not only decreases but undergoes a genetic revolution, as exemplified by differentiation of northern European populations of *Phycodrys rubens* into two distinct genetic groups (van Oppen *et al*., 1995).

Malinowski (1974) found that all *Codium fragile* subsp. *tomentosoides* individuals within a population were genetically identical. Ten of 14 loci analysed were monomorphic at all ten sites in Long Island Sound and southeastern Massachusetts. The remaining four loci showed identical allele frequencies in all individuals (thus accounting for the high heterozygosity detected in this species) (Table 3). The most plausible explanation was that the entire Western Atlantic population was founded by only a few colonizers of identical genotype. An asexual reproductive strategy of these colonizers (Carlton & Scanlon, 1985) coupled with high dispersibility of the flagellated parthenogenetic gametes yielded a clone. Additional samples from Maine, California, British Columbia and England revealed some genotypic diversity, and the pattern of genetic differentiation grouped the California and British Columbia samples together, with the remaining samples forming a second group, suggesting that Europe was the source of introduction of this species to eastern North America (Malinowski, 1974).

Gene flow

Non-random distribution of genetic variation is often referred to as the genetic structure of a population (Loveless & Hamrick, 1984). In the absence of strong selection, gene flow is the primary determinant of genetic differentiation within and among populations (Futuyma, 1986; Lacy, 1987; Neigel, 1997; Hartl & Clark, 1997). Gene flow is defined as the movement of genes from one location to another, and thus it includes all movements of gametes, propagules and individuals that are effective in changing the spatial distributions of genes (Slatkin, 1985; Neigel, 1997). Gene flow tends to homogenize populations and eliminate local differences. In contrast, the consequence of limited gene flow is to produce structured populations composed of patches of genetically similar individuals. Moreover, if gene flow is sufficiently restricted, even large continuously distributed assemblages of conspecifics may come to consist of many small breeding units, where deviations from random mating among the units will favour genetic divergence. If gene flow is the only factor operating (i.e. if population sizes are so large that genetic drift may be ignored and if the alleles are selectively neutral), all the populations will homogenize to the same allele frequency. However, if population sizes are sufficiently small, homogenization of gene frequencies by gene flow is counteracted by random divergence caused by genetic drift.

The estimation of gene flow from the distribution of genetic markers in populations requires an indirect approach and remains an area of great interest and discussion (Neigel, 1997). We will not attempt to summarize the large literature on this topic but instead refer the reader to reviews by Slatkin (1985, 1987, 1993), Futuyma (1986), Weir (1996), Hartl & Clark (1997) and Neigel (1997). A useful method is Wright's statistic for estimating the standardized variance in allele frequencies among different populations (F_{ST}). F_{ST} can be interpreted as an index of genetic differentiation (Hartl & Clark, 1997); ideally, it has a theoretical minimum of 0 (indicating no genetic divergence) and a theoretical maximum of 1 (indicating fixation for alternative alleles in different populations). Wright suggested qualitative guidelines for the interpretation of F_{ST} (Hartl & Clark, 1997). Thus, a range from 0 to 0·05 is considered to indicate little genetic differentiation, and values above of 0·25 to indicate a great deal of genetic differentiation.

Estimates of F_{ST} for natural populations of seaweeds vary widely (Table 4), presumably because F_{ST} is influenced by the size of the populations, by the amount and pattern of migration, and by other factors. However, estimates of F_{ST} values are expected to be similar across loci (if each locus studied is neutral). A high discrepancy between loci can be explained by the occurrence of artefacts, by problems in the interpretation of the loci, or by selection at one of the loci studied. This problem can easily be overcome when many loci are studied. Some species, such as *Caulerpa peltata* (Benzie *et al*., 1997) and *Halidrys dioica* (Lu & Williams, 1994), exhibit little genetic differentiation (low F_{ST} values), even over very large geographical areas, while other species, such as *Silvetia compressa* (as *Pelvetia fastigiata*) (Williams & Di Fiori, 1996), are highly differentiated (Table 4).

Table 4. Indices of genetic differentiation (F_{ST} value) and approximated geographic distance (GD) described in natural populations of seaweed

Species	NP	F_{ST}	GD (km)	Reference
Caulerpa cupressoides (d)	2	0·912	15	Benzie *et al.* (1997)
Caulerpa peltata	1	0·000	35	Benzie *et al.* (1997)
C. racemosa var. *laetevirens* (d)	5	0·266	–	Benzie *et al.* (1997)
C. racemosa var. *racemosa* (d)	2	0·886	0·3–600	Benzie *et al.* (1997)
C. serrulata (d)	5	0·635	0·3–600	Benzie *et al.* (1997)
C. taxifolia (d)	7	0·418	15	Benzie *et al.* (1997)
Halidrys dioica (d)	3	0·039	1	Lu & Williams (1994)
		0·018	4	
		0·194	90	
Silvetia compressa (as *Pelvetia fastigiata*) (d)	2	0·005	0·003–0·64	Williams & Di Fiori (1996)
		0·806	80–130	
Gelidium arbuscula (d)	7	0·280	30–120	Sosa *et al.* (1998)
G. canariensis (d)	5	0·130	30–120	Sosa *et al.* (1998)
Gracilaria cervicornis (h)	2	0·009	20	Sosa *et al.* (1996)
		0·186	> 130	
Lithothrix aspergillum (d)	8–11	0·004	0·03	Pearson & Murray (1997)
		0·03	5·5	
		0·398	70	
		0·618	> 750	
Porphyra yezoensis (h)	8	0·472	–	Miura *et al.* (1979) in Fujio *et al.* (1985)
Porphyra yezoensis (h)	6	0·623	–	Fujio *et al.* (1985)
Porphyra yezoensis (h)	11	0·125	0·003–0·6	Fujio *et al.* (1987)

NP, number of polymorphic loci used to calculate F_{ST}; (d), diploid; (h), haploid; –, not available.

Mating systems and dispersal capabilities influence gene flow, and therefore they are excellent predictors of genetic diversity and population structuring. In higher plants, genetic differentiation is far more extensive in selfing and asexual species (with low mobility) than in preferentially outcrossing species (Hamrick, 1989; Linhart & Grant, 1996), and some reviews have focused on correlations between life-history traits and genetic differentiation among populations (Hamrick *et al.*, 1979, 1991; Hamrick & Godt, 1997). Although it is still premature to conclude that life-history characteristics are good predictors of genetic variation in natural populations of seaweeds (Lu & Williams, 1994), available isozyme data suggest a correlation between genetic variation or differentiation in populations or species and the reproductive strategy of the species. For example, Pearson & Murray (1997) found a clear relationship between genetic differentiation and geographic distance in Californian populations of *Lithothrix aspergillum*. The F_{ST} values were very low (F_{ST} = 0·004 and 0·030) over a spatial scale of 30 m and 5·5 km, but these values increased to 0·583–0·683 for populations separated by more than 700 km, which agrees well with the limited long-distance dispersal of the species (Pearson & Murray, 1997; Table 4). Lu & Williams (1994) analysed five loci of *Halidrys dioica* from five localities in southern California. This species is dioecious, perennial, and has buoyant reproductive fronds that confer on it the capacity for high gene flow between populations, especially over short distances. Lu & Williams found very high genetic variation within populations, and moderate population differentiation (F_{ST} = 0·194) over considerable geographic distances (90 km; Table 4). Conversely, Benzie *et al.* (1997) found a strong spatial differentiation (highly significant F_{ST} values) for populations of *Caulerpa* spp. from Australia (Table 4). These values suggest that gene flow among populations of *Caulerpa* spp. is low, although most of the *Caulerpa* species are thought to be outcrossing. Most analysed populations approached conditions of random mating. Thus, the bulk of the populations were outbreeding, but geographical isolation led to differences in gene frequencies between sites (Benzie *et al.*, 1997). Such high F_{ST} values for conspecific populations warrant caution: these could be due to the low number of polymorphic loci analysed.

