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PARTHENOGENESIS IN THE BROWN ALGA *LESSONIA NIGRESCENS* (LAMINARIALES, PHAEOPHYCEAE) FROM CENTRAL CHILE[1*]

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Parthenogenesis, the development of female gametes without fertilization, is relatively common in brown algae, although limited quantitative information on the phenomenon is available. Its occurrence is reported for the first time in *Lessonia nigrescens* Bory, a member of the Laminariales and a key ecological component of the benthic algal communities along the Chilean coast. Isolated female gametophytes developed into parthenosporophytes throughout the year, with a maximum in spring to early summer. Isolated male gametophytes, on the other hand, never developed fronds. Parthenosporophytes obtained in the laboratory developed normally when cultivated under greenhouse conditions, and the resulting individuals were indistinguishable in size, shape, texture, and color from heterozygous sporophytes. Quantification of DNA of various tissues demonstrated that early during their development, parthenosporophytes duplicated their DNA content, displaying levels similar to heterozygous sporophytes and almost twice the level found in gametophytes. One out of 45 individuals from a field population yielded only female gametophytes, strongly suggesting that parthenogenesis does occur in wild stands of *L. nigrescens*.

Key index words: **DNA quantification; flow cytometry; *Lessonia nigrescens;* parthenogenesis; Phaeophyceae**

Abbreviation: **SFC, sterile filtered enriched seawater medium C**

Parthenogenesis is a form of reproduction in which a gamete develops into a new individual without fertilization. This apomictic process has been interpreted as being advantageous because it avoids some of the costs of sexual reproduction, such as finding the gamete of the opposite sex for fertilization (Judson and Normark 1996). Parthenogenesis has been observed in a wide diversity of organisms, including animals, plants, and algae (Maynard-Smith 1986, Judson and Normark 1996, Vielle Calzada et al. 1996), but the frequency of the phenomenon varies among species (Judson and Normark 1996). The phenomenon has been recently reviewed in the context of the adaptive potential that parthenogenesis provides to a wide range of living organisms (Lushai et al. 2003).

Parthenogenesis occurs in both micro- and macroalgae and in the latter group has been reported in Chlorophyta, Rhodophyta, and Phaeophyceae. Within the brown algae, parthenogenetic development of both female and male gametes (the latter also being referred to as androgenesis) is the rule in isogamous species. In anisogamous taxa, usually only the female gametes are capable of parthenogenesis, although exceptions may exist. In oogamous brown algae, such as *Dictyota* (Dictyotales), parthenogenetic development of unfertilized eggs appears to be related to the presence of centrioles in oocytes: unfertilized eggs missing a centriole do not develop (Motomura 1994, Nagasato et al. 1998), whereas eggs of members of the order Laminariales, which possess a vestigial flagellum and contain a centriole (Motomura and Nagasato 2004), may show parthenogenetic development. However, parthenogenetic sporophytes of *Laminaria saccharina* (L.) J. V. Lamour., *Lam. longicruris* Bach. Pyl., *Lam. ochotensis* Miyabe, *Lam. digitata* (Huds.) J. V. Lamour., *Eisenia arborea* Aresch., *Nereocystis luetkeana* (K. Mert.) Postels et Rupr., *Macrocystis pyrifera, M. integrifolia* (L.) C. Agardh, *Alaria marginata* Postels et Rupr., *Lessoniopsis littoralis* (Farl. et Setch. ex Tilden) Reinke, and *Costaria costata* (C. Agardh) D. A. Saunders—all originated in cultures containing only female gametophytes—have been described to develop into abnormally shaped and fragile plants, with abnormal levels of ploidy and high mortality rates (Kemp and Cole 1961, Nakahara 1984, Yabu and Notoya 1985, Yabu and Sanbonsuga 1985, 1987, 1990, Yabu and Taniguchi 1990, Lewis et al. 1993, Druehl et al. 2005). Furthermore, these parthenogenetic plants never grew more than a few

