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Observations on the Chromosome Numbers of *Porphyra* (Bangiales, Rhodophyta) Populations from Long Island Sound to the Canadian Maritimes

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As part of an ongoing investigation into the species delineation and distribution of *Porphyra* from Long Island Sound to the Canadian Maritimes, cytological investigations are being undertaken. A variety of methods have been attempted and of the techniques used, DAPI fluorescent-dye staining was found to be most preferable. Three different *Porphyra purpurea* (Roth) C. Agardh populations were found within this geographic range, each with different chromosome numbers or arrangements. Different populations of *P. leucosticta* Thuret in Le Jolis were also delineated using this cytological method. The use of chromosome counts as an aid to traditional taxonomic methods has proved useful in this species-distribution investigation of *Porphyra* in the Northwest Atlantic.

Key Words: Bangiales, chromosomes, DAPI staining, *Porphyra*

INTRODUCTION

The red alga *Porphyra* (Bangiales, Rhodophyta) is among the most economically important marine algae in the world today, with an annual value of over US$1.4 billion (Hanisak 1998). It is a valuable food-stuff and a rich source of protein, vitamins and essential minerals. Cultivation has been ongoing in the Orient for hundreds of years with commercial cultivation being undertaken on a large scale since the 1950's. Developments into cultivation in the US have been underway since the 1970's (Merrill 1989).

While the species is widespread in temperate waters around the world, only a few North Pacific species are commercially utilised. Due to their economic potential, these species have been widely studied. Over 45 species have been investigated cytologically (Mumford and Cole 1977). The majority of these investigations have been focused on the Pacific species with North Atlantic species being investigated to a lesser degree (e.g. Kapraun and Freshwater 1987; Lindstrom and Cole 1992; Mitman 1991).

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As part of a large-scale investigation into the commercial potential and domestication of North Atlantic *Porphyra*, cytological investigations are being undertaken on populations from Long Island Sound to the Canadian Maritimes (Yarish *et al.* 1998, 1999). The use of chromosome number as an aid to taxonomy is well recognised (Mumford 1975; Mumford and Cole 1977; Cole 1990) and these cytological studies are being used, in conjunction with traditional morphometric investigations and ecophysiological experimentation, to help delineate the species composition and distribution of *Porphyra* throughout this geographical range (Chopin *et al.* 1999). Attempts at cultivation in the Northeastern United States have had limited success so far, in part due to the use of poorly adapted, introduced *Porphyra* species (Yarish *et al.* 1998). A better knowledge of the species distribution of *Porphyra* will help to identify and isolate more suitable candidates for commercial cultivation. This is a major step in the development of an indigenous *Porphyra* industry.

MATERIALS AND METHODS

Field collections were made from sites along the coasts of New England and the Canadian Maritimes (Table 1). Collected thalli were blotted dry, rolled in cheesecloth

Table 1. Collection sites and chromosome numbers of *Porphyra* populations from New England and the Canadian Maritimes

Species	Collection site	Chromosome number
P. purpurea	A: Avonport, Nova Scotia, Canada	5
P. purpurea	B: Hopewell Rocks, New Brunswick, Canada	5
P. purpurea	C: South Manursing Island, Rye, NY, USA	5
P. purpurea	D: Cape Breton Island, North Bay, Nova Scotia, Canada	2
P. purpurea	E: Dipper Harbour, New Brunswick, Canada	2
P. purpurea	F: Montauk Point, Montauk, NY, USA	2
P. leucosticta	G: Millstone Point, Waterford, CT, USA	2
P. leucosticta	H: Westport, MA, USA	2
P. leucosticta	I: Digby Neck, Sandy Cove West, Nova Scotia, Canada	3
P. miniata	J: Digby Neck, Sandy Cove West, Nova Scotia, Canada	3
P. miniata	K: Cole Harbour, Nova Scotia, Canada	3

and stored on ice. Species were identified using traditional morphometric parameters and taxonomic references such as Bird and McLachlan (1992) and Kornmann and Sahling (1991).

Spermatangial tissue was fixed in 3 parts absolute ethanol:1 part glacial acetic acid for 12-24 hours and then transferred to 70% ethanol for storage. Chromosomes were stained by modified procedures after Austin(1959), using acetocarmine, after Wittmann (1965), using iron-hematoxylin-chloral hydrate and after Hull *et al.* (1982), using the fluorescent dye DAPI (4',6-diamindino-2-phenylindole, Sigma).