In *Porphyra*, Hwang *et al.* (1998) and Lindstrom (1993*b*, unpublished; Table 5) observed significantly higher average expected heterozygosity among species that are dioecious or sectored compared with species with the sexes intermixed on the thallus. Significantly higher average heterozygosity was also the case for species with a narrower geographic range and species occurring higher in the intertidal. There were no significant differences between levels of population differentiation based on season of appearance or persistence of the thalli.

Most seaweed species reproduce asexually during at least part of their life cycle. Reduced genetic diversity is an expected consequence of this type of reproduction. Asexual reproduction causes many individuals within a population to be genetically identical (van Oppen *et al.*, 1995). From the viewpoint of the population dynamics of genes, asexual reproduction transmits the whole set of genes of an individual to its offspring. Since recombination of genes is absent, alleles at different loci are fixed, and the genotypes in the populations do not change. Therefore,

Table 5. Comparison of mean levels of population differentiation in species of *Porphyra* with different ecological, phenological and other attributes

Attribute	Mean heterozygosity	Per cent polymorphic loci	Species
Geographic range	*	NS	
1000–3500 km	0·077 ± 0·041	0·272 ± 0·202	Pe(2), Pf, Pk, Pm(2)
5000–8500 km	0·018 ± 0·021	0·078 ± 0·094	Pa, Pp, Pt(2)
Habitat	*	*	
High intertidal	0·072 ± 0·039	0·270 ± 0·176	Pe(2), Pf, Pm(2), Pt(2)
Mid-intertidal	0·010 ± 0·017	0·019 ± 0·032	Pa, Pk, Pp
Season	NS	NS	
Winter	0·063 ± 0·045	0·236 ± 0·189	Pe(2), Pf, Pm(2), Pp, Pt(2)
Spring (and summer)	0·015 ± 0·021	0·028 ± 0·040	Pa, Pk
Mating system	**	**	
Sexes mixed	0·022 ± 0·017	0·072 ± 0·073	Pa, Pf, Pk, Pp, Pt(2)
Sexes separate	0·101 ± 0·024	0·378 ± 0·151	Pe(2), Pm(2)
Persistence	NS	NS	
⩽ Four months	0·073 ± 0·066	0·274 ± 0·296	Pa, Pf, Pm(2)
⩾ Five months	0·045 ± 0·036	0·160 ± 0·140	Pe(2), Pk, Pp, Pt(2)

Data from Lindstrom (1993*a*, *b*). Significant differences were determined using the Tukey HSD test.
*$0{\cdot}01 < p < 0{\cdot}05$; **$0{\cdot}001 < p < 0{\cdot}005$; NS, not significant.
Species abbreviations: Pa, *P. abbottae*; Pe, *P. pseudolanceolata*; Pf, *P. fallax*; Pk, *P. kanakaensis*; Pm, *P. mumfordii*; Pp, *P. perforata*; Pt, *P. torta*. (2) indicates that data from two different populations were used.

effective population size is probably small for many algal species although the number of individuals may be large, and genetic drift is more efficient.

Reproductive strategy

Asexual reproduction may explain the lack of genetic variation in some species. Cheney & Babbel (1978) observed that most new individuals in Florida populations of *Eucheuma* were found in proximity to parent plants, which implied limited dispersal. Vegetative reproduction in these populations probably accounted for the observed heterozygote deficiency. Innes & Yarish (1984) observed only 13 out of a possible 5400 multi-locus phenotypes in more than 1000 individuals of *Enteromorpha linza* from Long Island Sound and suggested that this was due to asexual reproduction. Genetic variation in New Zealand *Gracilaria chilensis* was also very low (Intasuwan *et al*., 1993; Table 3), with most alleles in a population being fixed. Asexual reproduction by vegetative fragmentation, which occurs in this species, could account for such low genetic variation. Similarly, Sosa *et al*. (1996) concluded that asexual reproduction was the most important factor responsible for low genetic variation in three Canary Island populations of *Gracilaria cervicornis*, although a low effective population size could not be discarded as an explanation; fertile tetrasporophytes were not detected in two of the three populations over several years, supporting the notion that these populations are clonal.

As discussed by Hunter (1993), locally adapted clones may propagate asexually and become dominant through competitive advantage in stable habitats, thereby minimizing the availability of unoccupied substratum and limiting opportunity for recruitment of new genotypes. Populations such as these are expected to be almost wholly structured by the processes of clonal replication. Genetic drift is more efficient in a species characterized by asexual reproduction and a reduced population size (Sosa *et al*., 1998), and therefore species with vegetative reproduction are expected to exhibit a higher level of differentiation among populations. Thus, Pearson & Murray (1997) observed high genetic differentiation between southern and northern California populations of *Lithothrix aspergillum*. The southern populations reproduced sexually and exhibited relatively high genetic diversity for red algae. The northern population, isolated from a previously continuous population (bottleneck event) or introduced through a founder event, persisted by vegetative propagation, perennation and asexual bispore reproduction and showed no genetic diversity.

There are exceptions to the generalization that asexual reproduction is correlated with low levels of within-population genetic diversity and high levels of population differentiation. If an asexual species is characterized by a high migration rate, however, gene flow could compensate for the genetic drift, and a higher level of genetic diversity would occur. Other exceptions have been reported. *Porphyra mumfordii*, which is monoecious but sectored and probably selfed at least some of the time, is as polymorphic as *P. pseudolanceolata* and *P. pseudolinearis*, which are dioecious obligate outcrossers (Lindstrom & Cole, 1992*c*; Lindstrom, 1993*a*). *Porphyra yezoensis*, which is also monoecious and reportedly largely selfed (Shin & Miura, 1990), has one of the highest levels of genetic variation described in seaweeds (Table 3; Hwang *et al*., 1998). Genetic variation in *P. yezoensis* may indeed be as high as

studies indicate. Japan and Korea were not glaciated during the Pleistocene and have rather steep environmental gradients, particularly of temperature. These ecological fluctuations may have provided conditions conducive to the development of high levels of polymorphism without accompanying speciation. However, such high genetic diversity led Lindstrom (1993*a*) to question whether Japanese researchers had confused morphologically similar but genetically distinct species. Reported morphological and phenological differences (Miura *et al*., 1978*b*) and linkages of seven polymorphic enzymes (Fujio *et al*., 1987) provide evidence for cryptic species rather than genetic differentiation within a single species. Not surprisingly, cultured populations of *Porphyra yezoensis* showed little genetic differentiation (Miura *et al*., 1979; Gil-Kodaka *et al*., 1988).