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millimeters (Bolton et al. 1983, Le Gall et al. 1996). An exception to the apparently negative effect of parthenogenesis on development seems to be *Lam. japonica* Aresch., which was reported to produce normal, fertile, haploid parthenosporophytes from unisexual female gametophytes cultured in the laboratory (Fang et al. 1978, Nakahara 1984, Lewis et al. 1993). Fang also noted that spontaneous chromosome doubling occasionally occurred in haploid cell cultures producing homozygous diploid cells, from which fertile plants could be obtained (Chen and Lin 1976, Fang 1984). Moreover, female gametophytes of *Lam. japonica* derived from mature parthenosporophytes were again capable of parthenogenesis (Fang and Dai 1980). A similar phenomenon was reported for *Undaria pinnatifida* (Harv.) Suringar, in which gametophytes derived from mature parthenosporophytes experienced parthenogenesis (Fang et al. 1983).

Lessonia nigrescens belongs to the Laminariales, and it is a species that dominates in cover and biomass the lower-intertidal and shallow-subtidal areas, which are wave exposed and rocky, along most of the temperate Pacific coasts of South America, between 18 and 56° S (Kim 1971, Santelices et al. 1980, Hoffmann and Santelices 1997). This species is economically important because it is used as raw material for alginate extraction (Cancino and Santelices 1984). From an ecological point of view, *L. nigrescens* is used as food, shelter, and area for larval settlement by invertebrates and fish (Santelices et al. 1980, Cancino and Santelices 1981, Ojeda and Santelices 1984, Vásquez and Santelices 1984). *Lessonia nigrescens* has been described to have the typical *Laminaria*-type life history with dioecious, morphologically different microscopic gametophytes, and where the female-borne oocytes, after fertilization by the male-produced spermatozoids, develop into macroscopic sporophytes (Olivari 1974, Searles 1978, Avila et al. 1985). As both male and female gametophytes of *L. nigrescens* are commonly present and easily recognized in laboratory cultures due to their sexual dimorphism even at very early stages of development, the occurrence of sexual reproduction has never been questioned. Following the same rationale, the occurrence of alternative ways of producing the macroscopic sporophytes has never been tested in this species. However, our preliminary studies on the early stages of development in gametophytes and sporophytes of *L. nigrescens* revealed that male gametophytes were rarely mature, and, in spite of that, juvenile sporophytes always developed in cultures.

Thus, the objectives of the present work were to (i) assess the occurrence of parthenogenesis in *L. nigrescens*, (ii) characterize and quantify the phenomenon, (iii) assess the eventual development of parthenosporophytes, (iv) quantify DNA content of parthenosporophytes, and (v) test the occurrence of parthenogenesis in a natural population.

MATERIALS AND METHODS

Sampling and cultures. Fertile sporophytic fronds from at least five individuals of *L. nigrescens* were collected monthly from March to December 2003 in Las Cruces, central Chile (33°27′ S; 71°38′ W).

Mature sori were cleaned with running tap water, rinsed several times with sterile seawater, and incubated in 250 mL Erlenmeyer flasks with sterile seawater for 2–4 h at 16°C to obtain a spore suspension. Three drops of this suspension were inoculated into petri dishes with 4 mL of sterile filtered enriched seawater medium C (SFC; Correa et al. 1988). Spores were cultured at 10°C and a 12:12 light:dark (L:D) photoperiod, with cool-white fluorescent tubes (tlt 20W/54 RS Day Light; Philips, Sao Paulo, Brazil) yielding a photon fluence rate of 35–45 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Culture medium was changed once a week, and observations were made every 3 d.

After 12–15 d in culture, gametophytes with two to six cells and typical female morphology were selected and isolated from the starting culture using a micropipette manipulated on a Nikon Eclipse TE300 inverted microscope (Nikon Corp., Tokyo, Japan). Monthly, a total of 100 female gametophytes isolated as indicated were cultivated individually in plastic multiplates under the same conditions as the starting culture. After 50 d in culture, the isolated female gametophytes were monitored to record the presence of parthenogenetic sporophytes.

