Demography of genets of clonal red seaweeds: current limitations and proposed solutions using genetic markers from experimental populations

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ABSTRACT
For clonal red seaweeds, the genet is defined as the thallus that develops from a single spore. The demography of genets for these organisms is poorly known. This results from the inability of visually detecting genets when ramets (fronds) are abundant, when separate thalli actually represent fragments of one original genet, and when holdfast coalescence occurs between neighboring genets. The use of genetic markers, such as allozymes and certain DNA regions, has allowed for the identification of genets in natural populations of clonal terrestrial plants. However, this may not work for natural populations of clonal red seaweeds, because several genets may be produced by asexual spores from the same individual, rendering those genets unidentifiable with certain genetic markers. A useful approach could be to study experimental populations under controlled laboratory conditions. The position and shape of genets could be monitored as they develop from genetically distinct spores, selected as such on purpose. Once the visual identification of genets becomes impossible due to the increasing density of ramets, mapping the different ramet genotypes should allow for the continuous identification of genets. Some genetic variation could arise within genets through somatic mutation and genetic transposition. Rates of these processes are largely unknown for clonal seaweeds, so they should be first estimated to determine their potential effects on demographic studies based on genetic markers.

Key words: clonal; demography; Gelidiales; genet; genetic markers; Gigartinales; Gratilariales; ramet; Rhodophyta.

RESUMEN
Para algas rojas clonales, define el genet como el talo que se desarrolla a partir de una espora. La demografía de genets de estos organismos está muy poco estudiada. Esto se debe a nuestra incapacidad para diferenciar genets visualmente cuando la densidad de ramets (frondas) es alta, cuando vemos talos distintos que, en realidad, son fragmentos de un mismo genet original y cuando algunos genets vecinos se fusionan. El mapeo del genotipo de ramets, usando marcadores genéticos como alocromas y ADN, ha permitido identificar a los genets de plantas clonales terrestres en poblaciones naturales. Sin embargo, esto no servirá para poblaciones naturales de algas rojas clonales, pues varios genets pueden derivar de un mismo individuo original mediante esporas asexuales, lo que determina que esos genets hijos sean genéticamente iguales para ciertos marcadores. La demografía de genets de algas rojas clonales podría estudiarse mejor en poblaciones experimentales de laboratorio. Por ejemplo, se podría seleccionar esporas genéticamente distintas y monitorear la posición de los genets resultantes mientras crecen. Cuando su identificación visual se torna imposible, al aumentar la densidad de ramets, el mapeo del genotipo de los ramets debería permitir identificar la posición de los genets y, por lo tanto, estudiar su demografía. Algun grado de variación genética podría ocurrir en algunos genets por mutación somática o por transposición genética. La frecuencia de estos fenómenos es generalmente desconocida para algas rojas, por lo que debería primordialmente estimarse para determinar su posible efecto en estudios demográficos basados en marcadores genéticos.

Palabras clave: clonal; demografía; Gelidiales; genet; Gigartinales; Gratilariales; marcadores genéticos; ramet; Rhodophyta.
INTRODUCTION

Clonal plants are those that vegetatively produce similar functional units (e.g., shoots) that are potentially able to live on their own if physically separated from the parent plant. Such vegetative units are termed ramets, whereas the entire plant is termed genet (Harper and White, 1974). The demography of genets of clonal plants is less understood than those of nonclonal plants. Genets of clonal plants are difficult, and frequently impossible, to identify in the field, mainly because of their frequent fragmentation and of the difficulty in determining the association of ramets with their parent genet when they are produced by rhizomes or by intermingled stolons. These limitations seem to explain why research on plant demography has been principally done with nonclonal plants (Eriksson, 1993). Additionally, these problems apparently resulted in demographic studies on clonal plants being mostly done at the ramet level (de Kroon, 1983; Hara, 1984; Peterson and Jones, 1987; Suzuki and Hutchings, 1997).

Although limiting a demographic study to the ramet level may seem enough to understand the population dynamics of clonal plants, there is essential information that results only from genet demography. The ecological and evolutionary dynamics of clonal plants depend on the interactions between their hierarchical levels of organization (Eriksson and Jerling, 1990; Vuorisalo et al., 1997). For example, demographic rates of ramets may depend on the position of ramets within a genet, on their distance to a neighboring genet, on the dynamics of a disease within a genet, or on genet age or size. Genet identification is also important in making inferences about the evolution of foraging and reproductive strategies of clonal plants, especially when selection pressures act mainly on genets.