The relationship between reproductive strategy and genetic variation can also be analysed by determining deviation of the genotype from Hardy-Weinberg equilibrium or by F-statistics (Weir & Cockerham, 1984; Weir, 1996; Hartl & Clark, 1997). In a strictly sexually reproducing species, the distribution of genotype frequencies at a locus should approximate Hardy-Weinberg proportions (Hartl & Clark, 1997). In contrast, a population of asexually reproducing individuals is expected to show large deviations from Hardy-Weinberg equilibrium, non-random association among loci and reduced genotypic diversity compared with a population of sexually reproducing individuals. Deviations from this equilibrium have been shown in several species of seaweeds with primarily asexual reproduction. Innes & Yarish (1984) thought that asexual reproduction was responsible for a non-random association of genotypes and a significant deviation from Hardy-Weinberg equilibrium for five loci in *Enteromorpha linza*. Similar observations have been made in *Eucheuma nudum* (= *E. isiforme* var. *denudatum*) and *E. isiforme* (Cheney & Babbel, 1978), *Gelidium arbuscula* (Sosa & Garcia-Reina, 1992; Sosa *et al*., 1998), *Caulerpa taxifolia* (Benzie *et al*., 1997) and *Lithothrix aspergillum* (Pearson & Murray, 1997). In contrast, most loci analysed in the obligate outcrosser *Halidrys dioica* (Lu & Williams, 1994) and several outcrossing *Caulerpa* species (Benzie *et al*., 1997) exhibited Hardy-Weinberg equilibrium.

Assumptions required for Hardy-Weinberg equilibrium are rarely met in most seaweed populations (Pearson & Murray, 1997), because of non-random mating and asexual reproduction, without regard to natural selection. Moreover, deviations of gene frequencies from Hardy-Weinberg equilibrium can result from the Wahlund effect, i.e. two genetically different populations being sampled and pooled (Hartl & Clark, 1997). This latter problem can be avoided by using a hierarchical and stratified sampling strategy to determine more accurately spatial-scale factors influencing within-population structure and to discriminate between heterozygote deficiencies due to breeding behaviour of a species (e.g. inbreeding) and deficits due to the Wahlund effect (Goudet *et al*., 1994). Since most authors have not used an appropriate sampling strategy for discriminating among causes of the observed population structure, conclusions about Hardy-Weinberg equilibrium should be accepted with caution (Benzie *et al*., 1997; Sosa *et al*., 1998).

Use of isozymes in systematics

Isozymes are particularly useful as taxonomic characters because they are relatively easily observed, provide reliable data, and have the power to discriminate among taxa. They provide a valuable source of data because of the direct connection between the enzymes, as revealed in zymograms, and the genome. However, like other forms of systematic data, isozyme characters are also subject to certain limitations. Not all data will be useful for all taxa: enzymes useful for one group of species may be useless for another. For maximum value, comparisons need to be made between closely related taxa, where perceived similarities are most likely due to homology and not to convergence.

Mallery & Richardson (1972) were the first to apply protein electrophoresis to look for relationships among seaweeds. They compared zymograms for three multi-substrate enzymes (acid phosphatase, esterase and aminopeptidase) for 11 genera across the Rhodophyta. Although the resulting complex banding patterns could not be interpreted genetically, the authors nonetheless opened the door to the application of isozymes as a systematic tool for macroalgae.

As in population studies, allelic frequencies provide the basis for comparisons among species in systematic approaches. A number of coefficients have been developed that summarize these frequencies into indices that measure the degree of identity or the converse, distance, between pairs of populations or species. The three most commonly used indices are Nei's genetic identity (Nei, 1972), the distance measure of Rogers (Rogers, 1972) and Hedrick's genotypic identity (Hedrick, 1971). All indices equal 1 when pairs of populations or species share the same alleles in exactly the same proportions, i.e. the distance between populations or species is zero when they are identical. Likewise, all identities equal zero when the populations or species being compared share no alleles. All indices utilize data from all loci, not just the polymorphic loci. Both Nei's and Roger's coefficients are skewed by within-taxon heterozygosity, and a correction has been proposed (Hillis, 1984). Innes (1987) has suggested that Hedrick's identity provides a better measure of similarity than coefficients based on allelic frequencies for populations not in Hardy-Weinberg equilibrium, as is often the case for asexually reproducing seaweeds. This is so because two populations with the same allelic frequencies may have different genotype frequencies. Although there is merit to this idea, the lack of genetic interpretation of the banding patterns limits the usefulness of Hedrick's measure, and its values cannot be compared between life-cycle stages with different ploidy levels. Hedrick's identity has been used by

Table 6. Mean genetic identities (I) and distances (D, in parentheses) (Nei, 1972) among conspecific populations of seaweeds

Species	I (D)	I Range	Reference
CHLOROPHYTA			
Caulerpa cupressoides	1·000 (0·000)	–	Benzie *et al.* (1997)*
C. racemosa var. *laetevirens*	0·913 (0·091)	0·808–1·000	Benzie *et al.* (1997)*
Caulerpa racemosa var. *racemosa*	0·897 (0·109)	0·845–1·000	Benzie *et al.* (1997)*
Caulerpa serrulata	1·000 (0·000)	–	Benzie *et al.* (1997)*
Caulerpa taxifolia	0·873 (0·136)	0·795–0·958	Benzie *et al.* (1997)*
Codium fragile	0·927 (0·076)	0·623–1·000	Malinowski (1974)
Enteromorpha linza	0·908 (0·097)	0·695–0·999	Innes & Yarish (1984)*
PHAEOPHYCEAE			
Halidrys dioica	0·924 (0·079)	0·823–0·999	Lu & Williams (1994)*
Laminaria saccharina[a]	0·998 (0·002)	–	Neefus *et al.* (1993)*
Silvetia compressa[b]	0·944 (0·058)	0·863–0·999	Williams & Di Fiori (1996)*
RHODOPHYTA			
Eucheuma isiforme var. *isiforme*	0·996 (0·004)	0·993–0·998	Cheney & Babbel (1978)
E. isiforme var. *denudatum*[c]	0·895 (0·111)	0·830–0·952	Cheney & Babbel (1978)
Gelidium arbuscula	0·983 (0·017)	0·954–0·991	Sosa & Garcia-Reina (1992)
Gelidium canariensis	0·972 (0·028)	0·960–0·997	Sosa & Garcia-Reina (1993)
Gracilaria cervicornis	0·991 (0·009)	0·985–0·999	Sosa *et al.* (1996)
Gracilaria chilensis	–	0·650–1·000	Intasuwan *et al.* (1993)
Lithothrix aspergillum	0·820 (0·198)	0·714–0·999	Pearson & Murray (1997)
Meristiella gelidium[d]	0·857 (0·154)	–	Cheney & Babbel (1978)
Palmaria palmata	0·886 (0·121)	–	Lindstrom & South (1989)
Porphyra abbottae	–	0·933–1·000	Lindstrom & Cole (1992*c*)
Porphyra smithii	–	0·938–1·000	Lindstrom (1993*b*)
Porphyra yezoensis	0·898 (0·107)	0·667–1·000	Miura *et al.* (1979)
Porphyra yezoensis	0·919 (0·084)	0·785–0·998	Fujio *et al.* (1987)*
Average	0·936 (0·066)	0·836–0·993	
Self-fertilizing plants	0·975 (0·025)		Gottlieb (1981)
Outcrosser plants	0·956 (0·045)		Gottlieb (1981)
Bryophytes	0·900 (0·105)		Wyatt *et al.* (1989)
Pteridophytes	0·911 (0·093)		Soltis & Soltis (1989*b*)

Values are from original authors, or were calculated from allelic frequencies provided by authors (*).