Development of the parthenosporophytic progeny. Parthenosporophytes obtained during April–May were sent to indoor culture facilities available at Universidad Austral in Puerto Montt, southern Chile. These plants were fastened to plastic frames and maintained in 100 L tanks with semicontinuous water exchange and aeration. The maximum length of each individual was recorded weekly. Heterozygous sporophytes (obtained from cultures containing mature gametophytes of both sexes) were used as controls.

DNA content. The ploidy level of parthenosporophytes and of heterozygous sporophytes was assessed by measurements of the DNA content using flow cytometry of isolated nuclei, according to a methodology described elsewhere (Le Gall et al. 1993, 1996, Asensi et al. 2001). Briefly, nuclei obtained by chopping tissue were stained with SYBR Green I (Molecular Probes Inc., Eugene, OR, USA), and DNA quantification was performed in a FACSort flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with an adjustable laser (coherent innova 90) emitting at 353–357 nm with the appropriate emission filters. Stained nuclei of *Ectocarpus siliculosus* (Dillwyn) Lyngb. (Phaeophyceae) gametes and of *Chondrus crispus* Stackh. (Rhodophyta) gametophytes were added to the samples as internal standards.

The experimental ratios of fluorescence—that is, the ratio of the intensity of fluorescence emitted by the sample population of nuclei to that of reference nuclei—were taken into account. Using this procedure, the relative DNA content was estimated for 100–1200 nuclei per sample. The ploidy level of sporophytes was estimated on the basis of their nuclear DNA content relative to that of haploid cells (Le Gall et al. 1996).

Parthenogenesis in natural populations. A total of 46 mature sori, each from a different plant of *L. nigrescens,* were collected in April 2004 from the same population sampled to obtain the reproductive material used in the first part of the study. They were prepared as explained above prior to incubation. Once cleaned, each sorus was inoculated into a 15 mL plastic tube with sterile seawater and incubated for 2–4 h to obtain a spore suspension. Three drops from each suspension were inoculated into individual petri dishes to create individual cultures for each parental plant. These cultures were maintained under the same conditions as described above. After 25 d in culture,

the ratio between male and female gametophytes was recorded for each plant.

RESULTS

Shortly after release, spores of *L. nigrescens* withdrew their flagella, became rounded, and initiated the process of settlement (Fig. 1A). Large-celled early female gametophytes (Fig. 1B) developed rapidly and within 7–12 d were clearly different from male gametophytes, which appeared at this time as smaller and thinner unbranched filaments. Female gametophytes became reproductive, both in mixed and clonal cultures, by developing at least one apical cell of their radiating vegetative filaments into an oogonium (Fig. 1C). Oocytes (Fig. 1C) were clearly distinguishable in female-only cultures after 7 d of isolation. These egg cells underwent mitosis and gave rise to erect fronds (Fig. 1D), representing the beginning of the development of parthenogenetic sporophytes. The presence of small bladelike formations attached to the female gametophytes was recorded as early as 4 d after the development of the oocyte was completed. The parthenogenetic fronds remained attached to the gametophyte (Fig. 1E), and it was possible to observe multiple parthenogenetic fronds attached to a single gametophyte (Fig. 1F). Isolated male gametophytes, maintained for up to 50 d under the same culture conditions as their female counterparts, only developed into spherical masses of thin filaments that never developed fronds. Furthermore, we found no microscopic evidence of maturation (i.e., mature male gametangia) in these isolated male gametophytes.

Parthenogenesis in cultures of *L. nigrescens* occurred during most of the year, with a maximum in spring to early summer (October–December; Fig. 2) when almost all female gametophytes produced parthenosporophytic fronds. During April and May, the same protocol used to isolate female gametophytes of *L. nigrescens* was used to isolate 100 male gametophytes each month. Androgenesis, however, was not observed in these cultures.

Parthenosporophytic and heterozygous sporophytes from April, May, and June (raised in the greenhouse) grew at similar rates, reached similar sizes, and had the same shape, texture, and pigmentation after 3 months of indoor cultivation (Fig. 3, A–C).

