Alternation of nuclear phases and chromosome numbers in Porphyra linearis (Bangiales, Rhodophyta) from western Ireland and Maine, USA

ELENA VARELA-ÁLVAREZ†, DAGMAR B. STENGEL*, FABIO RINDI AND MICHAEL D. GUIRY

Department of Botany, Martin Ryan Institute, National University of Ireland, Galway, Ireland

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Observations were made on chromosome numbers of isolates of Porphyra linearis (Rhodophyta) from western Ireland and Maine, USA. Chromosome numbers differed between specimens from East and West Atlantic populations. In Irish isolates the haploid chromosome number was \( n = 4 \) for all the specimens examined. Conchocelis filament cells and conchosporangial cells were diploid with \( 2n = 8 \), but zygotosporangial cells and some conchospores released had a diploid chromosome number which varied between 8 and 10. By contrast, in isolates from Maine, USA, the haploid chromosome number was mainly 3, with variations between 4 and 2. The loss or gain of one chromosome, probably caused by aneuploidy in the US isolates, could be explained as a step on the evolution of the chromosome number to 4. The results suggest that the P. linearis populations from the East and West Atlantic represent two separate entities.

INTRODUCTION

Knowledge of the chromosomes of red algae is basic to an understanding of their life-histories and provides valuable corroborative data in systematic studies (e.g. Cole 1990). Drew (1949) defined a life history as the recurring sequence of somatic and nuclear phases characteristic of a species. Both morphological and cytological information, including chromosome numbers and the occurrence and position of meiosis, are prerequisites to a thorough understanding of a life-history (Dixon 1982). However, complete data of chromosome numbers are available for only a relatively low percentage of red algal species (about 5% in 1990: Cole 1990).

The use of chromosome number as an aid to taxonomy in red algae is well recognized (Mumford & Cole 1977; Cole 1990). Chromosome information has been used as a critical taxonomic feature in distinguishing morphologically similar species within several genera, including Gracilaria Greville and Porphyra C. Agardh, and also in noting that morphologically similar populations of one species may exhibit karyotype changes (Cole 1990). Chromosome numbers have been determined for many red algal species, and the genus Porphyra in particular has been investigated cytologically more than any other genus of the Rhodophyta (Mumford & Cole 1977). Species of Bangiales (especially Bangia Lyngbye and Porphyra) have the advantage of having small numbers of chromosomes (\( n = 2–7 \)) contained in uninucleate cells. Chromosomes in spermatangial material are usually condensed during an extended prophase stage and readily stained and spread, even in specimens that have been conserved on herbarium sheets for a number of years (Cole et al. 1983).

In spite of difficulties in dealing with very small chromosomes, recent detailed studies of Bangia and Porphyra provided data leading to hypotheses on mechanisms of karyotype evolution within these genera, such as chromosome deletion, centric fission and fusion, inversions and translations, as well as meiotic nondisjunction (Cole 1990).

The taxonomy of the genus Porphyra is notoriously problematic. Species circumscriptions based on gross morphology do not provide clear solutions to taxonomic problems in the genus Porphyra (Nelson & Knight 1996). Taxonomic confusion surrounds the relationships of many morphological forms referred to P. linearis Greville. It is likely that linear specimens of several species, such as P. dioica Brodie & L.M. Irvine, P. leucosticta Thuret in Le Jolis, P. laciniata (Lightfoot) C. Agardh and P. purpurea (Roth) C. Agardh, have been erroneously identified as P. linearis (Varela-Álvarez 2002). Previous studies on the alternation of the nuclear phase in the life-history of P. linearis have been carried out by different authors who obtained different results. Magne (1952) first investigated the phenomenon in P. linearis and found the haploid chromosome number in material from the north-western coast of France to be 4, whereas Yabu (1978), working with material from Nova Scotia, found that the haploid number was 5. Dangeard (1927) reported that in P. umbilicalis (Linnaeus) Kützing f. linearis (Greville) Rosenvinge the haploid chromosome number was 2. A haploid chromosome number \( n = 4 \) has been reported for British P. linearis (M. Holmes personal communication in Brodie & Irvine 2003).

The present study was undertaken to determine the number of chromosomes (haploid and diploid) of P. linearis from western Ireland and Maine, USA, in order to resolve discrepancies in the previous records of chromosome numbers in this species.