[a] As *Laminaria longicruris* and *L. saccharina*.

[b] As *Pelvetia fastigiata*.

[c] As *Eucheuma nudum*.

[d] As *Eucheuma gelidium* and *E. acanthocladum*.

Cheney & Babbel (1978), Cheney & Mathieson (1979) and Blair *et al.* (1982).

Table 6 presents mean genetic identities and distances using Nei's measure for conspecific populations of seaweeds. These identity values are generally high and are comparable to those reported for higher plants. Overall, values average above 0·90, and are similar in all three algal phyla. Instances where values dipped just below this level may represent cases of incipient speciation. For example, the two populations of *Palmaria palmata* sampled by Lindstrom & South (1989), with an identity of 0·886, came from opposite sides of the North Atlantic; van der Meer (1987) reported abnormal meiosis in crosses between *P. palmata* populations from the eastern and western Atlantic, suggesting that speciation is in progress for these disjunct populations. Not included in the table are the data of Blair *et al.* (1982) for *Chaetomorpha* spp.; they obtained phenotypic identities of 0·84 and 0·87 between two pairs of species that have since been considered conspecific. Also not included are the recently published data for Korean species of *Porphyra* for which Hwang *et al.* (1998) observed phenotypic identities between populations of the same species to range from 0·529 to 0·998. Values below 0·80 may represent comparisons of cryptic species rather than populations of a single species. For example, the low values observed by Malinowski (1974) in *Codium fragile* were between northeast Pacific and northwest Atlantic populations, which are currently recognized to represent different subspecies if not species (Carlton & Scanlon, 1985; Goff *et al.*, 1992). Similar, low values among populations of *Enteromorpha linza*, *Gracilaria chilensis* and *Porphyra yezoensis* suggest that more than one species may have been involved in those studies.

These examples highlight one of the strengths of isozyme electrophoresis: its ability to call into question specific or even generic identification of taxa based on levels of similarity of their genotypes. Lindstrom & Cole (1990*a*) used isozymes to confirm the distinctness of a suite of species that had earlier been included in *Porphyra perforata*; based on distinct zymograms as well as other characters, they identified and described one additional species that previously had not been segregated from the complex (Lindstrom & Cole, 1990*b*). Among specimens that had been included earlier in *P. lanceolata* or *P.*

Table 7. Mean genetic identities (I) and distances (D) (Nei, 1972) among congeneric species of seaweeds

Species	I (D)	Reference
CHLOROPHYTA		
Caulerpa cupressoides–C. racemosa var. *imbricata*	0·600 (0·511)	Benzie *et al.* (1997)*
Caulerpa cupressoides–Caulerpa sp.	0·800 (0·223)	Benzie *et al.* (1997)*
Caulerpa cupressoides–Caulerpa serrulata	0·600 (0·511)	Benzie *et al.* (1997)*
Caulerpa cupressoides–C. sertularioides	0·800 (0·223)	Benzie *et al.* (1997)*
Caulerpa racemosa var. *imbricata–C. racemosa* var. *laetevirens*	0·383 (0·958)	Benzie *et al.* (1997)*
Caulerpa racemosa var. *imbricata–Caulerpa* sp.	0·600 (0·511)	Benzie *et al.* (1997)*
Caulerpa racemosa var. *imbricata–C. serrulata*	0·400 (0·916)	Benzie *et al.* (1997)*
Caulerpa racemosa var. *imbricata–C. sertularioides*	0·600 (0·511)	Benzie *et al.* (1997)*
Caulerpa racemosa var. *imbricata–C. taxifolia*	0·402 (0·911)	Benzie *et al.* (1997)*
Caulerpa racemosa var. *laetevirens–Caulerpa* sp.	0·398 (0·921)	Benzie *et al.* (1997)*
Caulerpa racemosa var. *laetevirens–C. serrulata*	0·563 (0·574)	Benzie *et al.* (1997)*
Caulerpa racemosa var. *laetevirens–C. taxifolia*	0·441 (0·819)	Benzie *et al.* (1997)*
Caulerpa sp.–*C. serrulata*	0·782 (0·246)	Benzie *et al.* (1997)*
Caulerpa sp.–*C. sertularioides*	0·982 (0·018)	Benzie *et al.* (1997)*
Caulerpa serrulata–C. sertularioides	0·800 (0·223)	Benzie *et al.* (1997)*
PHAEOPHYCEAE		
Laminaria digitata–L. 'groenlandica'	0·399 (0·919)	Neefus *et al.* (1993)*
Laminaria digitata–L. longicruris	0·562 (0·576)	Neefus *et al.* (1993)*
Laminaria digitata–L. saccharina	0·600 (0·511)	Neefus *et al.* (1993)*
Laminaria 'groenlandica'–L. longicruris	0·500 (0·693)	Neefus *et al.* (1993)*
Laminaria 'groenlandica'–L. saccharina	0·500 (0·693)	Neefus *et al.* (1993)*
RHODOPHYTA		
Gelidium arbuscula–G. canariensis	0·848 (0·165)	Sosa *et al.* (1998)
Palmaria hecatensis–P. callophylloides	0·400 (0·916)	Lindstrom & South (1989)
Palmaria mollis–P. callophylloides	0·391 (0·938)	Lindstrom & South (1989)
Porphyra abbottae–P. torta	0.469 (0·757)	Lindstrom & Cole (1993)
Porphyra amplissima–P. cuneiformis	0·692 (0·368)	Lindstrom & Cole (1993)
Porphyra brumalis–P. kurogii	0·467 (0·761)	Lindstrom & Cole (1993)
Porphyra fallax–P. fallax subsp. *conwayae*	0·745 (0·294)	Lindstrom & Cole (1993)
Porphyra kurogii-P. linearis	0·599 (0·512)	Lindstrom & Cole (1993)
Porphyra kurogii–P. pseudolinearis	0·531 (0·633)	Lindstrom & Cole (1993)
Porphyra kurogii–P. 'purpurea'	0·600 (0·511)	Lindstrom & Cole (1993)
Porphyra 'leucosticta'–P. fucicola	0·436 (0·830)	Lindstrom & Cole (1993)
Porphyra linearis–P. pseudolinearis	0·433 (0·837)	Lindstrom & Cole (1993)
Porphyra linearis–P. 'purpurea'	0·439 (0·823)	Lindstrom & Cole (1993)
Porphyra miniata–P. variegata	0·385 (0·955)	Lindstrom & Cole (1993)
Porphyra pseudolinearis–P. 'purpurea'	0·600 (0·511)	Lindstrom & Cole (1993)
Porphyra 'umbilicalis'–P. 'umbilicalis'	0·457 (0·783)	Lindstrom & Cole (1993)
Porphyra yezoensis–Porphyra sp.	0·886 (0·121)	Gil-Kodaka & Fujio (1990)
Porphyra yezoensis–P. pseudolinearis	0·377 (0·974)	Gil-Kodaka & Fujio (1990)
Porphyra sp.–*P. pseudolinearis*	0·408 (0·896)	Gil-Kodaka & Fujio (1990)