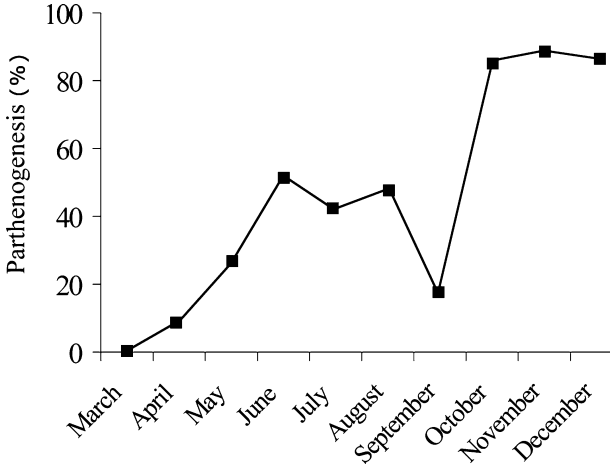

FIG. 2. Frequency of parthenogenesis in female gametophyte cultures of *Lessonia nigrescens* isolated from March to December 2003.

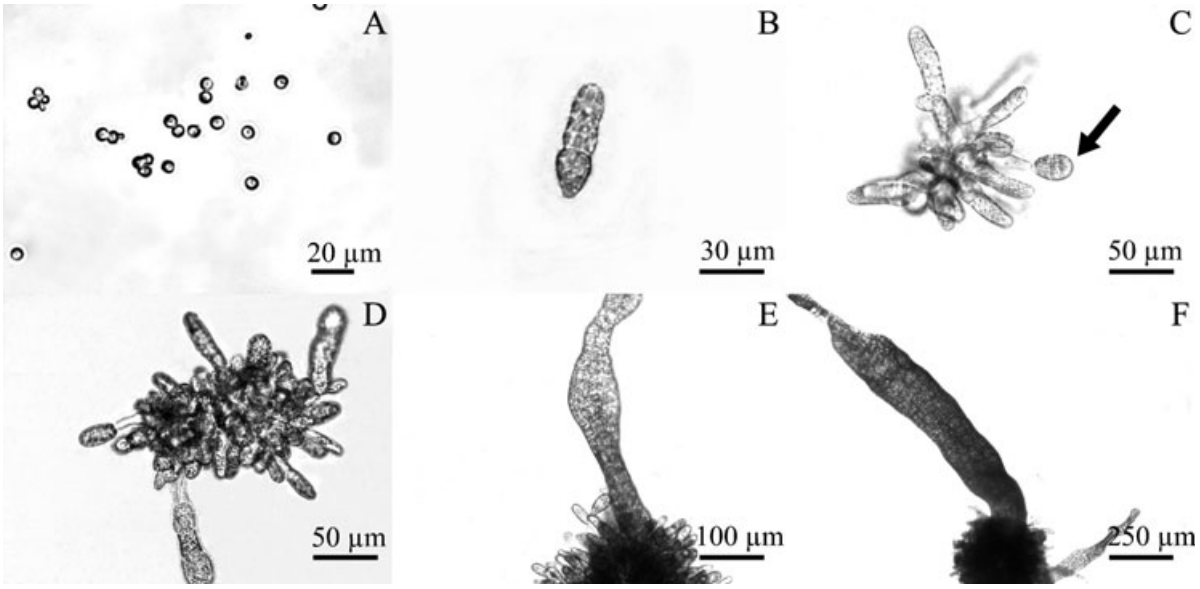

FIG. 1. Parthenogenetic development of sporophytes in *Lessonia nigrescens*. (A) One-day-old meiospores. (B) Four-celled female gametophyte at day 12. (C) Mature female gametophyte with egg-bearing oogonium (arrow) at day 21. (D) Young parthenosporophyte attached to a female gametophyte at day 31. (E) Parthenosporophyte still attached to a female gametophyte in a 43-day-old culture. (F) Two parthenosporophytes attached to a single female gametophyte at day 50.