Seaweeds are important components of several coastal marine ecosystems. Many species are nonclonal, such as some kelps and fucooids (Phaeophyceae - brown algae -), and their individuals are usually easy to identify. Their basic density-dependent patterns are similar to those for nonclonal terrestrial plants (Black, 1974; Chapman and Goudy, 1983; Dean et al., 1988; Reed, 1990; Ang and DeWreede, 1992; Creed, 1995; Flores-Moya et al., 1997; Creed et al., 1998; Arenas and Fernández, 2000), with some special circumstances, such as the recruitment of intertidal fucooids (Ang and DeWreede, 1992). There are also several clonal seaweeds, which may be found among the Rhodophyta (red algae), the Chlorophyta (green algae), and the Phaeophyceae. This paper will focus on clonal red seaweeds, many of which are dominant in their communities and have economic value as well.

The genet of clonal red seaweeds is interpreted here as the entire thallus that develops from a single spore, whether a carpospore or a tetraspore. The word “genet” was originally used for clonal terrestrial plants to refer to the genetic individual that develops from a seed and that produces a number of ramets (Harper and White, 1974; Kays and Harper, 1974). Recent definitions of the genet refer specifically to the zygote as its initial cell (de Kroon and van Groenendaal, 1997). The term “genetic individual” has been coined to mean that all of the parts of the genet are genetically identical. However, somatic mutations can occur during the growth of the genet (Klokowski, 1997), so such interpretation should be abandoned. On the other hand, considering the zygote as the only initial cell of the genet is perhaps too limiting. The main purpose of having a definition of genet is to make the distinction between individuals that develop from a single reproductive cell and individuals that result from the vegetative fragmentation (clonal fragments, sensu Eriksson and Jerling, 1990) of a single original individual. Thus, the genet may be better defined as the free-living individual that develops from one zygote, parthenogenetic gametes, or spore and that produces ramets during growth. In this way, the definition of genet can be applied appropriately to the equivalent structure found in clonal seaweeds, bryophytes, and vascular plants. For more details on this discussion, see Scrosati (2002).

In many species of clonal red seaweeds, the genet is composed of a crustose (e.g., Gigartinales) or stoloniferous (e.g., Gelidiales) holdfast and of several fronds that vary greatly in form across species. Fronds can be considered as ramets, because of their capacity for independent life when separated from the parent thallus, provided that they remain attached to the substratum. Such separation could result from rock dislodgment, partial bleaching and subsequent death of certain regions of a genet, or partial herbivory on a genet. Despite the ecological and economic importance of these seaweeds, their genets have rarely been the subject of demographic studies, exceptions including those on the genus Mazzaella, in the Gigartinales (May, 1986; Dyck and DeWreede, 1995; Scrosati, 1998). As is the case for clonal terrestrial plants, for these seaweeds more is known about ramet demography, although for only a few species. There are important similarities with many clonal terrestrial plants, such as the general lack of self-thinning among growing ramets of seasonal herbar (Suzuki and Hutchings, 1997), which was observed for the seaweeds Gelidium sesquipedale (Clemente) Bornet et Thuret (Gelidiales; Santos, 1995). Mazzaella cornucopiae (Postels et Ruprecht) Hammersand (Scrosati and DeWreede, 1997), Chondrus crispus Stockhouse (Gigartinales), and Pterocladia capillacea (Gmelin) Santelices et Hammersand (Gelidiales; Scrosati and Servière-Zaragoza, 2000).

CURRENT LIMITATIONS

Why has genet demography been so poorly investigated for clonal red seaweeds? There seem to be three reasons.
The first one is the difficulty of identifying neighboring genets when fronds are dense, up to 21 cm$^{-2}$ for *Mazzaella cornucopiae* (Scrosati and Servière-Zaragoza, 2000) and up to 22 cm$^{-2}$ for *Pterocladia capillacea* (Scrosati, 2000), for example. High frond densities generally prevent the accurate detection of boundaries of crustose holdfasts and especially of stoloniferous ones, which often intermingle. The second reason is that a genet may break up into clonal fragments (sensu Eriksson and Jerling, 1990), each one composed of two or more ramets. Even if such clonal fragments are distant, one cannot tell whether they represent one or more genets without knowing their history (Fig. 1). The third reason is holdfast coalescence: for some members of the Gigartinales and the Gracilariaceae, holdfasts of young, neighboring genets may coalesce during growth (Tveten and Mathieson, 1976; Tveten-Gallagher and Mathieson, 1980; Maggs and Cheney, 1980; Santelices et al., 1996, 1999). At the adult stage, it is generally impossible to recognize the chimeric nature of a thallus after coalescence or, in other words, that such a thallus is composed of different genotypes.

