Molecular genetic analysis of variation in *Costaria costata* (Turner) Saunders

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Introduction

Kelps have been shown to exhibit significant intraspecific morphological variation (Sundene, 1958; Druehl, 1978; Mathieson et al., 1981). The possible causes (i.e., environmental and genetic) of this polymorphism have been discussed thoroughly (Norton et al., 1982; Innes, 1984) and investigated experimentally (Sundene, 1962; Chapman, 1973). Statistical analysis of morphological variation of contrasting forms has also been carried out (Widdowson, 1971; Druehl & Kemp, 1982). Generally, the above studies have indicated a high degree of phenotypic plasticity. This suggests the desirability of utilizing a method for delineating subspecific groups which provides data reflecting evolutionary and not morphological divergence.

Recently, techniques allowing the molecular analysis of DNA have been applied to the characterization of specific portions of chloroplast and nuclear DNA in kelps (Fain, 1986; Lim et al., 1986 respectively). These papers have dealt with taxonomic applications of these techniques. With the exception of an exploratory study by Fain (1986), no data have yet appeared regarding the characterization of within- and between-population genetic divergence in kelp. The primary aim of this paper will be to outline molecular genetic techniques briefly and then to present some preliminary data assessing two stands of *Costaria costata* (Turner) Saunders. The goal of this work is to ascertain the existence of stand-specific genetic markers which may be used to establish phylogenies of groups within and between stands.

Variations in DNA sequence for a given locus are heritable. They may be detected through restriction fragment length difference (RFLD) and primary sequence analysis. Since restriction endonucleases recognize specific cleavage sites they can be used to discriminate between DNA sequences which may have lost or gained one or more of these sites through mutational change (Lewin, 1983). Applications of RFLD analysis have spanned the spectrum from finding the phylogeny of human populations (Wainscoat et al., 1986) to finding the phylogeny of salmonids (Wilson et al., 1985) and maize (Johns et al., 1983). Two main classes of probes (DNA fragments used for analysis) are commonly used to discover RFLDs: probes of known sequence and function (Appels & Dvorak, 1982) and anonymous probes drawn at random from genomic libraries (Rose et al., 1983). The second type of probe is valuable in the search for RFLDs since they may encode any region of DNA either coding or non-coding (Gusella, 1986). Noncoding sequences such as intervening sequences (introns) and spacers (nontranscribed, or transcribed but not translated) are known to accumulate mutations more rapidly than conserved sequences (Long & Dawid, 1980). They are therefore more likely to show DNA differences. It is not necessary, for this type of study, to know the identity of the probe DNA sequence. Phylogenetic studies, analyzing population structure, require only that the sequence studied be heritable and that sufficient variation (although not too much) exist within it to expose the structure of the population.

Primary sequence analysis is the most powerful
existing method for detecting DNA divergence (e.g., Lim et al., 1986). This technique involves establishing cloned homologous fragments of DNA of known or anonymous function from individuals of choice. These fragments may then be biochemically sequenced by a method such as dideoxynucleotide chain termination (Sanger et al., 1977). With such data, regions of DNA can be compared in totality, resulting in a higher resolution of DNA differences than revealed by RFLD analysis. In conjunction with RFLD analysis one may utilize direct sequence analysis to establish the nature of mutations detected by the former method.

Costaria costata is a kelp native to the North Pacific and forms discontinuous stands in the low intertidal and subtidal regions. The phenotype of this species is highly polymorphic and it exists in a broad range of wave exposures from sheltered to highly exposed (Obrien, 1972). Multivariate analysis of two morphologically distinct stands of C. costata occurring on the coast of British Columbia, Canada has been carried out (Bhattacharya & Druehl, unpubl.). These data have indicated a statistically significant degree of morphological variation between plants in the sheltered site and exposed site stands. A preliminary study conducted by Obrien (1972) on C. costata suggested that such morphological variability may be environmentally induced. In the present study the sheltered site was off Brockton Point in Stanley Park, Vancouver (49°18' N, 123°07'30" W) and the exposed site was off Cape Beale, Vancouver Island (48°47'15" N, 125°12'45" W).

Materials and methods

The general technique of detecting RFLDs involves the digestion of genomic DNA with a restriction endonuclease. The resulting fragments are then size-fractionated on a gel, rendered single-stranded, transferred onto a solid support (Southern, 1975) and then hybridized to a radioactively labelled probe (Rigby et al., 1977). The areas of homology are visualized by exposure of X-ray film with the probe-bound genomic DNA.

Using the method of Fain & Druehl (1984) genomic DNA was extracted from individuals of C. costata arising from Stanley Park and Cape Beale. These DNA samples were purified with the phenol-extraction method (Maniatis et al., 1982) and digested with restriction enzymes at the conditions recommended by the manufacturers. Fragments were run on 0.7% agarose gels (Fig. 1), transferred onto nitrocellulose filters and hybridized to probes radioactively labelled with 32P by the nick-translation method (Maniatis et al., 1982).

Randomly cloned probes were developed from a digest of a Stanley Park plant with the endonuclease EcoRI. Resulting fragments were placed in a mixture containing the plasmid pUC 19 also digested with EcoRI. The inserts and plasmids were allowed to anneal and then ligated (T4 DNA

![Fig. 1. Restriction-endonuclease digest fragment patterns of DNA from plants from Cape Beale and Stanley Park. Lanes 1-4 represent two individuals from Stanley Park followed by two individuals from Cape Beale cut with Hind III. Lanes 5-8 represent these same DNAs cut with PstI. Lane A contains lambda DNA cut with EcoRI and HindIII as a fragment-size standard.]}