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Reproduction in *Sphacelaria biradiata* Askenasy (Sphacelariales, Phaeophyceae) in Southern Australia

Maria Gibson¹

Abstract
*Sphacelaria biradiata* Askenasy is a little known but common brown alga of southern Australian coasts. This paper describes its reproduction. Populations of *S. biradiata* reproduced asexually by vegetative propagules in all localities examined. The propagules were produced throughout the year and did not appear to be affected by season, although, if sexual structures occurred, propagule numbers declined. Sexual reproduction can occur and involves an alternation of generations, but it is a rare event. Gametophytes may produce male gametangia, female gametangia or both. Male gametangia, however, are extremely rare and are described for the first time. Production of female gametangia was influenced by season and occurred at all areas studied. The sexual behaviour of the male and female gametes is described. *(The Victorian Naturalist, 120* (5), 2003, 171-178)

Introduction
*Sphacelaria biradiata* Askenasy (Fig. 1) is a small brown alga which is mainly epiphytic on larger algae or seagrasses but occasionally is epilithic (grows on rock) or epizoic (grows on animals). Many erect filaments arise from monostromatic or polystromatic basal discs (Fig. 2) forming soft, isolated to densely aggregated tufts.

*Sphacelaria biradiata* is a common and yet little known alga of southern Australian coasts. Askenasy (1894), Sauvageau (1900-1914) and Womersley (1967, 1987) provided brief descriptions of the species but nothing is known of its ecology or reproduction.

Sexual reproduction in the Sphacelariales typically involves an ‘alternation of generations’ where a diploid plant, the sporophyte generation, produces reproductive

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¹ School of Biological and Chemical Sciences, Deakin University, Burwood, Victoria 3125
structures (sporangia) in which haploid spores are produced by meiosis. These haploid spores, sometimes called meiospores, germinate and grow into haploid plants, which constitute the gametophyte generation. Gametophytes produce reproductive structures (gametangia) in which haploid gametes are produced by mitosis. The male gametes fuse with the female gametes resulting in a diploid zygote, which grows into another sporophyte. An alternation of generations occurs in most brown algal orders. In most species, gametangia are plurilocular (having many compartments) and sporangia are

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Fig. 1. Dense tufts of *Sphacelaria biradiata* epiphytic on *Caulocystis uvifera*. Fig. 2. *Sphacelaria biradiata* (a) monostromatic and (b) polystromatic discs with erect filaments. Fig. 3. Propagules (P). Fig. 4. Unilocular sporangia.
Fig. 5. Reproduction of a population of *Sphacelaria biradiata*, Point Lonsdale, Victoria, showing the percentage of plants with **A.** unilocular sporangia, **B.** plurilocular organs, **C.** propagules, **D.** plurilocular and unilocular organs, **E.** plurilocular organs and propagules, **F.** sporangia and propagules, **G.** sporangia, propagules and plurilocular organs, and **H.** no reproductive structures.
Unilocular (having one compartment) (Clayton and King 1990), however, plurilocular organs producing asexual zooids (motile reproductive cells) also occur, for example, in *Ectocarpus siliculosus* (Dillwyn) Lyngbye (Ectocarpales) (Müller 1967), *Sphacelaria plumosa* Lyngbye, *Sphacelaria cirrosa* (Roth) C.A. Agardh and *Sphacelaria plumigera* Holmes (Prud'homme van Reine 1982).

Askenasy (1894), Sauvageau (1900-1914) and Womersley (1967, 1987) described plurilocular organs for *S. biradiata* but they did not state whether zooids were gametes or spores. Askenasy (1894) and Womersley (1967, 1987) also described unilocular sporangia and propagules, deciduous vegetative reproductive structures (Fig. 3). The presence of plurilocular and unilocular organs suggest that an alternation of generations may occur, but in the Sphacelariales a sporophyte generation may follow another sporophyte generation as meiosis fails to occur (Gibson 1989), for example in *Haloperis pseudospicata* Sauvageau, or a gametophyte generation may follow another gametophyte generation, for example in *Cladophyta spongiosa* (Hudson) C. Agardh (Gibson 1994).