Values are from original authors, or are calculated from allelic frequencies provided by authors (*).

pseudolanceolata, Lindstrom & Cole (1992*b*) recognized three new species using isozymes in addition to morphological, ecological and chromosome characters. Van Oppen *et al*. (1995) interpreted fixed allelic differences at three of four loci as indicating specific differences between samples all originally identified as *Phycodrys rubens*. A low level of similarity ($I_p = 0{\cdot}372$) between pairs of *Eucheuma* spp. (Cheney & Babbel, 1978) provided part of the evidence for including one of the pairs of species in a new genus of Solieriaceae, *Meristiella* (Gabrielson & Cheney, 1987).

The converse is also true: isozymes appear especially well suited to recognizing conspecific and closely related species. Lindstrom & Cole (1990*a*) used lack of differences in zymograms to synonymize the asexual *Porphyra sanjuanensis* with the sexual *P. perforata*. Later, they used isozymes, morphology and chromosomes to identify pairs of disjunct sibling species of *Porphyra* occurring in the northeast Pacific and northwest Atlantic (Lindstrom & Cole, 1992*a*). Nei's identities for these species pairs ranged from 0·385 to 0·692 (Table 7). Subsequently, they reported three additional pairs of sibling species, two of which were restricted to the northeast Pacific; two of the three pairs had identities of 0·457 and 0·469, whereas the third, recognized at the rank of subspecies, had an identity of 0·745 (Lindstrom & Cole, 1993). In contrast to animals and higher plants, macroalgal sibling species tend to display fixation for different alleles rather than different allelic frequencies. These alleles can be considered diagnostic for identifying the species. Allelic fixation has been observed in species of *Palmaria* (Lindstrom & South, 1989), *Porphyra* (Lindstrom & Cole, 1992*a*, 1993), *Gracilaria* (Intasuwan *et*

al., 1993) and *Phycodrys* (van Oppen *et al.*, 1995) in the Rhodophyta, for species of *Ulva* (Doi *et al.*, 1993) and *Caulerpa* (Benzie *et al.*, 1997) in the Chlorophyta, and for species of *Laminaria* (Neefus *et al.*, 1993) in the Phaeophyta.

Nei's genetic identities for species pairs for which genetic identity or allelic frequency data have been published are given in Table 7. Since, as a rule of thumb, if $D_N > 1{\cdot}0$ ($I_N < 0{\cdot}37$), divergence has probably exceeded the resolution of the technique because of the masking effect of parallel or back mutations (Nei, 1987), we have included only pairs with identities exceeding 0·37. This cut-off value may in fact have some meaning in seaweeds: all of Lindstrom & Cole's (1992*a*, 1993) sibling species had values exceeding this level; Cheney & Babbel's (1978) species that were eventually segregated into different genera had an identity at just this level.

Isozymes can serve as the first approach to identifying species in morphologically difficult genera. Brostoff & Gordon (1997) examined allozyme variation in *Porphyra* specimens from 27 sites around the coast of New Zealand and concluded that more species existed than were previously reported. Their study was too preliminary to delimit taxa clearly, however. Their results are especially cautionary for population biologists, who need to remain open to the possibility that variation in isozymes in the seaweeds they are studying may be a reflection of interspecific rather than intraspecific differences. This is particularly important for genera or species with few distinctive morphological features. Moreover, generalities that have emerged from studies of isozymes in other groups of organisms may not be appropriate for seaweeds. For example, seaweeds have a relatively large number of enzymes that are coded at only one locus observable with current techniques (Table 1), and monomorphism is common in populations and species of seaweeds whereas polymorphism is low relative to other groups of organisms (Table 3).

As with measures of population parameters, one must keep in mind the sensitivity of genetic identities and distances to the numbers and kinds of loci on which the indices are based: the more individuals per population or species and the more single substrate enzymes sampled, the stronger the data. Difficulties with techniques or interpretations that hampered earlier studies (Richardson & Mallery, 1973; Kemp *et al.*, 1980; Küppers & Weidner, 1980; Marsden *et al.*, 1981, 1984*a*, *b*; Matlock & Romeo, 1981; Mohammad & Shaalan, 1985, 1991; Shaalan & Mohammad, 1985, 1986; Penniman, 1987; Rice & Crowden, 1987; Shaalan, 1991, 1992) are being overcome, and we expect future studies utilizing isozyme electrophoresis to provide insight into important population and evolutionary processes in seaweeds. Understanding patterns of genetic variability and population structure within a species using molecular markers is of fundamental importance to studies of ecology and evolution, and isozyme (including allozyme) loci provide one of the most useful genetic markers for obtaining these data.

References

Abo, K., Egoshi, K. & Noda, H. (1996). Isozyme from conchocelis and leafy thalli of *Porphyra* species. *Fish. Sci.*, **62**: 367–371.

Benzie, J.A.H., Price, I.R. & Ballment, E. (1997). Population genetics and taxonomy of *Caulerpa* (Chlorophyta) from the Great Barrier Reef, Australia. *J. Phycol.*, **33**: 491–504.

Blair, S., Mathieson, A.C. & Cheney, D.P. (1982). Morphological and electrophoretic investigations of selected species of *Chaetomorpha* (Chlorophyta, Cladophorales). *Phycologia*, **21**: 164–172.

Brostoff, W.N. & Gordon, M.E. (1997). Allozyme variation in New Zealand *Porphyra* (Rhodophyta). *N.Z.J. Bot.*, **35**: 203–220.

Carlton, J.T. & Scanlon, J.A. (1985). Progression and dispersal of an introduced alga: *Codium fragile* ssp. *tomentosoides* (Chlorophyta) on the Atlantic Coast of North America. *Bot. Mar.*, **28**: 155–165.

Cheney, D.P. (1985). Electrophoresis. In *Handbook of Phycological Methods, Ecological Field Methods*: *Macroalgae* (Littler, M.N. & Littler, D.S., editors), 87–119. Cambridge University Press, Cambridge.

Cheney, D.P. & Babbel, G.R. (1978). Biosystematic studies of the red algal genus *Eucheuma*. 1. Electrophoretic variation among Florida populations. *Mar. Biol.*, **47**: 251–264.

Cheney, D.P. & Mathieson, A.C. (1979). Population differentiation in the seaweed *Chondrus crispus*: preliminary results. *Isozyme Bull.*, **12**: 57.

