Journal of Applied Phycology **11:** 241–246, 1999.
© 1999 *Kluwer Academic Publishers. Printed in the Netherlands.*

Strain selection and genetic variation in *Gracilaria chilensis* (Gracilariales, Rhodophyta)

I. Meneses & B. Santelices*
Departamento de Ecología, Facultad de Ciencias Biológicas, Pontifica Universidad Católica de Chile, Casilla 114-D, Santiago, Chile

(*Author for correspondence)

Received 30 October 1998; revised January 1999; accepted 26 January 1999

Key words: DNA polymorphism, *Gracilaria chilensis,* genetic variability, strain selection

Abstract

Strain selection processes in seaweed often have assumed that sterile clones could be maintained for long periods in a diversity of environments without major genetic changes. However, clonal species such as *Gracilaria chilensis* exhibit intra-clonal variation in performance and ongoing studies suggest such changes may be due to rapid changes in DNA composition associated with growth, via mitotic recombinations. Therefore performance of a given ramet in this type of seaweed should be understood as the dynamic outcome of rapid reactions between the environment and the changing genotype of the selected strain. To evaluate this idea, we measured changes in genetic variability, as detected by DNA-fragment polymorphism using RAPDs-PCR, exhibited by clones of *G. chilensis* after two transfers to different environmental conditions (from field to controlled laboratory conditions and from the laboratory to large-scale tank culture). The transfer to laboratory conditions reduced the frequency of low similarity values and increased the frequency of intermediate similarity values in DNA banding patterns, suggesting the branchlets produced under controlled laboratory conditions have less genetic variability (evaluated as total DNA polymorphism) than plants recently collected in the field. Tank incubation reduced the total range of similarity and significantly increased the frequency of high similarity values. Results thus suggest the dynamic of genetic changes in vegetative clones of *Gracilaria chilensis* that is fast and strongly affected by the external environment.

Introduction

Vegetative propagation through thallus fragmentation is an advantageous aspect of commercial algal species. Simple thallus fragments are likely to grow and spread faster than spores or other types of microscopic propagules and the features selected in one particular strain are maintained by growing self-replicating fragments, which are supposedly identical genetic units. However, strain selection processes in seaweed (see Santelices, 1992 for review) often have assumed that: (a) selection processes could be completed under environmental conditions (*e.g.,* laboratory conditions or small tank facilities) not necessarily representative of the farm conditions where the selected strain is to be propagated and (b) that sterile clones could be maintained for long periods of time without major changes in their genetic make up.

Current conceptual and empirical knowledge of strain selection and genetic variation of clones suggests that these two assumptions may not be valid. Since a particular gene combination is selected under a given set of environmental conditions, the optimal performance of the selected strain is intimately related to the specific culture conditions under which selection was performed. Strain selection ultimately means the fixation of particular gene combinations to be later propagated intensively and extensively in a given mariculture setting. Given the close relationship between strain performance and culture conditions

used in the selection process, it would be expected that clones selected for their superior performances under a set of conditions will perform poorly when transplanted to other habitats.

Despite the importance of clonal propagation in maintaining particular genetic strains in mariculture of seaweeds, spontaneous intraclonal variation does occur in certain clones (Lignell & Peterson, 1989; Santelices, 1992; Santelices & Varela, 1993), which challenges the idea of genetic stability over time. The most frequently described types of change are somatic mutations and mitotic recombinations which can occur through branch (ramet) replication (van der Meer & Todd, 1979). In addition, motile genetic elements (*i.e.*, transposons) further increase the probabilities of genetic variation within a clone (van der Meer & Zhang, 1988).