This paper describes asexual reproduction by propagules and sexual reproduction in southern Australian *S. biradiata* and establishes that the zooids released by the plurilocular structures are gametes.

**Methods**

Monthly collections of 30 plants of *Sphacelaria biradiata* were made from Point Lonsdale, Victoria from June 1982 to July 1983 and transported to the laboratory in plastic bags on ice and in the dark. Fertile plants also were collected from Point Lonsdale on two other occasions (7/7/1985 and 9/9/1988), from Flinders (22/9/1981, 9/2/1982), Sorrento (9/2/1983), Apollo Bay (3/7/1984), Wilsons Promontory (2/8/1984) and Walkerville (3/8/1984), all in Victoria, from Cape Rabelais (January 1984) in South Australia and Wedgebay (26/10/1983) in Tasmania. The type of reproductive structures was noted and the behaviour of zooids from the plurilocular structures observed to determine any sexual nature.

Unialgal cultures were established from apical tips of filaments, propagules, spores and zooids from plurilocular organs and maintained in Provasoli's (1968) enriched seawater medium, initially in 10 cm diameter glass petri-dishes and, as plants became larger, in 250 ml pyrex culture dishes. Before use, petri-dishes and culture dishes were thoroughly washed in tap water and then in a dishwasher (without detergent) with five rinses, the last being distilled water. They were then autoclaved. Cultures were illuminated by Sylvania Grolux WS fluorescent tubes emitting a photon fluence of 60-70 μmol m⁻² s⁻¹. Controlled environment cabinets were used to provide temperature/daylength regimes corresponding approximately to conditions prevailing at various times of the year (Table 1) (Clayton 1980). Cultures were examined daily for the first week and then weekly for development of propagules, unilocular and plurilocular structure.

**Results**

**Sexual reproduction**

Plants of *Sphacelaria biradiata* from Point Lonsdale bearing unilocular sporangia (Fig. 4) were common throughout the year but most abundant during summer and autumn (Fig. 5). Plants bearing plurilocular structures also occurred throughout this period but were more common during winter and spring although they did not occur in very high numbers. Both plurilocular and unilocular structures were occasionally found on what appeared to be the same plant but were never found on the same filament. Plants having unilocular and/or plurilocular structures commonly also had propagules but, generally, fewer than plants without unilocular or plurilocular structures. Plants that were not reproductive, that is, without propagules and unilocular or plurilocular structures, were most frequent during winter.

Unilocular and plurilocular structures were borne on single celled pedicels that curved upward (Figs 4, 6-9). They were scattered singly along axes or in rows on either or both sides of axes.

Mature unilocular sporangia were spherical and measured 48-67 μm in diameter. Spore release was observed a number of times on several different occasions. Zoospores

ranged from 3-10 µm in length, had one chloroplast with an eyespot and two flagella. Some remained motile for up to two hours but the majority settled after a few minutes. Germination did not occur. Plurilocular gametangia were ovoid. Their length ranged from 37-74 µm, width 36-63 µm.

Plurilocular structures proved to be of two types, male and female. Male gametangia were found only twice, on 7/7/1985 and 9/9/1988, at Point Lonsdale. Female (Figs 6 and 7) and male (Figs 8 and 9) gametangia were easily distinguished. Male gametangia had smaller loculi (2.5-6 µm) than female gametangia (5-8 µm in diameter). Male gametangia were extremely pale and had one small chloroplast with an eyespot whereas female gametangia had one large to several small chloroplasts and one eyespot (occasionally two). Female gametangia were pyriform but only 2-6 µm long. Male and female gametangia could occur on one filament or on separate plants.

Fertilization was observed several times on 9/9/1988. Gametes were released through pores present in the outer wall of each locale. The female gametes initially moved rapidly in straight lines but then slowed and moved in ever decreasing circles. As they slowed they rounded up until, finally, they became stationary (Figs 10 and 11). Male gametangia were then attracted to them and fertilization occurred (Figs 12 and 13). Germination and subsequent development of zygotes was not followed.