De Wreede, R.E. & Klinger, T. (1988). Reproductive strategies in algae. In *Plant Reproductive Ecology* (Doust, J.L. & Doust, L.L., editors), 267–284. Oxford University Press, New York.

Doi, K., Kamiya, M., Hara, Y. & Chihara, M. (1993). Taxonomic studies on *Ulva pertusa* (Ulvophyceae). II. Preliminary isozyme analysis. *Jpn. J. Phycol.*, **41**: 199–206.

Frankel, O.H., Brown, A.H.D. & Burdon, J.J. (1995). *The Conservation of Plant Biodiversity*. Cambridge University Press, Cambridge.

Fujio, Y., Kodaka, P.L.G. & Hara, M. (1985). Genetic differentiation and amount of genetic variability in natural populations of the haploid laver *Porphyra yezoensis*. *Jpn J. Genet.*, **60**: 347–354.

Fujio, Y., Tanaka, M.Y., Hara, M. & Akiyama, K. (1987). Enzyme polymorphism and population structure of the haploid laver *Porphyra yezoensis*. *Nippon Suisan Gakkaishi*, **53**: 357–362.

Fujio, Y., Kodaka, P.L.G., Hara, M. & Akiyama, K. (1988). Electrophoretic variants characteristic of heterozygotes in haploid laver *Porphyra* sp. *Nippon Suisan Gakkaishi*, **54**: 969–974.

Futuyma, D.J. (1986). *Evolutionary Biology*. Sinauer Associates, Sunderland, MA.

Gabrielson, P.W. & Cheney, D.P. (1987). Morphology and taxonomy of *Meristiella* gen. nov. (Solieriaceae, Rhodophyta). *J. Phycol.*, **23**: 481–493.

Gil-Kodaka, P.L. & Fujio, Y. (1990). Genetic similarity and distance among 3 species of *Porphyra*. *Fish Genet. Breeding Sci.*, **15**: 31–34.

Gil-Kodaka, P.L., Hara, M., Akiyama, K. & Fujio, Y. (1988). Genetic differences between natural and cultured populations of *Porphyra yezoensis*. *Tohoku J. Agric. Res.*, **38**: 27–34.

Goff, L.J., Liddle, L., Silva, P.C., Voytek, M. & Coleman, A.W. (1992). Tracing species invasions in *Codium*, a siphonous green alga, using molecular tools. *Am. J. Bot.*, **79**: 1279–1285.

Gottlieb, L.D. (1977). Electrophoretic evidence and plant systematics. *Ann. Mo. Bot. Gdn.*, **64**: 161–180.

Gottlieb, L.D. (1981). Electrophoretic evidence and plant populations. *Prog. Phytochem.*, **7**: 1–46.

Goudet, J., De Meeüs, T., Day, A.J. & Gliddon, C. (1994). The different levels of population structuring of dogwhelks, *Nucella lapillus*, along the south Devon coast. In *Genetics and Evolution of Aquatic Organisms* (Beaumont, A., editor), 81–95. Chapman & Hall, London.

Hamrick, J.L. (1989). Isozymes are the analysis of genetic structure in plant populations. In *Isozymes in Plant Biology* (Soltis, D.E., Soltis, P.S., editors), 87–105. Dioscorides Press, Portland, OR.

Hamrick, J.L., Linhart, Y.B. & Mitton, B. (1979). Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annu. Rev. Ecol. Syst.*, **10**: 173–200.

Hamrick, J.L., Godt, M.J.W., Murawski, D.A. & Loveless, M.D. (1991). Correlations between species traits and allozyme diversity: implications for conservation biology. In *Genetics and Conservation of Rare Plants* (Falk, DA., Holsinger, KE., editors) 75–86. Oxford University Press, Oxford.

Hamrick, JL. & Godt, M.J.W. (1997). Effects of life history traits on genetic diversity in plant species. In *Plant Life Histories*: *Ecology, Phylogeny and*

Evolution (Silverstown, J., Franco, M. & Harper, J.L., editors), 102–118. The Royal Society/Cambridge University Press, London/Cambridge.

Hara, M., Tanaka, M.Y. & Fujio, Y. (1986). Linkage of an additional isozyme genes in *Porphyra yezoensis*. *Fish Genet. Breeding Sci.*, **11**: 25–28. (In Japanese.)

Hartl, D.L. & Clark, A.G. (1997). *Principles of Population Genetics*, 3rd edition. Sinauer Associates, Sunderland, MA.

Hedrick, P.W. (1971). A new approach to measuring genetic similarity. *Evolution*, **25**: 276–280.

Hillis, D.M. (1984). Misuse and modification of Nei's genetic distance. *Syst. Zool.*, **33**: 238–240.

Hunter, C.L. (1993). Genotypic variation and clonal structure in coral populations with different disturbance histories. *Evolution*, **47**: 1213–1228.

Hunter, R.L. & Markert, C.L. (1957). Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. *Science*, **125**: 1294–1295.

Hwang, M.S., Han, M. & Lee, I.K. (1998). Allozyme variation and species relationships in the genus *Porphyra* (Bangiales, Rhodophyta) from Korea. *Algae*, **13**: 447–459.

Innes, D.J. (1984). Genetic differentiation among populations of marine algae. *Helgoländer Meeresunters.*, **38**: 401–417.

Innes, D.J. (1987). Genetic structure of asexually reproducing *Enteromorpha linza* (Ulvales: Chlorophyta) in Long Island Sound. *Mar. Biol.*, **94**: 459–467.

Innes, D.J. (1988). Genetic differentiation in the intertidal zone in populations of the alga *Enteromorpha linza* (Ulvales: Chlorophyta). *Mar. Biol.*, **97**: 9–16.

Innes, D.J. & Yarish, C. (1984). Genetic evidence for the occurrence of asexual reproduction in populations of *Enteromorpha linza* (Chlorophyta) from Long Island Sound. *Phycologia*, **23**: 311–320.

Intasuwan, S., Gordon, M.E., Daugherty, C.H. & Lindsay, G.C. (1993). Assessment of allozyme variation among New Zealand populations of *Gracilaria chilensis* (Gracilariales, Rhodophyta) using starch gel electrophoresis. *Hydrobiologia*, **260/261** (*Proc. Int. Seaweed Symp.*, **14**): 159–165.

Kemp, L., Riabowol, R. & Druehl, L. (1980). Isoenzyme variation in *Macrocystis integrifolia* Bory. *J. Phycol.*, **16** (Suppl.): 23.

Kephart, S.R. (1990). Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. *Am. J. Bot.*, **77**: 693–712.

Kiang, Y.T. & Gorman, M.B. (1983). Soybean. In *Isozymes in Plant Genetics and Breeding*, part B (Tanksley, S. & Orton, T.J., editors), 295–328. Elsevier, Amsterdam.

Küppers, U. & Weidner, M. (1980). Seasonal variation of enzyme activities in *Laminaria hyperborea*. *Planta*, **148**: 222–230.

Lacy, R.C. (1987). Loss of genetic diversity from managed populations: Interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conserv. Biol.*, **1**: 143–158.