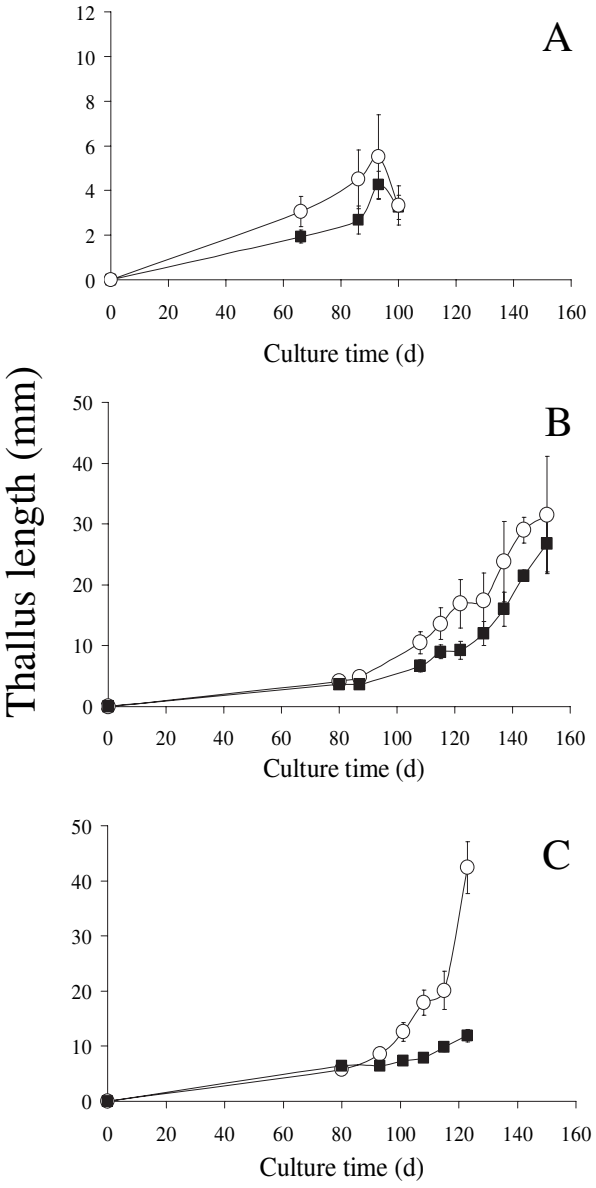

FIG. 3. Growth of tank-cultivated *Lessonia nigrescens* sporophytes from spores isolated in April (A), May (B), and June (C). ○, parthenosporophytes ($n = 5$); ■, control thalli (heterozygous sporophytes, $n = 5$). Error bars show standard error.

DNA content. The ratio between mean values of nuclear fluorescence emission of parthenosporophytic tissue from five different thalli and gametophyte filaments ($n = 5$) ranged from 1.75 to 1.86 (Table 1). The ratio between mean values of nuclear fluorescence emission of heterozygous sporophytic tissue ($n = 5$) and gametophytes ($n = 5$) was 1.8–1.91 (Table 2).

Parthenogenesis in natural populations. The ratio between male and female gametophytes obtained from 45 wild individuals was 41:59, a ratio not significantly different from 1:1 (Kruskal–Wallis test, $H = 1.08$; df = 1, $P = 0.299$), indicating that the sampled population of *L. nigrescens* consists almost exclusively of heterozygote individuals. However, one individual produced only female offspring.

TABLE 1. Mean values of fluorescence emitted from nuclei from five different parthenosporophytic individuals of *Lessonia nigrescens*.

Measurement	Individuals	No. nuclei	Emission	2*n*:*n*
1	PS-1	445	181.8	1.81
2	PS-2	567	199.7	1.75
3	PS-3	437	229.0	1.83
4	PS-4	888	231.7	1.85
5	PS-5	183	217.4	1.86

2*n*:*n* = parthenosporophytic:gamethophyte emission ratio.

TABLE 2. Mean values of fluorescence emitted from nuclei from five different heterozygous sporophyes of *Lessonia nigrescens*.

