

Sources of morphological variation in populations of *Gracilaria chilensis* Bird, McLachlan & Oliveira of Chile

Fuentes de variación morfológica en poblaciones de
Gracilaria chilensis Bird, McLachlan & Oliveira de Chile

ISABEL MENESES

Departamento de Ecología, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago

ABSTRACT

Gracilaria chilensis Bird, McLachlan & Oliveira is generally recognized as a variable species whose morphological changes are the result of the environmental conditions under which it grows. However, no experimental evaluation has ever been done and few descriptive studies have focused on matching particular morphologies with distinct populations. Five localities with *G. chilensis* populations along Chilean coasts were sampled. The posterior analysis of vegetative characters indicated that populations from Lenga are easily segregated from other localities. Meanwhile, localities such as Maullín, Coquimbo and Niebla can be grouped apparently due to their similar growth habit, i.e. plants originated from spores, and not due to characteristics of their habitat. Results also showed that variability of most characters within populations, originated through vegetative propagation, is not significantly different from that of populations of sporic origin where higher genetic variability is expected. Finally, other variables such as reproductive stage and life history phase of the plants exhibited differences in few characters.

Key words: vegetative propagation, sporic origin, genetic variability.

RESUMEN

Gracilaria chilensis es reconocida, en términos generales, como una especie variable cuyos cambios morfológicos son resultado de las condiciones ambientales bajo las cuales crece. Sin embargo no se han realizado evaluaciones experimentales que pongan a prueba lo anterior, y los pocos estudios existentes son descriptivos y están enfocados a relacionar morfologías particulares con las poblaciones de origen. En el presente estudio se muestrearon cinco localidades a lo largo de Chile que albergan poblaciones de *G. chilensis*. El análisis posterior de los caracteres vegetativos de los talos provenientes de dichas localidades indicó que la población de Lenga es fácilmente separables de aquellas de otras localidades. Al mismo tiempo, poblaciones de Maullín, Coquimbo y Niebla pueden ser agrupadas aparentemente en base a presentar talos de *Gracilaria* provenientes de esporas, a diferencia de aquellas poblaciones en que los talos se originan por fragmentación. Los resultados indican además que la variabilidad detectada al interior de poblaciones originadas por propagación vegetativa no difiere significativamente de la de poblaciones originadas de esporas, donde se esperaba una variabilidad genética mas alta. Por último, otras variables tales como el estado reproductivo y la fase del ciclo de vida de las plantas mostraron diferencias en unos pocos caracteres, en su mayoría relacionados con el desarrollo de estructuras reproductivas.

Palabras clave: propagación vegetativa, origen esporico, variabilidad genética.

INTRODUCTION

In the absence of gametangial reproductive structures *Gracilaria* species are difficult to distinguish, except for some habit features which are able to separate among major species groups, (i.e. thallus cylindrical vs. flattened, Taylor 1957). This difficulty is due to the high morphological variability of *Gracilaria* thalli (Dawson 1949, Ohmi 1958, Yamamoto 1984) that usually conceals species limits. *Gracilaria chilensis* Bird,

McLachlan et Oliveira is locally considered no exception to this rule. Previous records of the genus along Chilean coasts reported the presence of *Gracilaria lemaneiformis* (Bory) Weber van Bosse (now *Gracilariopsis*), *G. verrucosa* (Hudson) Papenfuss, *G. edulis* (Gmelin) Silva, *G. pulvinata* Skottsberg and *G. peruana* Piccone et Grunow. The three last records are uncertain and the species have not been reported again since Levring (1960), at the same time, two of them belong to the group of "flat"

Gracilarias while *G. edulis* has a cylindrical thallus similar to *G. verrucosa* and *G. chilensis*. *G. verrucosa* records most likely correspond to *G. chilensis* and *Gracilariopsis lemaneiformis* apparently has a disjunct bathimetric distribution regarding the *Gracilaria* representatives in Chile. *G. lemaneiformis* and *Gracilaria chilensis* so far are the only two species unmistakably recorded for Chile, although the frequent transplanting of thalli from one locality to another, with the purpose of starting or maintaining massive cultures of this agar-producer (Pizarro 1986, Pizarro & Barrales 1986) have masked the possibility of recognizing other species of similar morphology.