Clones of the economically important *Gracilaria chilensis* exhibit intraclonal variability (Santelices & Varela, 1993; Santelices et al., 1995, 1996). Therefore, whenever strain selection is conducted in this species, selected strains may exhibit major changes in performance with time. One of the source of such variability found in this species may involve genetic changes coupled to growth, due to the probability of mitotic recombination and similar mechanisms (Meneses et al., 1999). Thus, fragments from a single thallus that initially showed no traces of genetic differences showed significant changes in genetic constitution as measured by RAPDs just by the fact of growing, even under constant environmental conditions. It follows that if the external environment strongly influences the gene combination to be fixed by selection and if such a combination, in turn, is constantly changing due to growth, the performance of ramets within a seaweed clone becomes a continuous reaction between genotype and environment, expressed at any particular time or place. Such a situation has been suggested for other clonal organisms (*e.g.*, Bryozoa, Boardman et al., 1969), but it has not been addressed for seaweeds.

In this study we evaluate the genetic variability expressed by clones of *Gracilaria chilensis* when grown under three different growing conditions. Genetic variability, as detected by DNA fragment polymorphism using RAPDs-PCR, was first measured in wild samples collected from a particular site. Then, thalli were transplanted to grow under controlled, small-scale, laboratory condition. After a month of growth genetic variability was measured again. Finally, the same thalli were transferred to large scale-tank culture conditions, measuring again genetic variability after a month of growth under tank conditions.

Materials and methods

Seven clumps of *Gracilaria chilensis*, each with 7–10 axes arising from a common holdfast were randomly collected from a natural population growing between 1 and 4 m deep in Maullín (41° 40′ S, 73° 45′ W), southern Chile. Three independent axes from each clump were selected and permanently labeled using plastic tags wrapped around them. From each of these 3 axes, three randomly chosen branches were cut off and their apices (approximately 2 mm long) were excised in order to extract total DNA. DNA from the three branches from a similar axis were pooled together. Thus, there were 3 samples of DNA (one of each axis) per a total of seven clumps, for a total of 21 samples.

Afterwards, the labeled plants were placed in seven 4.5 L glass tanks and cultured for 30 days under constant aeration in sterilized enriched seawater with SFC medium (Correa & McLachlan, 1991). Constant culture conditions were 40–50 μmol·m^{-2}·s^{-1}, 14 $\pm$ 1°; 12 h of daily light and pH 7.7. Culture medium was changed once a week. After a month under these conditions, a second DNA sampling was undertaken.

Thalli were then moved to 1000-L outdoor tanks at the Metri Marine Station (41° 36′ S, 72° 42′ W). Two clumps per tank remained for the following month under conditions of constant aeration, temperature between 12.8–14.02°C, pH 8.3–8.4 and 1600–1800 W m^{-2} of solar radiation (see Buschmann et al., 1994 for other cultivation conditions). At the end of the tank incubation period, a third DNA extraction from 3 apical branches of each previously tagged erect axes was completed.

DNA extraction and PCR methods followed Meneses (1996). The RAPDs method applied to the DNA samples segregates non-identified genetic loci generated by PCR amplification of the genomic DNA strands using short (10-mer) random primers. The segregation marker is a co-dominant. Since in diploid organisms the double amount of DNA may compete for annealing generating variable bands, only haploid (female gametophytes) thalli were used. Ten-mer primers of the OPX-series: 09, 10, 11, 12 and 13 were used for PCR amplification. Amplifications were repeated twice for each set of samples in order to ensure reproducibility of RAPDs results. Negative controls consisted of the entire reaction solution without the

DNA template, filling up the volume with distilled water or TE buffer.

Results from band polymorphism obtained from the agarose gel electrophoresis of the PCR products were recorded as 0s and 1s, depending on the absence or presence of bands respectively. These data matrices were the basis for similarity index calculations (Jaccard's) between pairs of samples. The process results in a symmetric similarity mathrix with 420 values, half of which are deleted because they correspond to comparisons of each sample with itself or to comparisons included in the other half of the mathrix. Indices were then grouped in interval of classes according to their values, ranging from 0.25 up to 1.0. Similarities values of less than 0.25 were added all together. Similarity values were calculated for each pair of sample (each pair of erect axes) during each phase of the experiment. Phase one corresponded to similarity comparisons among axes of the 7 clumps collected directly from the field (= 210 comparisons). Phase two corresponded to similarity comparisons among the same erect axes of the 7 clumps, now incubated for a month under constant laboratory culture conditions (n = 210 comparisons). Phase three corresponded to similarity comparisons among the same erect axes of the 7 clumps which had been transferred and incubated for a month under large tank cultivation conditions. In this last experiment, however, a number of clumps and erect axes were lost, reducing the number of potential comparisons to 78.