Tissue to be stained with DAPI was first rehydrated in a series of ethanol solutions (75, 50, 30, 15%) and finally in distilled water. Samples were then stained in 0.5 μg/ml DAPI in McIlvaine's Buffer, pH 4.0 for 10 minutes. It was then left to destain for one hour before being mounted in buffer and examined. Further experimentation with this method found that rehydration for 10 minutes in deionised water and staining in 0.5 μg/ml DAPI/deionised water for ~30 minutes worked equally well. Samples were mounted in the DAPI solution for examination. Of all the methods attempted in this study the use of DAPI staining was found to be the easiest staining method and as such was used for the majority of samples.

Stained cells were examined and photographed under oil immersion using an Olympus BX50 microscope with an HBO 100W mercury vapour illumination epifluorescence attachment and also with a Zeiss Universal research microscope. Digital pictures were taken with an Olympix digital monochrome 2MHz camera using ImagePro software and photographs taken with Kodak Tmax100 film and Ilford XP1 400 film. Images were digitised and manipulated with ImagePro for clarity.

Chromosome counts were determined from multiple samples from each location and from multiple counts of each prepared sample.

RESULTS AND DISCUSSION

The chromosome numbers of the populations examined are shown in Table 1 and Figure 1. *Porphyra purpurea* (Roth) C. Agardh populations from this geographic region are grouped into two, or possibly three, different types.

The populations at Cape Breton Island, Dipper Harbour and Montauk Point, NY (Table 1) were found to have a chromosome number of n=2 (Fig. 1 D, E, F). The *P. purpurea* at each of these locations was broad, dark olive green in colour and was found growing epiphytically in the mid-intertidal.

The *P. purpurea* found at Avonport, Nova Scotia and Hopewell Rocks, New Brunswick were growing epilithically on boulders in the intertidal mudflats (sandstone). These thalli were elongate and much lighter in colour than those at the other locales. These specimens had a chromosome number of 5 (Fig. 1 A, B). Previous studies have also found *P. purpurea* with n=5 from this area (Mitman 1991; Kito *et al.* 1971; Krishnamurthy 1959).

The third population from Long Island Sound were also n=5 but with one of the chromosomes being grouped away from the other four. The morphology of this *P. purpurea* was more similar to the n=2 thalli but they were growing epilithically. This apparent n=4+1 was not found in any of the other population but may simply be due to the grouping of different sized chromosomes. A similar pattern of chromosome distribution can be seen in *P. katadae* Miura (Yabu 1972).