Measurement	Individuals	No. nuclei	Emission	2*n*:*n*
1	NS-1	747	183.6	1.84
2	NS-2	337	205.4	1.91
3	NS-3	187	169.4	1.8
4	NS-4	522	169.7	1.84
5	NS-5	274	167.5	1.82

2*n*:*n* = heterozygous sporophyte:gamethophyte emission ratio.

DISCUSSION

Our results demonstrate that the phenomenon of parthenogenesis takes place in *L. nigrescens*, both in nature and in culture. One out of 45 individuals sampled in the wild population studied yielded female gametophytes only. The latter condition has been used in *Lam. japonica* as a diagnostic feature of parthenogenesis (Fang 1983), and as Lewis et al. (1993) commented, it is consistent with earlier observations that sex determination in brown algae is genotypic (Schreiber 1930, 1935, Evans 1965, Müller 1967, Yabu and Sanbonsuga 1981). In culture, a surprisingly large proportion of clonal female gametophytes (up to 90%) produced sporophytes in the absence of male gametes. We rule out contamination by male gametophytes, mainly due to the differential development rate and time to reach maturity that was observed for female and male gametophytes. These differences allowed us to identify and isolate female gametophytes early in the process, well before any indication of maturity was detected in the co-occurring male gametophytes. Furthermore, microscopic observation found no signs of cryptic monoecism (i.e., male gametangia on female gametophytes).

Parthenosporophytes of *L. nigrescens* developed normally both in the laboratory and when cultivated in tanks, resembling previous findings in *Lam. japonica* (Fang 1983, 1984, Lewis et al. 1993), and *U. pinnatifida* (Yan 1984, Kawashima and Tokuda 1993), in which parthenosporophytes produced in

the laboratory were raised in culture. In *Lam. japonica*, such parthenosporophytes were even cultivated for up to nine successive generations in the sea (Lewis et al. 1993). The apparent normality in the development of parthenosporophytes of *L. nigrescens* and *Lam. japonica* contrasts with several reports on other Laminariales, suggesting that parthenogenesis is more of a rarity and that most of the resulting parthenosporophytes are abortive or abnormal (Sundene 1958, Kemp and Cole 1961, Svendsen and Kain 1971, Nakahara and Nakamura 1973, Chapman 1974, Lüning 1975, Fang et al. 1978, Sanbonsuga and Neushul 1978, Bolton et al. 1983, Fang 1983, Bharathan and Shinmura 1986, tom Dieck 1992, Lewis and Neushul 1994, Druehl et al. 2005). In a broader context, abnormal development of sporophytes in brown algae has been associated with their parthenogenetic origin (Clayton 1988), but there are also several reports of normal development as haploid individuals, as in *Arthrocladia villosa* (Huds.) Duby (Müller and Meel 1982), *Desmarestia viridis* (O. F. Müll.) J. V. Lamour. (Nakahara 1984), *Halosiphon tomentosus* (Lyngb.) Jaasund (Maier 1984), and *Haplospora globosa* Kjellm. (Kuhlenkamp and Müller 1985).

In the case of *L. nigrescens*, there are at least three elements that suggest that parthenogenesis is not a rarity. First, the phenomenon involved female gametophytes isolated through most of the year. Second, parthenogenesis was expressed in a large proportion of the female gametophytes, with levels close to 90% in spring gametophytes. Lastly, the phenomenon also occurs in the wild, as concluded from the presence of a sporophyte containing only the female sex factor.