The frequency distribution histograms of similarity values describe the dispersal (= variability) of similarity in DNA banding patterns among different samples and the frequency of such similarity values within that phase. The distribution histograms for successive phases (phases 1, 2 and 3) were compared using Rank Signed Wilcoxon test for large samples (Siegel & Castellan, 1988). In this test, the statistical T^+ becomes normally distributed when the number of samples are large and are appropriate for non-independent samples (*e.g.*, treatments in phases 1, 2 and 3 performed on the same individuals).

Results

Similarity values obtained with branchlets of recently collected clumps ranged from 25% to 95% (Figure 1A). Although intermediate similarity values (*e.g.*, 50–55%, 65–70%) were slightly more frequent than other values, most similarity values between 30 and 80% were similarly represented in the sample. High similarity values (80–95%) were represented with values of 2% or less.

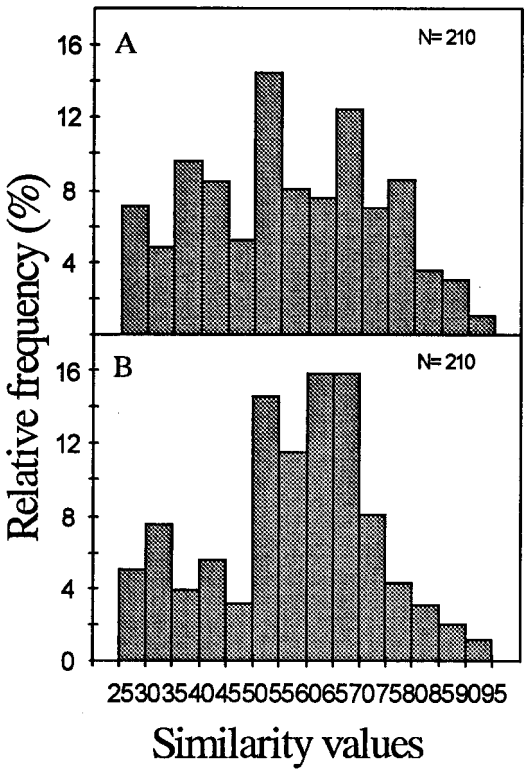

Figure 1. Frequency distribution of similarity indices of DNA sampled in erect axes of clumps of *Gracilaria chilensis*. A) Similarity values among axes of 7 clumps collected in Maullín River, southern Chile. B) Similarity values among the same axes incubated for a month under controlled laboratory conditions.

Cultivation of these thalli under controlled laboratory conditions for a month (Figure 1B) induces significant differences in similarity values ($Z = 4.46$; $N = 210$ $p < 0.001$; Wilcoxon test). There was a reduction in the frequency of the low similarity values as compared to the corresponding values among recently collected thalli (Figure 1A). On the other hand, there was an increase among intermediate similarity values (55–70%), showing that the branches produced under controlled laboratory conditions have less genetic variability (evaluated as total DNA polymorphism) than plants recently collected in the field.

Large-scale tank cultivation means plant exposure to variable temperature, light irradiance and nutrient conditions. Not all axis of all clumps could survive such variability. Clump N^o 4, 1 axes of clumps N^o 1 and N^o 2 and 2 axes of clump N^o 3 were completely

lost. In order to analyze the remaining material and compare it with results obtained in phases 1 and 2, we excluded data from clump 4 and from the corresponding axes that were ultimately lost. In Figure 2 we have recalculated the corresponding similarity values of the two first phases of this experiment without considering the lost axes.