In addition to the possible existence of other species among stands of *G. chilensis*, this species itself possess a high morphological variability (pers. com. from E. Fonck, R. Noranbuena and R. Westermeier) from one stand to another. These observations have led to the generalized assumption that the extent of the morphological variation within populations is such that accounts for much of the variation among populations. Nevertheless, such assumption has few documented basis in the literature since, on one hand, much of the studies on variation of *Gracilaria* in Chile have focused upon physiological (Westermeier et al. 1991, Santelices & Ugarte 1990, Santelices & Varela 1993) rather than morphological responses to physical and biotic factors. On the other hand, the few studies addressing morphological variation, confirm the distinction among populations upon morphological characters and their persistence through time and changes in growing conditions (Buschmann et al. 1992).

Populations of *Gracilaria chilensis* are conspicuous along the Chilean coast. The stands of this alga are common in protected bays often interrupted by considerable extensions of non-suitable habitats of high wave exposure. Although the massive stands of *G. chilensis* grow in sandy bottoms, the origin of these stands are man-made plantations initiated and maintained by the regeneration capacity of thalli fragments (Santelices & Doty 1989). This process occurs naturally in certain areas. Therefore, the ultimate origin of these populations is unknown and

it is expected that morphological variability will be low since a few thalli may give rise to an entire stand subjected to similar growth conditions. A few other populations grow on rocky or other hard substrata. These latter stands share the characteristic of being originated from spores that settle and form adhesion discs from which thalli arise. Plants grown in this manner reach reproductive maturity and thus, fertile material in these stands is frequent, whereas most thalli of cultivated populations are sterile.

The access to extensive collections of thalli from populations generated by both spores and thallus fragments, allow comparisons of morphological variation within and between populations of both origins. Thus, the goal of this study is to test the following hypotheses, (1) Populations of *Gracilaria chilensis* along the coast of Chile can be differentiated based on vegetative characters, (2) vegetative characters of reproductively mature plants do not differ significantly from those of sterile plants within populations of *G. chilensis*, (3) vegetative characters of gametophytic thalli do not differ significantly from those of sporophytic thalli within populations of *G. chilensis* and, (4) variability in vegetative characters of *G. chilensis* plants grown from thallus fragments is less than that of plants grown from spores.

MATERIALS AND METHODS

Gracilaria plants were obtained from five different localities along the coast of Chile (Fig. 1), Lenga (36° 45' S, 73° 11' W), Mautllín (41° 40' S, 73° 45' W), two stands from Coquimbo (Bahía La Herradura, 29° 58' S, 71° 22' W) and Chañar (27° 33' S, 70° 01' W). A minimum of 20 plants were collected at each stand. Each stand was considered a population since they are located in geographically distant localities with the exception of Coquimbo, where the two sampled stands differed in their habitats. When plants were growing on sandy bottom and neighbor thalli were connected underground, only one emergent thallus was collected within a 2 m² area. In cases where the stand was clearly restricted to a small area, thalli were collect-