Quantification of DNA contents indicated that both parthenosporophytes and heterozygous sporophytes of *L. nigrescens* were diploid. The results obtained from flow cytometry were in agreement with microfluorometric measurements, in which DAPI-stained nuclei of parthenogenetic sporophytes showed 1.7 times the fluorescence of those in gametophyte filaments (data not shown in detail). These results suggest that parthenosporophytes of *L. nigrescens* acquired diploid DNA content during the development of the frond. In this context, it seems possible that an early diploidization of parthenosporophytes in *L. nigrescens* is responsible for their vigor. The evidence available in the literature is controversial on this issue. On the basis of chromosome counts and the fact that parthenosporophytes reproduced normally in farms, Fang (1983, 1984) and Fang et al. (1978) concluded that sporophytes of *Lam. japonica* developed from female gametophytes were diploid and that spores from these parthenosporophytes were the result of meiosis during maturation of the sporophytes (Fang and Dai 1980). Likewise, Lewis et al. (1993) recognized that a large proportion of their parthenogenetic kelp sporophytes were diploid, but they also showed that young and mature parthenogenetic sporophytes of *Lam. japonica* could be haploid and that sporogenesis in those individuals did not involve a reduction in the chromosome numbers. The same study demonstrated that in spite of the fact that parthenosporophytes could display up to hexaploidy, the three strains that became reproductive were haploid (Lewis et al. 1993).

There is no consistent developmental pattern following parthenogenesis in isogamous brown algae. In *E. siliculosus*, Müller (1967) observed that nonfused gametes developed into parthenosporophytes, but chromosome counts showed that parthenosporophytes remained either haploid or underwent vegetative diploidization. Nonfused gametes of other isogamous brown algae, whose chromosomes were counted, may develop into haploid sporophytes (Henry and Müller 1983), haploid gametophytes (Müller 1984), either haploid gametophytes or sporophytes (Nakahara 1984, Clayton 1986), or either haploid gametophytes or haploid or diploid sporophytes (Peters 1988). Thus, the mechanisms regulating the development into sporophyte or gametophyte generations in brown algae remain to be elucidated.

Even though our study was not focused on the fate of the male gametophytes, the only experiment performed with this purpose did not reveal any signs of frond development. This response differs from that of *Lam. japonica*, where androgenesis (Laviolette and Grassé 1971), or the formation of the so-called male sporophytes, has been reported. It was recognized, however, that these individuals were abnormal in morphology, grew very slowly, and rarely reached a normal size when farmed (Fang 1983). Furthermore, even in cases where *Lam. japonica* male sporophytes reached normal size, they never became reproductive. Androgenesis regularly occurs in isogamous Phaeophyceae such as *E. siliculosus* (Müller 1967). In the anisogamous brown alga *Colpomenia peregrina* Sauv., both female and to a much lesser extent male nonfused gametes produced partenosporophytes (Yamagishi and Kogame 1998). In fact, this greater success of female parthenosporophytes in culture was considered responsible for the female dominance observed in wild stands of *C. peregrina* in Japan. Androgenesis in oogamous species appears to be rare. In dioecious *Desmarestia firma* (C. Agardh) Skottsb. from Chile, Ramírez et al. (1986) observed that sporophytes developed from settled male gametes. Chromosome counts showed that the ploidy of such androgenetic sporophytes was either haploid or diploid.

For technical reasons, we were not able to transplant parthenosporophytes of *L. nigrescens* to their natural habitat (i.e., the wave-swept lower intertidal zone of exposed rocky shores), and we do not know whether they could reproduce like the cultivated strains of *Lam. japonica* (Lewis et al. 1993).

However, indirect evidence that parthenosporophytes of *L. nigrescens* may reach maturity is provided by the finding of a parthenosporophyte from the wild stand at Las Cruces. Certainly, more experimental work is needed, particularly to address the ecological consequences of this reproductive strategy. In this context, it is worthwhile to note that some studies have reported the dominance of female individuals in wild populations of brown algae, including *C. peregrina* (Yamagishi and Kogame 1998), *Cutleria multifida* (Turner) Grev. (Fletcher 1987, Womersley 1987), and *Cutleria cylindrica* Okamura (Kitayama et al. 1992). No information on this aspect is available for Laminariales, let alone for *L. nigrescens*. Unless large-scale germination experiments with sporophyte-derived spores are undertaken, there is little hope of unmasking hidden parthenosporophytes in wild stands of *L. nigrescens*. However, molecular markers designed to assess the level of homozygosity or to directly identify sex loci could be useful tools to quantify the frequency of parthenogenetic sporophytes in natural kelp populations.

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