Comparisons of DNA similarities (Figure 2) between tank and laboratory cultivated clumps indicated statistically significant differences in frequency distribution ($Z = 3.55$; $p < 0.001$; $N = 78$; Wilcoxon test). Compared with the laboratory cultivated branches, the tank cultivated branches exhibit slightly less genetic variability and an increase in the frequency of relatively high similarity values (75% similarity values and above).

The general pattern emerging from these experiments is one of changing genetic structure as plants and axes are moved from one to another culture condition. This can be read in Figure 2 where the similarity values in experimental thalli have been re-calculated only for those axes surviving through the three phases. Field cultivated axes have a broader range of genetic variability while most of the similarity values have approximately equal frequencies (Figure 2). Cultivation under laboratory conditions decreased the frequency of low similarity values and increased the frequency of medium similarity values, showing a slight reduction of total genetic variability. The subsequent transfer to tank cultivation effectively reduces the range of similarity values and increases the frequency of high similarity indices, showing further reduction of genetic variability among the branchlets produced under this last type of culture.

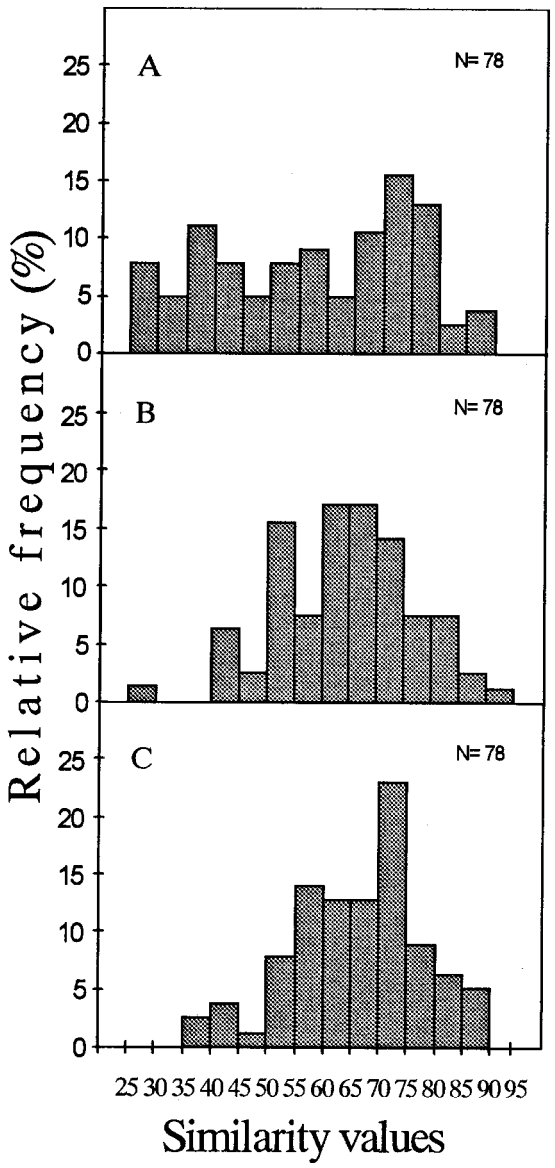

Figure 2. Frequency distribution of similarity indices of DNA sampled in erect axes of clumps of *Gracilaria chilensis*. A) Similarity values among the corresponding axes of *Gracilaria chilensis* collected in the field. Axes considered have been restricted only to those surviving in tank culture. Similarity values have been re-calculated to consider only those axes. B) Similarity values among the corresponding axes of the 6 clumps of *G. chilensis* after cultivation under controlled conditions. Clumps and axes considered have been restricted only to those surviving in tank culture. Similarity values have been re-calculated to consider only those axes. C) Similarity values among axes of 7 clumps of *G. chilensis* surviving large-scale tank culture.