Two different types of *P. leucosticta* Thuret in Le Jolis

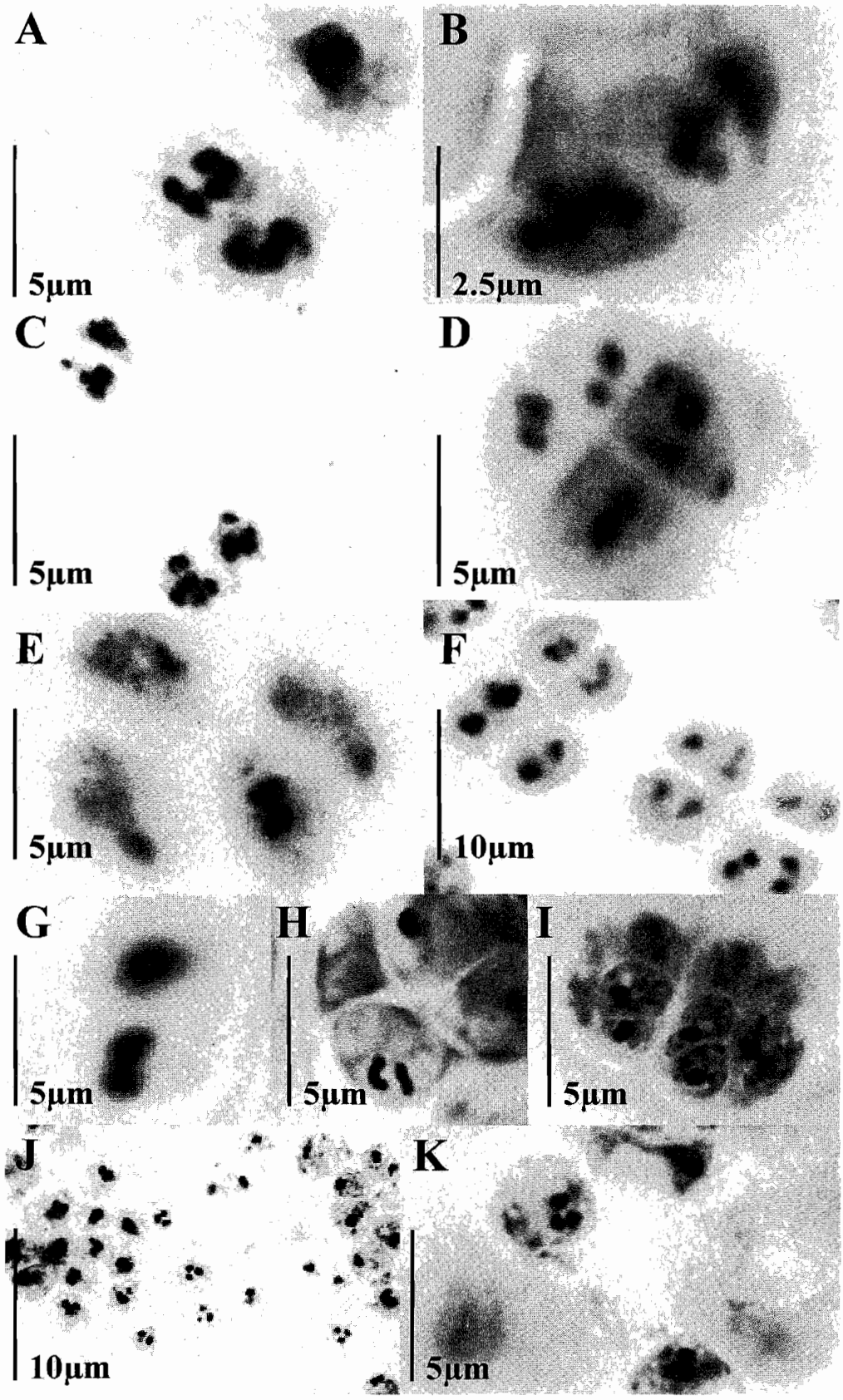

Fig. 1. Photomicrographs of spermatangial *Porphyra* tissue. Images have been digitally manipulated for clarity and contrast. A: *P. purpurea*; n=5. (DAPI) B: *P. purpurea*; n=5. (DAPI) C: *P. purpurea*; n=5. (DAPI) D: *P. purpurea*; n=2. (DAPI) E: *P. purpurea*; n=2. (DAPI) F: *P. purpurea*; n=2. (DAPI) G: *P. leucosticta*; n=2. (DAPI) H: *P. leucosticta*; n=2. (Wittmann's) I: *P. leucosticta*; n=3. (Wittmann's) J: *P. miniata*; n=3. (Wittmann's) K: *P. miniata*; n=3. (Wittmann's) See table 1 for sample details.

were uncovered. Populations from Westport, MA and Waterford, CT both had two large chromosomes. *P. leucosticta* from Nova Scotia had three. While both population were morphologically similar, chromosomal variation is a noted feature of this species. *P. leucosticta* has also been found with n=4 (Coll and Olivera Filho 1977). Kapraun *et al.* (1991) hypothesised that the wide distribution of *P. leucosticta* consists of a number of different geographical populations, an hypothesis supported by our present findings.

The populations of *Porphyra miniata* (C. Agardh) C. Agardh studied throughout this geographical range showed little variation and both locales studied cytologically were found with a chromosome number of 3. This is supported by previous studies (Lindstrom and Cole 1992; Kito *et al.* 1971).

The use of chromosome counts has proved useful in helping delineate the population distribution of different species throughout the geographical range of this study. It is clear that *Porphyra purpurea* is a more widespread and complicated entity than previously thought. It is present all along the Northeast coast of America but consists of several different ecological populations. More detailed studies are underway to further elucidate the composition and distribution of this species. The apparent presence of different population of *P. purpurea* is similar to the ecological distribution of more intensively investigated species such as *P. leucosticta*.

Alongside the more traditional taxonomic techniques, chromosome counts have proved very useful in mapping the *Porphyra* species distribution from the Canadian Maritimes to Long Island Sound.

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