The extent of holdfast coalescence may be significant in natural populations (Santelices et al., 1999). The demography of genets of coalescent clonal seaweeds may differ from that of nonclonal seaweeds and even clonal terrestrial plants, which normally do not coalesce. Would coalescing genets of clonal seaweeds grow according to density-dependent patterns, as nonclonal plants generally do? Would a physical encounter between growing genets always result in coalescence? Or would it result in competition, depending on the life-history phase or genet size, age, or genotype? To answer these questions, it is necessary to develop techniques to identify genets through time.

**INTEGRATING GENETIC MARKERS**

The basic technique, which has been used for the genus *Mazzaella* (May, 1986; Dyck and DeWreede, 1995; Scrosati, 1998), is the periodic mapping of genets while they are visually identifiable. For dense stands, additional techniques are needed. One approach might be to determine the genetic identity of ramets from a given area and, thus, to gain the ability to identify genets and clonal fragments. From mosses to flowering plants, allozyme electrophoresis, the RAPD (random amplified polymorphic DNA) technique, and DNA fingerprinting have been applied to samples of ramets to distinguish different neighboring genets (Ellstrand and Roose, 1987; Schaal et al., 1991; Widén et al., 1994; Cronberg, 1996; Harada et al., 1997; McLellan et al., 1997; Mayes et al., 1998; Reusch et al., 1998; Sydes and Peacock, 1998; Tyson et al., 1998; Bush and Mulcahy, 1999; Bushakra et al., 1999; Davis et al., 1999; Kudoh et al., 1999; Reusch et al., 1999; Sawada, 1999; Suzuki et al., 1999; Jónsdóttir et al., 2000; Pappert et al., 2000; Reusch et al., 2000). The level of resolution of these molecular techniques depends on the intraspecific variability of the enzyme system or DNA region selected for the study (Avise, 1984; Parker et al., 1998). At first glance, the analysis of the spatial distribution of ramet genotypes with a high-resolution molecular technique would appear to be enough to identify the genets of a clonal seaweed population. However, there are certain restrictive factors.

As part of the life cycle of most red seaweeds, many carpospores are produced within a female gametophyte, each carpospore potentially giving rise to a tetrasporophyte (Hawkes, 1990; Hømmersand and Fredericq, 1990). Carpospores are produced by a special reproductive phase referred to as the carposporophyte (gonimocarp, sensu van den Hoek et al., 1995), which develops within the female gametophyte after repeated mitotic events from a single zygote. Therefore, all of the tetrasporophytes that result from carpospores from a single carposporophyte would be genetically identical. As a consequence, even the most refined molecular technique will not be able to distinguish among these tetrasporophytes. This may result in the underestimation of genet density if these tetrasporophytes were wrongly interpreted as clonal fragments from one original genet. This problem may also occur when gametophytes are produced by other gametophytes directly through carpospores. Some gametophytes of *Mastocarpus papillatus* (C. Agardh) Kitzing (Gigartinales) are thought to produce carpospores through parthenogenesis from a single cell (Pöllnäs and West, 1977; Zupan and West, 1988) and some gametophytes of *Mastocarpus stellatus* (Stackhouse) Guiry are thought to produce carpospores from a zygote originated through self-fertilization, lacking male-determining alleles (Maggs, 1988). When coming from the same carposporophyte, both kinds of carpospores will likely produce geneti-
cally identical gametophytes, which may ultimately also result in unrealistically low numbers of genets identified. Additionally, a given tetrasporophyte may produce tetraspores that are genetically identical for certain markers even after meiotic recombination, which could also result in the underestimation of genet (each resulting gametophyte) density. The opposite situation, that is, the overestimation of genet density, could occur when clonal fragments of a genet are wrongly interpreted as sibling tetrasporophytes or parthenogenetically derived gametophytes. The possible under- or overestimation of genet density may also weaken evolutionary considerations about reproductive strategies, since the relative importance of reproduction and clonal fragmentation would be incorrectly assessed.

Additional factors that may limit the accuracy of genet identification through spatio-temporal mapping of ramet genotypes are somatic mutation, mitotic recombination, apomeiosis, and genetic transposition. These processes might induce genetic variation within a genet and result in an overestimation of genet density (not to be confused with overestimating genetic diversity, which would not occur) after sampling genetically different ramets from such a genet. Genetic mosaicism has been detected for a few species of clonal terrestrial plants (Gill et al., 1995).