MATERIAL AND METHODS

Plant material

Chromosome counts were obtained from cultures, field-collected material and herbarium specimens of P. linearis from

* Corresponding author (dagmar.stengel@nuigalway.ie).
† Present address: CCMAR, Centre for Marine Science, University of the Algarve, Campus Gambelas, P8005-139 Faro, Portugal.
two locations on different sides of the Atlantic: Galway Bay, Ireland and Maine, USA.

**Strains from Ireland:** Gametophytes of *P. linearis* were collected from two sites on upper intertidal rock in Salthill, Co. Galway, Ireland (9°05′W, 53°15′N; GALW 011331, GALW 011338) on 9 April 1997. Fronds normally appear in October; spermatangial and zygotosporangial sori usually develop in January–February; gametophytes begin to degenerate in April and disappear completely by June. From May to September blades do not occur on the shore.

*Porphyra linearis* at Salthill had a light red-brown to reddish colour. It had a linear frond, typically pear-shaped at the base. Reproduction occurred at the margin of the blade. Male sori appeared as pale yellow patches at the top or on the sides of the blade. Female sori were dark red and occurred in the basal parts of the blade. Usually, plants were dioecious or, less frequently, monoecious, with a distinct separation between male and female sori. This distinct separation was either between male and female sori.

**Strain from Maine:** Blades were produced in culture from conchocelis solution of *P. linearis* obtained from wild *P. linearis* blades collected in winter at Ocean Point, Boothbay Harbour, ME, USA, by Prof. C. Yarish. The material was sent to Ireland on 8 June 1999. Plants were cultivated at four temperatures (10°C, 13°C, 15°C and 20°C), two daylengths, 8:16 h and 16:8 h light–dark, and an irradiance of 10 μmol photons m⁻² s⁻¹ at all conditions. The optimum growth temperature was 10°C (Varela-Alvarez 2002). The blades produced in culture were red to olive-green or -brown, linear to pear-shaped. They were monoecious, with the reproductive parts located in small patches at the apical margin (Galway Herbarium GALW 011355).

**Fixation of specimens**

**Strains from Ireland:** At least 20 specimens collected from two sites at Salthill were fixed with Carnoy’s fluid (3:1 ethanol:acetic acid) at 12 different h over a day in an attempt to find the prophase stage of mitosis that usually occurs every 6 h. Conchocelis cultures were initiated from fertile plants collected at both sites at 10°C, 13:11 h light–dark, 10–20 μmol photons m⁻² s⁻¹. Fronds collected from the field were washed and cleaned in sterile seawater enriched using Guiry & Cunningham’s (1984) modification of von Stosch–enriched seawater medium (von Stosch 1964). After being air-dried, small fragments of thalli were placed into a Petri dish with fresh medium, at 15°C, 16:8 h light–dark, 5 μmol photons m⁻² s⁻¹. After release, zygotospores were removed and pipetted into flasks with fresh medium and incubated at 15°C, 10–20 μmol photons m⁻² s⁻¹, 16:8 h light–dark to obtain vegetative conchocelis filaments. Conchocelis plants were fixed at hourly intervals from 5 to 6 h before the initiation of the dark period (Kito et al. 1971).

**Strain from Maine:** Complete life-history cycle in culture was obtained from vegetative conchocelis (Varela-Alvarez 2002). Blades and conchocelis filaments were fixed at hourly intervals from 5 to 6 h before the initiation of the dark period (Kito et al. 1971). Chromosome counts were made from at least 20 cultured blades.

In both Irish and American strains, observations of fixed conchocelis and conchosporangial filaments, and conchospores were made in at least 20 dividing cells.

**Staining protocols**

Two different chromosome-staining protocols were used: (1) for material from the field, an aceto-orcein procedure staining was used according to a modified method described in Gargiulo et al. (1991); (2) for conchocelis cultures and herbarium specimens, Wittman’s (1965) staining procedure was used.