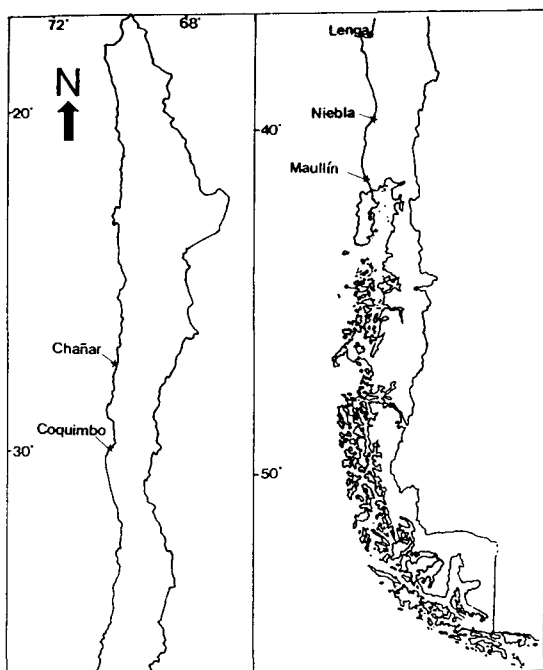


Fig. 1: Localities sampled along the Chilean coast.
Localidades muestreadas a lo largo de Chile.

ed as distant from each other as possible. Populations characterized by vegetative propagation are hereafter called artificial populations while those consisting of plants originated from spores (recognizable by having adhesion discs) and growing on hard substrata (rocks, boulders, pebbles and mollusk shells) are called natural populations.

Plants cleaned from epiphytes were pressed on herbarium paper for several days until dried. These dried specimens were examined under a dissecting scope looking for reproductive structures (tetrasporangia, spermatangia and cystocarps) and portions suitable for cross sectioning. Cross sections were obtained either by hand with a razor blade or using a freezing microtome. Sections were stained with methylene blue and mounted with 50% Karo corn syrup. The vegetative characters evaluated appear in the tables of the results section, and are expressed as the average of 10 measurements per each plant except for total thallus length. Thallus length was used to standardize other variables (e.g. number of primary branches per cm). Normality was assessed for each of these characters with a small sample ($n < 50$).

Shapiro-Wilks test (Shapiro & Wilks 1965). When characters were not normally distributed comparisons between populations were performed using a Kruskal-Wallis one way analysis of variance (Siegel & Castellan 1988). In order to compare the variability of each morphological character between populations the F-test for variance ratio (Zar 1984) was applied only to those sets of observations in which the logarithms of the data were normally distributed according to the requirements of the test (Zar 1984). A cluster analysis was performed including all characters measured. In order to avoid the excessive weight of any of the characters in most coefficients of similarity (or dissimilarity), these were equalized using Gower's ranging procedure which take care of both size and variability of the states of each character (Sneath & Sokal 1963). The resulting matrix was analyzed applying UPGMA clustering method. All analyses were performed using SAS (SAS Institute, Inc. 1985).

RESULTS

Cluster analysis

Maullín and Niebla showed the highest resemblance among populations forming the first group (at 0.43 average distance) to which plants from the natural population of Coquimbo joined at 0.62 average distance (Fig. 2). A second group formed at higher average distance (0.75) included plants belonging to the two artificial populations of Coquimbo and Chañar. *Gracilaria* from Lenga did not cluster with any of the previous groups. These preliminary results led to a closer examination of the variation of each of the morphological characters among populations in order to explain the differences.

Comparison between populations

Table 1 summarizes the results of comparing external and internal characters between populations. Figure 3 is a diagram based on these results depicting graphically the appearance of plants from different populations. Plants originated from spores (natu-

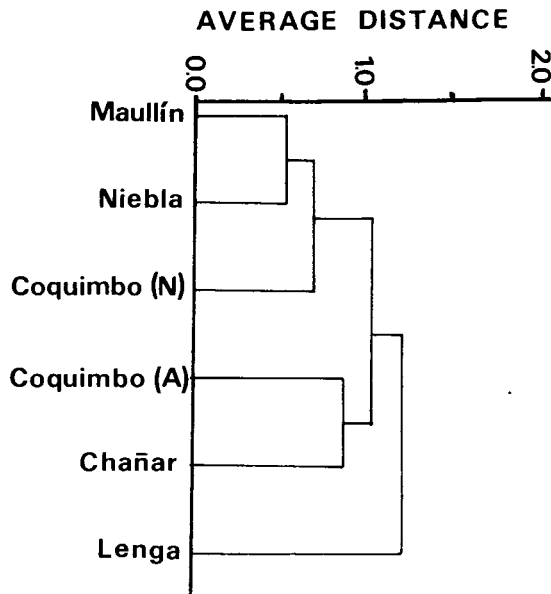


Fig. 2: Dendrogram depicting the results of the UPGMA Cluster analysis of the morphological characteristics in *Gracilaria* populations.