Lindstrom, S.C. (1993*a*). Intraspecific and interspecific genetic variation in species of *Porphyra* (Rhodophyta, Bangiales) from British Columbia and adjacent waters. *J. Appl. Phycol.*, **5**: 53–62.

Lindstrom, S.C. (1993*b*). Intra- and interpopulation genetic variation in species of *Porphyra* (Rhodophyta: Bangiales) from British Columbia and adjacent areas. In *Program & Abstracts, Western Society of Naturalists, 73rd Annual Meeting*, Newport, Oregon, 7–10 Jan. 1993.

Lindstrom, S.C. & Cole, K.M. (1990*a*). An evaluation of species relationships in the *Porphyra perforata* complex (Bangiales, Rhodophyta) using starch gel electrophoresis. *Hydrobiologia*, **204/205** (*Proc. Int. Seaweed Symp.*, **13**): 179–183.

Lindstrom, S.C. & Cole, K.M. (1990*b*). *Porphyra fallax*, a new species of Rhodophyta from British Columbia and northern Washington. *Jpn. J. Phycol.*, **38**: 371–376.

Lindstrom, S.C. & Cole, K.M. (1992*a*). Relationships between some North Atlantic and North Pacific species of *Porphyra* (Bangiales, Rhodophyta): evidence from isozymes, morphology, and chromosomes. *Can. J. Bot.*, **70**: 1355–1363.

Lindstrom, S.C. & Cole, K.M. (1992*b*). The *Porphyra lanceolata-P. pseudolanceolata* (Bangiales, Rhodophyta) complex unmasked: recognition of new species based on isozymes, morphology, chromosomes and distributions. *Phycologia*, **31**: 431–448.

Lindstrom, S.C. & Cole, K.M. (1992*c*). A revision of the species of *Porphyra* (Rhodophyta: Bangiales) occurring in British Columbia and adjacent waters. *Can. J. Bot.*, **70**: 2066–2075.

Lindstrom, S.C. & Cole, K.M. (1993). The systematics of *Porphyra*: character evolution in closely related species. *Hydrobiologia*, **260/261** (*Proc. Int. Seaweed Symp.*, **14**): 151–157.

Lindstrom, S.C. & South, G.R. (1989). Evidence of species relationships in the Palmariaceae (Palmariales, Rhodophyta) based on starch gel electrophoresis. *Crypt. Bot.*, **1**: 32–41.

Linhart, Y.B. & Grant, M.C. (1996). Evolutionary significance of local genetic differentiation in plants. *Annu. Rev. Ecol. Syst.*, **27**: 237–277.

Loveless, M.D. & Hamrick, J.L. (1984). Ecological determinants of genetic structure in plant populations. *Annu. Rev. Ecol. Syst.*, **15**: 65–95.

Lu, T.T. & Williams, S.L. (1994). Genetic diversity and genetic structure in the brown alga *Halidrys dioica* (Fucales: Cystoseiraceae) in Southern California. *Mar. Biol.*, **121**: 363–371.

Malinowski, K. (1974). '*Codium fragile*': the ecology and population biology of a colonizing species. PhD dissertation, Yale University, New Haven, CN.

Mallery, C. & Richardson, N. (1972). Disc gel electrophoresis of proteins of Rhodophyta. In *Proceedings of the Seventh International Seaweed Symposium* (Nishizawa, K., editor), 292–300. University of Tokyo Press, Japan.

Markert, C.L. & Möller, F. (1959). Multiple forms of enzymes: tissue, ontogenetic and species specific patterns. *Proc. Natl. Acad. Sci. USA*, **45**: 753–763.

Marsden, W.J.N., Callow, J.A. & Evans, L.V. (1981). A novel and comprehensive approach to the extraction of enzymes from brown algae and their separation by polyacrylamide gel electrophoresis. *Mar. Biol. Lett.*, **2**: 353–362.

Marsden, W.J.N., Callow, J.A. & Evans, L.V. (1984*a*). A preliminary investigation of the electrophoretic characteristics of enzymes from a range of macroscopic brown algae. *Bot. Mar.*, **27**: 521–526.

Marsden, W.J.N., Evans, L.V., Callow, J.A. & Keen, J.N. (1984*b*). A preliminary electrophoretic comparison of *Fucus serratus* and *Fucus vesiculosus*. *Bot. Mar.*, **27**: 79–83.

Matlock, D.B. & Romeo, C. (1981). Interspecific variability within the genus *Gracilaria* (Rhodophyta) on Guam. *Proc. 4th Int. Coral Reef Symp.*, **2**: 415–417.

May, B. (1992). Starch gel electrophoresis of allozymes. In *Molecular Genetic Analysis of Populations: a Practical Approach* (Hoelzel, A.R., editor), 1–28. IRL Press, Boca Raton, FL.

Miura, W., Fujio, Y. & Suto, S. (1978*a*). Isozymes from individual thallus of *Porphyra* species. *Jpn. J. Phycol.*, **26**: 139–143. (In Japanese.)

Miura, W., Fujio, Y. & Suto, S. (1978*b*). Polymorphism of catalase isozyme in *Porphyra*. *Tohoku J. Agric. Res.*, **29**: 159–166.

Miura, W., Fujio, Y. & Suto, S. (1979). Genetic differentiation between the wild and cultured populations of *Porphyra yezoensis*. *Tohoku J. Agric. Res.*, **30**: 114–125.

Mohammad, Y.A. & Shaalan, S.H. (1985). Electrophoretic studies of isoenzymes in some Rhodophycean marine algae from Alexandria. *Bull. Fac. Sci., Alex. Univ.*, **25**: 10–22.

Mohammad, Y.A. & Shaalan, S.H. (1991). Classical and biochemical taxonomy of some *Sargassum* species from the seashore of Alexandria, Egypt. *Bull. Fac. Sci., Alex. Univ.*, **31**: 118–141.

Murphy, R.W., Sites, J.W. Jr, Buth, D.G. & Haufler, C.H. (1996). Proteins: isozyme electrophoresis. In *Molecular Systematics*, 2nd edition (Hillis, D.M., Moritz, C. & Mable, B.K., editors), 51–120. Sinauer Associates, Sunderland, MA.

Neefus, C.D., Allen, B.P., Baldwin, H.P., Mathieson, A.C., Eckert, R.T., Yarish, C. & Miller, M.A. (1993). An examination of the population genetics of *Laminaria* and other brown algae in the Laminariales using starch gel electrophoresis. *Hydrobiologia*, **260/261** (*Proc. Int. Seaweed Symp.*, **14**): 67–79.

Nei, M. (1972). Genetic distance between populations. *Am. Nat.*, **106**: 283–292.

Nei, M. (1987). *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Neigel, J.E. (1997). A comparison of alternative strategies for estimating gene flow from genetic markers. *Annu. Rev. Ecol. Syst.*, **28**: 105–128.

Oelshlegel, F.J. & Stahman, M.A. (1973). The electrophoretic technique: a practical guide for its application. *Bull. Torr. Bot. Club*, **100**: 260–271.