**Modified aceto-orcein staining method:** Material was fixed in Carnoy’s Fixative (three parts absolute alcohol, one part glacial acetic acid, made up immediately before fixation) for at least 24 h before staining. After 24 h, material was removed from the fixative and washed thoroughly in distilled water for 10 min to remove traces of the fixative. Material was then placed in 1 N HCl for 10–15 min and washed again for 10–15 min in distilled water. Fixed plants were placed in 4% pectinase for at least 20–25 min and again washed in distilled water. Aceto-orcein stain was added and left for at least 3 h.

Material was then removed and placed on a slide with a few drops of stain and heated gently until a slight colour change took place. The material was then squashed using the tip of an eraser-tipped pencil applied to thin cover-slips (no. 0). Light microscopy (LM) was done using oil-immersion and a ×100 objective; a camera was attached for photography.

**Aceto-iron–hematoxylin–chloral hydrate (Wittman method):** A stock solution of Wittman stain was prepared (Wittman 1965): 4 g hematoxylin (C₁₆H₁₄O₆); 1 g ferric ammonium sulphate [FeNH₄(SO₄)·12H₂O]; 100 ml 45% glacial acetic acid (C₂H₄O₂), and left to stand for at least 24 h before use. A working solution of Wittman stain was made with 5 ml of stock solution and 2 g of chloral hydrate. A 1 mm³ piece of plant material fixed in 3:1 acetic alcohol was placed on a slide. A drop of the working stain solution was then added to the cells and the material was compressed where necessary with a flat object before the cover slip was placed in position. The preparation was then carefully heated until a slight colour-change occurred. Material was squashed out with an eraser-tipped pencil applied to thin cover-slips (no. 0). Oil-immersion (×100 objective) was used for LM.

**RESULTS**

**Chromosome numbers in Irish isolates**

Chromosomes of different nuclear phases of Irish isolates are shown in Figs 1–7. Most mitotic stages were observed in the...

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Figs 1–10. Chromosomes in *Porphyra linearis*, LM. Images have been digitally enhanced for clarity and contrast.

Figs 1–7. Specimens from west coast of Ireland.

Figs 1, 2. Spermatia, n = 4 (indicated by arrows).

Fig. 3. Zygotosporangia, n = 8–10.
Fig. 4. Conchosporangial filaments, n = 8.
Figs 5, 6. Chromosome moving to the poles of the cells in anaphase.
Fig. 7. Conchospore release, n = 8–10.
Figs 8–10. Photomicrographs of spermatangial tissue of *P. linearis* from Maine.
Fig. 8. n = 3.
Fig. 9. n = 2.
Fig. 10. n = 4.
conchocelis phase fixed 3–5 h before the start of the light period, and in spermatia and fertilized zygotosporangia of blades fixed on the shore during the early hours of the morning. Gametophytes of the two populations of *P. linearis* from Salthill had a haploid number of 4. Fertilized zygotosporangia and zygotospores had a diploid number that varied between 8 and 10. The conchocelis and conchosporangial filaments, as well as conchospores, also had a diploid number between 8 and 10 (Table 1).

### Chromosome numbers in isolates from Maine, USA

Figs 8–10 show chromosomes of spermatangial tissue of *P. linearis* from Maine. In most of the samples, chromosome number was 3. In some cases chromosome number appeared to vary between 4 and 2 (Table 1).

#### DISCUSSION

The haploid chromosome number of *P. linearis* from the west coast of Ireland was consistently n = 4, and no variation was observed between populations or specimens. Gametophytes of the population of *P. linearis* from Maine, mostly had a haploid chromosome number of 3, varying between 2 and 4 by loss or gain of one chromosome during irregular mitotic divisions. The results suggest that two separate taxa exist: an entity in Ireland with a mean haploid complement of n = 4, and a separate entity from Maine, with a mean haploid complement of n = 3.

Aneuploidy, originating from an increase or decrease in chromosome number by less than a complete genome (Jackson 1971), is often characteristic of polyploid evolution in plants (e.g. De Wet 1980); for example, in *Polysiphonia Greville* and *Callithamnion* Lyngbye species, aneuploidy is thought to be a significant feature of evolution at the species and population level (Kapraun 1978a, b). Kapraun & Freshwater (1987) proposed that the basic haploid chromosome number for North Atlantic species of *Porphyra* was 4 because it occurred in four of the five species they studied. Our studies of *P. linearis* from Maine, suggest that aneuploidy could be taking place in these populations. The loss or gain of one chromosome could be explained as a step on the evolution of the chromosome number to 4.