Dendrograma que describe los resultados del análisis de grupo (UPGMA) aplicado a las características morfológicas de las poblaciones de *Gracilaria*.

ral) did not show any significant difference either externally or internally except for plants from Niebla which were taller (Chi-square approximation of Kruskal-Wallis test, $X^2 = 22.57$, d.f. = 2, $P = 0.0001$) and from Coquimbo where branches arose in a wider angle than in the other two localities ($X^2 = 41.555$, d.f. = 2, $P = 0.0001$). In populations originated by vegetative propagation (artificial) only the number of proliferations varied ($X^2 = 30.859$, d.f. = 2, $P = 0.0001$).

When comparing plants from natural populations with plants originated by vegetative propagation, half of the characters differed (Table 1). Plants originated from spores had longer secondary branches ($X^2 = 52.142$, d.f. = 5, $P = 0.0001$) forming a closer angle with the main axis ($X^2 = 41.555$, d.f. = 5, $P = 0.0001$) and had more proliferations per unit of axis length ($X^2 = 30.859$, d.f. = 5, $P = 0.0001$) than artificial plants. Internally, plants from natural populations displayed smaller cortical and medullary cells ($X^2 = 23.015$, d.f. = 5, $P = 0.0001$ and $X^2 = 38.203$, d.f. = 5, $P = 0.0001$ respectively).

Lenga was initially considered as an artificial population because no adhesion discs were observed from collected plants at this locality. These plants separated themselves from other populations because of their apparent differences in morphology. Plants from Lenga were "simpler" than plants from natural populations, showing fewer and less developed branches ($X^2 = 14.175$, d.f. = 5, $P = 0.0027$ and $X^2 = 35.395$, d.f. = 5, $P = 0.0001$ respectively). These plants also had their branches forming wider angles with the main axes ($X^2 = 41.555$, $P = 0.0001$) than plants from other populations and had the largest medullary cells of all recorded plants (Fig. 3). In addition to these characters, plants from Lenga displayed cortical cells smaller than those shown by plants from artificial populations.

Comparison of reproductive and sterile plants within populations

Comparisons between reproductive and sterile thalli from Niebla and Maullín showed that in Maullín, sterile individuals were longer and thicker than reproductive thalli (Normal approximation of Wilcoxon two-sample test, $|z| = 2.816$, $P = 0.049$ and $|z| = 2.499$, $P = 0.0125$ respectively). In the same locality, reproductive thalli displayed the second and third cellular rows with larger cells ($|z| = 2.10459$, $P = 0.0353$ and $|z| = 2.83882$, $P = 0.0045$ respectively).

In Niebla, reproductive and sterile plants were externally indistinguishable. Nevertheless, reproductive plants had larger medullary cells and smaller cortical ones than sterile thalli ($|z| = 2.24094$, $P = 0.0250$). No comparisons were performed with material from Coquimbo since only reproductive plants were collected.

Comparison between phases

Populations of Niebla and Maullín had recognizable gametophytes and sporophytes among their reproductive plants. When compared within the populations no distinguishable morphologies between phases were detected in Maullín. Niebla showed however, longer sporophytic plants ($|z| = 2.54996$, $P = 0.0108$) with more elongated primary ($|z| = 2.35896$, $P = 0.0183$) and

TABLE 1

Comparison of vegetative characters between populations. (+) Difference between populations ($P < 0.05$, Kruskal -Wallis one way analysis of variance), (-) No significant difference detected, (n/a) no comparison made since material from artificial populations does not include the entire thallus