Discussion

Significant changes in DNA polymorphism in axes and branches of *Gracilaria chilensis* occur within relatively short periods of time (*i.e.*, 30 days) suggesting progressive decrease in genetic polymorphism as thalli are moved from the field into controlled laboratory conditions and from laboratory to open tank culture systems. DNA polymorphism detected here using RAPD-PCR methodology may reflect such minor changes as single changes in a base pair.

Habitats occupied by *Gracilaria chilensis* are characterized by diverse environmental conditions which probably allow for the occurrence of a wide polymorphism in DNA fragments within and between

thalli. Such polymorphism originates from meiotic recombinations, whenever the species exhibit sexual reproduction, and from mitotic recombinations, whenever the species is actively growing (Meneses et al., 1999). The transfer from the field to the laboratory conditions did not modify the total amount of genetic variability in the axes, but it increased the frequency of intermediate similarity values. This suggests that axes produced under constant culture conditions are leass variable than those gathered from the field.

The transfer of the experimental clumps to the tank cultivation systems also resulted in reduction of variability. In this case, however, the reduction was not only due to increased genetic similarity among axes grown in the tanks, but also due to loosing of branches. Growing conditions in tanks are much more variable than in the laboratory and certainly not optimal for all plants since portions of the plants suffered necrosis and disintegrated. In both these cases, we interpret the results as environmental selection that pressures on the genetic outcome between and within thalli groups. A similar case of clonal selection has been demonstrated in *Drosophila mercatorum* (Annest & Templeton, 1978) where parthenogenetic females initially heterozygous for 5 visible markers ended up in 99% of homozygous individuals for all of these gene markers. Similar processes could also be important in other seaweed species which reproduce mainly by asexual means, including vegetative propagation by fragmentation.

Fragmentation is frequent in clonal organisms. In corals this occurs by partial mortality of a group of intervening polyps in a colony or by breakage in branching species (Jackson, 1985). In land plants, propagation by fragments is an ancient practice and among seaweeds fragmentation is a frequent means of maintaining free-floating populations (Lobban & Harrison, 1994). In *Gracilaria chilensis*, propagation by thallus fragmentation is the most used method to farm the species.

Fragmentation has up to now been thought of as a way to spread a genet into multiple units without modifying its genetic make up. However, in terrestrial plants genetic and chromosomic changes occur in different parts of the plant, and they tend to be propagated when the parts become independent from the parent (Hartmann & Kester, 1975; Grant, 1975). In general, genetic mosaics of this type tend to be common in species that have multiple meristems and become more common when plants suffer physical damage (Whitham & Slobodchikoff, 1981). *Gracilaria chilensis* has meristems throughout the thallus, and it was expected (Santelices & Varella, 1993) that the genetic variation which originated within the thalli would be spread in the population or in the farm by natural or artificial cloning. The present results indicate that these genetic changes may occur with the emergence of new branches in a new environment. Therefore, the present farming methods, supposed to help to select thalli and to reduced variability, may in fact be doing exactly the opposite. That is, dispersing genetically different units, and introducing variability to the farmed population.

Data are too preliminary to make conclusions on the importance of the above genetic variation for the phenotypic responses of these seaweeds. However, the genetic changes measured in vegetative propagating clones of *Gracilaria chilensis* suggest two important conclusions related to strain selection of clonal seaweeds. The first is that in these clonal thalli the dynamics of genetic change seems to be fast. It is not known if these changes are faster than in sexually reproducing populations, where the changes are due to gene flow. However, the emerging picture is very different from that of genetic stability assumed for selected strains. The second point relates to the importance of the external environment in determining these changes. In the above experiments, branches produced in new culture conditons also exhibited changes in total DNA polymorphism, which suggests a continuous reaction between environment and genotype and confirms the need to conceive the selection process of *Gracilaria* as a continuous process that has to be performed under realistic culture conditions.

Acknowledgements

This study was supported by grant FONDECYT 1960646. Our gratitude to D. Aedo and P. Sánchez for laboratory help, to Dr R. Westermeier for the provision of clumps of *Gracilaria chilensis*, to Dr A. Buschmann, for allowing us to use his large-scale tank cultivation facility in southern Chile and to F. Smith for improving the manuscript.