Different opinions exist regarding the position of meiosis in species of *Porphyra*. For example, in *P. tenera* Kjellman meiosis has been observed variously during division of the fertilized carpospogon (Tseng & Chang 1955), during conchospore formation (Kito 1974, 1978) and during conchospore germination (Tseng & Sun 1989); in *P. yezoensis* Ueda, it has been observed during conchospore formation (Kito 1974, 1978) and conchospore germination (Ma & Miura 1984; Tseng & Sun 1989). In this study, the diploid chromosome number was determined only in the isolate from the Irish west coast. Diploid chromosome numbers were found in conchosporangia and conchocelis vegetative cells (2n = 8), as well as in zygotospores and conchospores (2n = 8 and 10). Haploid chromosome numbers (n = 4) were found in the blades. This suggests that meiosis takes place during conchospore germination.

Chromosome number has been very useful in mapping the *Porphyra* species distribution from the North Atlantic and the Mediterranean (Kito et al. 1971; Mumford & Cole 1977; Kapraun & Freshwater 1987; Lindstrom & Cole 1992). Krishnamurthy (1984) questioned the merging of several north-eastern Pacific *Porphyra* species with similar morphologies but different chromosome numbers.

The chromosome number of *P. linearis* from Ireland agrees with Magne’s (1952) report for French specimens of *P. linearis*. However, Yabu (1978) found a haploid chromosome number of 5 in material from Nova Scotia. Our population from Maine, which has a haploid chromosome number 2–4, represents another species or population. It is not possible to ascertain what type of material Yabu (1978) used, but it is evident that putative *P. linearis* isolates from the western Atlantic have different numbers of chromosomes from European populations, and probably represent a different species. The American strain we have examined here could represent the isolate of *P. linearis* analysed by Yabu (1978) or could even represent another cryptic winter species of *Porphyra*, which may have been misidentified or not yet recognized as an independent entity.

The possibility of alien species in the northeast Atlantic has been recently discussed in a comparison of the rbcL spacer sequence data for *Porphyrospora* sp. from the North Atlantic with species of *Porphyra* from the Pacific (Brodie et al. 1998). This study revealed that the *Porphyra* species fall into two distinct groupings: an Atlantic group containing *P. purpurea, P. dioica, P. amplissima* (Kjellman) Setchell & Hus ex Hus, *P. linearis, P. umbilicalis*, *P. miniata* (C. Agardh) C. Agardh and *Bangia atropurpurea* (Roth) C. Agardh, and a Pacific group containing *P. pseudolinearis* Ueda, *P. drachii* J. Feldmann, *P. leucosticta, P. yezoensis* and *P. insolita* P. Kornmann & P.-H. Sahling.

Important differences in chromosome numbers and temperature of release of conchospores between different populations of what is currently known as *P. linearis* have also been noted by S.C. Lindstrom (personal communication in Klein et al. 2003) and Varela-Alvarez et al. (2004). Whereas the release of conchospores takes place at only 13°C in Canadian isolates of *P. linearis* (Bird 1973), the Irish strains produced conchospores at a wider range of temperatures, with a distinct optimum at 20°C (Varela-Alvarez et al. 2004). The possibility of the separation and evolution of both strains could explain such differences in chromosome number and spore release.

### Table 1. Chromosome number in *Porphyra linearis.*

| Isolate                  | n  | 2n  | Tissue studied                      |
|-------------------------|----|-----|-------------------------------------|
| *P. linearis* from Ireland | 4 | 8–10 | G, S, FZ, ZY, C, CO                 |
| *P. linearis* from Maine  | 2–4| no data | S                                   |

1 G, gametophyte; S, spermatia; FZ, fertilized zygotosporangia; ZY, zygotospores or zygotosporangia; C, conchocelis filaments; CO, conchosporangia filaments and conchosporangia.
The results presented here provide further evidence of the utility of chromosome number as a taxonomic tool in the study of cryptic entities in the genus *Porphyra*. Detailed phylogenetic analyses based on DNA sequences of suitable molecular markers will be necessary for a complete clarification of the relationships of populations currently attributed to *P. linearis* from both sides of the Atlantic.

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