Comparación de caracteres vegetativos entre poblaciones. (+) Diferencia entre poblaciones ($P < 0.05$, Kruskal -Wallis análisis de varianza de una vía), (-) No se detecta diferencia significativa, (n/a) no se realizó comparación ya que el material de poblaciones artificiales no incluye el talo completo

CHARACTERS	Natural populations	Artificial populations	Natural & artificial populations	Natural populations & Lenga	Artificial populations & Lenga
Total thallus length	+	n/a	n/a	n/a	n/a
Main axis diameter	-	-	-	-	-
Primary branch length	-	-	-	+	+
Secondary branch length	-	-	+	+	+
Angle lateral branch/main axis	+	-	+	+	+
No. branches /cm	-	-	-	+	-
No. proliferations/cm	-	+	+	+	+
Branching orders	-	-	-	+	+
Cortical cell diameter	-	-	+	-	+
2nd cell row diameter	-	-	-	-	-
3rd cell row diameter	-	-	-	-	-
4th cell row diameter	-	-	+	-	+
Medullary cell diameter	-	-	+	+	+
Percentage of significantly different characters	15.4%	8.3%	50%	58.3%	66.7%

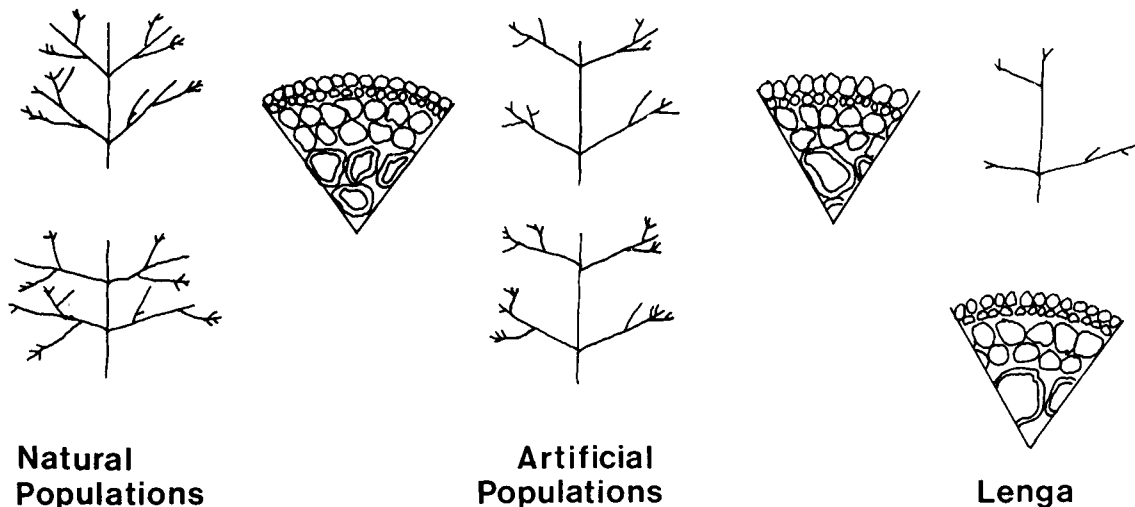


Fig. 3: Diagram showing the schematized external and internal vegetative anatomy (in cross section) of the three morphological groups in *Gracilaria*.

Diagrama que muestra esquematizada la anatomía vegetativa externa e interna (en sección transversal) de los tres grupos morfológicos de *Gracilaria*.

secondary branches ($|z| = 2.93486$, $P = 0.0033$) than gametophytes. Plants from this locality showed sporophytes with longer cortical cells than gametophytes ($|z| = 2.94836$, $P = 0.0032$).