Although unfertilized female gametes were frequently isolated and placed into culture, they did not germinate.

Plants grown in culture did not become fertile. Cultured plants did not attach to the culture vessel and as the unattached state was known to adversely affect fertility in some plants (Gibson 1989), the culture experiment was repeated using a variety of substrata in the hope that cultured plants would become attached. The various substrata used included a variety of filter papers of different pore sizes, glass wool, cotton wool, dental wax, beach sand, granite rocks, asbestos cement, coverslips and plastic and glass culture vessels. Attachment of cultured plants did not occur under any circumstances and cultured plants did not become sexually reproductive.

Vegetative reproduction

Propagules were found on the majority of specimens collected from all localities and did not show seasonality (Fig. 5). They were generally produced in the upper regions of plants. Of several thousand propagules isolated and placed into culture, approximately ten germinated. They did not attach to the culture vessels. The propagule arms and/or stem elongated and formed a prostrate or stolon-like system from which erect filaments developed. In shallow dishes, plants developed into large, flat, round mats, but in deeper dishes they formed into round balls, reaching about 10 cm in diameter.
Fig. 16. Possible reproductive cycle of *Sphacelaria biradiata*.

After two to six weeks in culture (depending on the conditions) secondary attachment discs formed (Figs 14 and 15), and often the thallus was a continuous mass of these discs which developed on first, second and even third order laterals. These formed the nucleus of new plants when the thallus deteriorated between discs. Plants never became fertile or produced propagules. Secondary attachment discs were not found on wild plants.

Tips with intact apical cells did not attach to the culture vessels. Elongation commonly occurred from the intact apical cell but a second apical cell or cells would frequently form from the cut end and growth would
occur in two directions. After seven days in culture, many erect filaments were produced from the original stem. Plants derived from apical tips formed free floating mats like those derived from propagules and did not develop propagules, sporangia or gametangia.

**Discussion**

The life history of *Sphacelaria biradiata* is still uncertain. The scarcity of male gametangia suggests that sexual reproduction and, therefore, the alternation of generations is a rare event. Gametophytes presumably develop from viable spores produced from diploid sporophytes but, they may, also, be derived from unfused gametes by parthenogenesis. The rarity of male plants suggests that parthenogenesis may occur in the life history of *S. biradiata*. However, female gametes did not germinate in cultures using various media, substrata, temperatures and daylengths. Parthenogenesis occurred readily in other members of the Sphacelarias (Gibson 1989, 1994; Hoek and Flinterman 1968; Prud’homme van Reine 1982). Also, plants of *S. biradiata* bearing female gametangia were common, although not numerous, in all localities examined in this study and release of gametes was achieved easily and gametes were active.

Plants bearing unilocular sporangia are typically diploid but haploid sporophytes are known in the Sphacelarias. In *Sphacelaria rigidula*, unfertilized female gametes developed into either haploid gametophytes, as would be expected from parthenogenesis, or haploid sporophytes (Gibson 1989, Hoek and Flinterman 1968). Haploid gametophytes produced viable gametes but the haploid sporophytes, which were morphologically identical to the normal diploid sporophytes, produced unilocular sporangia with infertile spores. Considering the number of wild plants of *S. biradiata* with unilocular sporangia in summer and autumn, more gametophytes would be expected in winter and spring, but gametophyte populations only ever reached half the size of sporophyte populations. This suggests the possible presence of haploid sporophytes with unilocular sporangia containing inviable spores, as occurs in *S. rigidula* (Gibson 1989).

Each generation produced propagules and, therefore, can vegetatively reproduce its own generation. However, few propagules germinated in culture. This also was noted by Prud’homme van Reine (1982) for *S. rigidula*. Germination of propagules in the field was not observed but this is not surprising considering their small size.

Askenasy (1894) and Womersley (1967, 1987) described plants with only one type of plurilocular organ. This study has demonstrated that two types of gametangia occur, those with small (male) and large (female) loculi, and also that plants may be monocious or dioecious. Fusion of gametes, observed for the first time, confirmed the sexual character of the gametes. A possible reproductive history is given in Fig. 16.

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