Mutations are thought to have little effect on allele frequencies in each generation of eukaryotic organisms (Gill et al., 1995; Slatkin et al., 1995). Spontaneous mutation rates are between 10^{-4} and 10^{-6} mutations per gene per generation (Hart, 1988). However, mutation rates depend on the physicochemical properties of the DNA, among other factors (Cockburn, 1991). For example, in special areas of the genome termed VNTR (variable number of tandem repeats), mutation rates are particularly high, up to 10^{-2} per generation (Slatkin et al., 1995). For seaweeds, mutation rates are largely unknown, although there is evidence suggesting that rates vary across taxonomic groups (Russell, 1986).

Mitotic recombination is common in Gracilaria tikvahiae McLachlan (Gracilariidae) under laboratory conditions and is thought to occur in other red seaweeds as well (van der Meer and Todd, 1977; van der Meer, 1981). Using genetic markers for thallus color, recombinant areas within thalli of G. tikvahiae were identified as patches of different color on the surface of fertile areas, although never on juvenile fronds. Mitotic recombination is apparently uncommon in other red seaweeds: for example, the patterns of recombination described for G. tikvahiae were not detected in Chondrus crispus (van der Meer, 1981). For terrestrial plants, rates of mitotic recombination have been estimated between 10^{-4} and 10^{-3} per cell (Gill et al., 1995).

Apomeiosis was observed in laboratory cultures of Gracilaria tikvahiae, for which cytokinetic failure during tetrasporre formation resulted in bi- and tetrancleate spores that could produce genets with both male and female frons (van der Meer, 1977). Rates of natural occurrence of apomeiosis are unknown for red seaweeds.

Genetic transposition has been proposed, but not yet established, as an explanation for the dynamics of unstable color mutations for three members of the Gracilariales (van der Meer and Zhang, 1989). Nevertheless, the spontaneous reversion of color reported for parts of the same genet represents another case of genetic variability within genets.

EXPERIMENTAL POPULATIONS

The problems discussed above may discourage demographic studies on clonal algal genets when the history of a stand is unknown, which is the case for most natural populations. However, genet demography may be studied under controlled conditions. For example, several aquaria could be seeded with spores that are all genetically different. By manipulating spore settlement densities, abiotic variables, and the proportions between carpospores and tetraspores, demographic patterns for genets could be studied by periodically mapping their shape and the position of ramet genotypes as genets grow. Mapping holdfast shape will be obviously easier for seaweeds that grow in hard substrates (e.g., Chondrus and Mazzaella) than for those that grow in sand (e.g., Gracilaria). Also, it will be easier for crustose holdfasts (e.g., Chondrus and Mazzaella) than for stoloniferous ones (e.g., Gelidium and Pilayaciella).

The continuous spatial mapping of ramet genotypes will enable one to determine if contact areas between neighboring genets remain static after contact or if overgrowth occurs. Possible coalescence between crustose holdfasts could be detected through microscopic observations on holdfast samples from contact areas. For coalescing genets, genetic sampling of ramets should be continuous to investigate if one genotype progressively prevails over the other within the chimeric thallus. For coalescing genets of Gracilaria chilensis Bird, McLachlan et Oliveira, different parts of a single frond may display the different original genotypes (Santelices et al., 1996). Thus, it may be necessary to analyze more than one part per frond around coalescent areas to ensure an accurate genotype mapping. For species whose holdfasts do not coalesce, but intermingle, the periodical mapping of ramet genotypes should enable one to determine patterns of intermingling.

If a new allelic combination arises through mitotic recombination in a ramet from contact areas between neighbo-
ring genets, knowledge of the original genotypes may enable one to associate the ramet to its parent if neighboring genets are genetically different enough. The production of male and female fronds within a genet resulting from an apomeiotic spore should not compromise genet identification either if the original genotypes are known. Somatic mutation might still complicate genet identification if it occurs in ramets arising in contact areas, especially if one analyzes rapidly evolving DNA areas, such as microsatellites, located in VNTR loci (Avis-
ese, 1994; Jarne and Lagoda, 1995). Genetic transposition might also be a problem if it occurs in contact areas. Genetic differentiation within a genet occurs during the growth of Gracilaria chilensis, as detected with RAPD markers, although the nature of such changes is still unknown (Meneses et al., 1999). Rates of somatic mutation and of genetic transposition should be estimated for red seaweeds, so their potential effects on the accuracy of genet identification using the methods suggested here can be assessed.

In conclusion, this paper proposes that determining the changing shape of genets and the position of ramet genotypes from experimental populations with known initial genotypes is a potentially useful method to upgrade our understanding of genet demography for clonal red seaweeds. Rates of genetic change within genets should be previously estimated to design adequate experiments.

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