Okumura, S. & Fujino, K. (1986). Thermostability of variations of enzymes in sea algae. *Bull. Jpn. Soc. Sci. Fish.*, **52**: 37–40.

Pearson, E.A. & Murray, S.N. (1997). Patterns of reproduction, genetic diversity and genetic differentiation in California populations of the geniculate coralline alga *Lithothrix aspergillum* (Rhodophyta). *J. Phycol.*, **33**: 753–763.

Penniman, C.A. (1987). Application of isozyme electrophoresis to systematic and genetic studies of marine macroalgae. *J. Phycol.*, **23** (suppl.): 12.

Rice, E.L. & Crowden, R.K. (1987). An improved method for the extraction and electrophoresis of proteins and active enzymes from fucalean macroalgae (Phaeophyta). *Phycologia*, **26**: 235–246.

Richardson, N. & Mallery, C.H. (1973). Some chemosystematic aspects of the *Audouinella* (*Acrochaetium-Rhodochorton*) complex. *Am. J. Bot.*, **60**: 1051–1056.

Rogers, J.S. (1972). Measures of genetic similarity and genetic distance. In *Studies in Genetics VII*, 145–153. University of Texas, Austin, TX.

Russell, G. (1986). Variation and natural selection in marine macroalgae. *Oceanogr. Mar. Biol. Annu. Rev.*, **24**: 309–377.

Shaalan, S.H. (1991). A collective taxonomic study of Eusiphonidae (Chlorophycophyta) from the Mediterranean seashore of Alexandria, Egypt. *Bull. Fac. Sci., Alex. Univ.*, **31**: 194–217.

Shaalan, S.H. (1992). Taxonomic studies of some Bryopsidophycean marine algae from the Mediterranean seashore of Alexandria: a cumulative approach. *Algol. Stud.*, **67**: 45–67.

Shaalan, S.H. & Mohammad, Y.A. (1985). Chemotaxonomy of some marine algae belonging to family Ulvaceae from Alexandria sea shore. *J. Fac. Mar. Sci.*, **4**: 183–191.

Shaalan, S.H. & Mohammad, Y.A. (1986). Biochemical taxonomy of some *Codium* species from Alexandria sea shore. *J. Univ. Kuwait Sci.*, **13**: 101–108.

Shin, J.A. & Miura, A. (1990). Estimation of the degree of self-fertilisation in *Porphyra yezoensis* (Bangiales, Rhodophyta). *Hydrobiologia*, **204/205** (*Proc. Int. Seaweed Symp.*, **13**): 397–400.

Slatkin, M. (1985). Gene flow in natural populations. *Annu. Rev. Ecol. Syst.*, **16**: 393–430.

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science*, **236**: 787–792.

Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**: 264–279.

Soltis, D.E. & Soltis, P.S. (Editors) (1989*a*). *Isozymes in Plant Population Biology*. Dioscorides Press, Portland, OR.

Soltis, D.E. & Soltis, P.S. (1989*b*). Ploidy, breeding systems, and genetic differentiation in homosporous pteridophytes. In *Isozymes in Plant Population Biology* (Soltis, D.E. & Soltis, P.S., editors), 241–258. Dioscorides Press, Portland, OR.

Sosa, P.A. & Garcia-Reina, G. (1991*a*). Isozymes in seaweeds. In *Seaweed Cellular Biotechnology, Physiology and Intensive Cultivation* (Garcia Reina, G. & Pedersén, M., editors), 257–264. COST-48, University of Las Palmas de Gran Canaria, Las Palmas.

Sosa, P.A. & Garcia-Reina, G. (1991*b*). Suggestion for analysing isoenzymes in agarophytes by starch electrophoresis. In *Seaweed Cellular Biotechnology, Physiology and Intensive Cultivation* (Garcia Reina, G. & Pedersén, M., editors), 309–312. COST-48, University of Las Palmas de Gran Canaria, Las Palmas.

Sosa, P.A. & Garcia-Reina, G. (1992). Genetic variability and differentiation of sporophytes and gametophytes in populations of *Gelidium arbuscula* (Gelidaceae: Rhodophyta) determined by isozyme electrophoresis. *Mar. Biol.*, **113**: 679–688.

Sosa, P.A. & Garcia-Reina, G. (1993). Genetic variability of *Gelidium canariensis* determined by isozyme electrophoresis. *J. Phycol.*, **29**: 118–124.

Sosa, P.A., Cabrera-Pérez, M.A. & Garcia-Reina, G. (1996). Genetic variation of *Gracilaria cervicornis* (Rhodophyta) gametophytes from the Canary Islands. *Eur. J. Phycol.*, **31**: 143–147.

Sosa, P.A., Valero, M., Batista, F. & Gonzalez-Perez, M.A. (1998). Genetic structure of natural populations of *Gelidium* species: a re-evaluation of results. *J. Appl. Phycol.*, **10**: 279–284.

Sytsma, K. & Schaal, B. (1985). Genetic variation, differentiation, and evolution in a species complex of tropical shrubs based on isozymic data. *Evolution*, **39**: 582–593.

Tanksley, S.D. & Orton, T.J. (1983). *Isozymes in Plant Genetics and Breeding*, parts A and B. Elsevier, Amsterdam.

Valero, M., Richerd, S., Perrot, V. & Destombe, C. (1992). Evolution of alternation of haploid and diploid phases in life cycles. *Trends Ecol. Evol.*, **7**: 25–29.

van der Meer, J.P. (1986). Genetic contributions to research on seaweeds. *Prog. Phycol. Res.*, **4**: 1–35.

van der Meer, J.P. (1987). Experimental hybridization of *Palmaria palmata* (Rhodophyta) from the northeast and northwest Atlantic Ocean. *Can. J. Bot.*, **65**: 1451–1458.

van Oppen, M.J.H., Olsen, J.L. & Stam, W.T. (1995). Genetic variation within and among North Atlantic and Baltic populations of the benthic alga *Phycodrys rubens* (Rhodophyta). *Eur. J. Phycol.*, **30**: 251–260.

Weeden, N. & Wendel, J. (1989). Genetics of plant isozymes. In *Isozymes in Plant Biology* (Soltis, D. & Soltis, P., editors), 46–72. Dioscorides Press, Portland, OR.

Weir, B.S. (1996). Genetic Data Analysis II. Methods for Discrete Population Genetic Data. Sinauer Associates, Sunderland. MA.

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

Wendel, J. & Weeden, N.F. (1989). Visualization and interpretation of plant isozymes. In *Isozymes in Plant Biology* (Soltis, D. & Soltis, P., editors), 5–45. Dioscorides Press, Portland, OR.

Williams, S.L. & Di Fiori, R.E. (1996). Genetic diversity and structure in *Pelvetia fastigiata* (Phaeophyta: Fucales): does a small effective neighborhood size explain fine-scale genetic structure? *Mar. Biol.*, **126**: 371–382.

Wyatt, R., Stoneburner, A. & Odrzykoski, J.O. (1989). Bryophyte isozymes: systematic and evolutionary implications. In *Isozymes in Plant Biology*, (Soltis, D.E. & Soltis, P.S., editors), 221–240. Dioscorides Press, Portland, OR.