References

Annest J, Templeton A (1978) Genetic recombination and clonal selection in *Drosophila mercatorum*. Genetics 89: 193–210.

Boardman RS, Cheetham AH, Cook PL (1969) Intracolony variation and the genus concept in Bryozoa. North American Paleontological Convention, part C, 294–300.

Buschmann AH, Mora OA, Gómez P, Böttger M, Buitano S, Retamales C, Vergara PA, Gutiérrez A (1994) *Gracilaria chilensis* outdoor tank cultivation in Chile: Use of land-base salmon culture effluents. Aquac. Eng. 13: 283–300.

Correa JA, McLachlan JL (1991) Endophytic algae of *Chondrus crispus* (Rhodophyta). III Host specificity. J. Phycol. 27: 448–459.

Ehrlich P, Raven P (1969) Differentiation of Population. Gene flow seems to be less important in speciation than the neo-Darwinians thought. Science 165: 1228–1231.

Grant V (1975) Genetics of Flowering Plants. Columbia Univ. Press, New York.

Hartmann HT, Kester DE (1975) Plant propagation. Prentice-Hall Inc. Englewood Cliffs, New Jersey.

Jackson JBC (1985) Distribution and Ecology of Clonal and Aclonal Benthic Invertebrates. In Jackson, Buss & Cook (eds), Population Biology and Evolution in Clonal Organisms. Yale University Press, New Haven and London, pp. 297–355.

Lignell A, Pedersen M (1989) Agar composition as a function of morphology and growth rate. Studies on some morphological strains of *Gracilaria secundata* and *Gracilaria verrucosa* (Rhodophyta). Bot. mar. 32: 219–277.

Lobban CS, Harrison PJ (1994) Seaweed Ecology and Physiology. Cambridge University Press, Cambridge, New York, USA. 366 pp.

Meneses I (1996) Assessment of population of *Gracilaria chilensis* (Gracilariales, Rhodophyta) utilizing RAPDs. J. appl. Phycol. 8: 185–192.

Meneses I, Aedo D, Sánchez P, Santelices B (1999) Intraclonal genetic change in *Gracilaria chilensis*: a mechanism ensuring variability in species with vegetative propagation (submitted).

Santelices B (1992) Strain selection of clonal seaweeds. In Round FE, Chapman DJ (eds), Progress in Phycological Research. Vol 8. Biopress, Bristol, UK, pp. 85–116.

Santelices B, Aedo D, Varela D (1995) Causes and implications of intra-clonal variation in *Gracilaria chilensis*. J. appl. Phycol. 7: 283–290.

Santelices B, Correa JA, Meneses I, Aedo D, Varela D (1996) Sporeling coalescence and intra-clonal variation in *Gracilaria chilensis* (Gracilariales: Rhodophyta). J. Phycol. 32: 313–322.

Santelices B, Varela D (1993) Intra-clonal variation in the red seaweed *Gracilaria chilensis*. Mar. Biol. 116: 543–552.

Siegel S, Castellon NJ Jr (1988) Non Parametric Statistics for the Behavioral Sciences. 2nd edition. McGraw-Hill, New York, 399 pp.

Van der Meer J, Tod ER (1977) Genetics of *Gracilaria* sp. (Rhodophycaea, Gigartinales). IV Mitotic recombinations and its relationship to mixed phases in the life history. Can J. Bot. 55: 2810–2817.

Van der Meer J, Zhang X (1988) Similar unstable mutations in three species of *Gracilaria chilensis* (Rhodophyta). J. Phycol. 24: 198–202.

Whitham TG, Slobodchikoff CN (1981) Evolution by individuals, Plant-Herbivore interactions, and mosaics of genetic variability: The adaptative significance of somatic mutations in plants. Oecologia (Berl.) 49: 287–292.