Variability of characters within and between populations

A statistical comparison of the morphological variability within and between

TABLE 2

Comparison of characters between reproductive and sterile plants and between gametophytes and sporophytes in natural populations. (+) Difference between conditions ($P < 0.05$, Wilcoxon Mann Whitney test), (-) No significant difference detected, * No comparison between sterile and reproductive material was made since only reproductive plants were collected. () Comparisons between phases

Comparación de caracteres entre plantas reproductivas y estériles y entre gametofitos y esporofitos de poblaciones naturales. (+) Diferencias entre condiciones ($P < 0.05$, Test Wilcoxon Mann Whitney), (-) No se detectan diferencias significativas, * No se realizaron comparaciones entre material reproductivo y estéril ya que sólo se colectó material reproductivo. () Comparaciones entre fases

CARACTERES	Maullín	Niebla	Coquimbo*
Total thallus length	+ (-)	- (+)	- (-)
Main axis diameter	+ (-)	- (-)	- (-)
Primary branch length	- (-)	- (+)	- (-)
Secondary branch length	- (-)	- (+)	- (+)
Angle lateral branch/ main axis	- (-)	- (-)	- (-)
Number of branches/cm	- (-)	- (-)	- (-)
No. proliferations/cm	- (-)	- (-)	- (-)
Branching orders	- (-)	- (-)	- (-)
Cortical cell diameter	- (-)	+ (+)	- (-)
2nd cell row diameter	+ (-)	+ (-)	- (-)
3rd cell row diameter	+ (+)	+ (-)	- (-)
4th cell row diameter	- (-)	+ (-)	- (-)
Medullary cell diameter	- (-)	- (-)	- (-)

populations for each character could only be done for those characters with log-transformed values normally distributed (Zar 1984). The analysis of those characters suitable to be included is summarized in Table 3. The extent of their variability fluctuated from one population to the other (Fig. 2). For example, plants from Lenga showed low variability for secondary branch length compared to the variability of the same character in plants from Chañar (Fig. 2) or Coquimbo. Again, plants from Lenga displayed a more restricted range of cortical cell diameter than plants from Maullín and Coquimbo. Comparisons of variability among natural populations (originated from spores) showed significant differences in three characters, one external and two internal, whereas comparisons between artificial populations (originated from thallus fragments) showed differences only in the variability of thallus length. Surprisingly,

no differences were detected between natural and artificial populations in terms of the range of variation of each character. However, Lenga was the only population that displayed a higher (or lower) variability of most of its characters compared to plants from either natural or artificial populations, indicating the least resemblance to populations of other localities.

DISCUSSION

Regardless of the variability that most vegetative characters displayed within populations, these are able to distinguish among populations and/or groups of populations. Although significant differences were expected to be found among all populations sampled in this study, this was not the case. Instead the combination of characters measured resulted in the clustering of some of these populations apparently based on the origin of the plants, i.e. spores versus thallus fragments. Thus, populations originated from spores were similar and distinct from populations originated vegetatively, whereas populations of vegetative origin were similar to each other.

Half of the characters that differ between natural and artificial populations relate to branching features while the other half correspond to characters of the inner anatomy of the plants. We cannot explain the reason for this particular pattern of population clustering. On one hand, populations of sporic origin share similar environments (intertidal, rocky or hard substrata), and so do populations originated from thallus fragments (subtidal, sandy bottom). Therefore, the clustering of populations could be a result of the similarity of the conditions under which they grow. On the other hand, the grouping of populations according to their morphology could be just a result of the origin of the thalli (spores versus thallus fragments).

A recent study on spore coalescence in *G. chilensis* (Santelices & Aedo 1994¹) showed

¹ Santelices B & D Aedo (1994) Coalescencia de esporas de *Gracilaria*: una reinterpretación de su valor adaptativo. Resúmenes XIV Jornadas de Ciencias de Mar, p. 73.

TABLE 3

Variability of each character analyzed between populations. F calculated from the \log_{10} of the character values. *Difference significant at the 5% level of confidence, **Difference significant at the 1% level of confidence, – No comparisons made

Variabilidad de cada carácter analizado entre poblaciones. F calculado a partir del \log_{10} de los valores de cada carácter. *Diferencia significativa a nivel de confianza del 5%, **Diferencia significativa a nivel de confianza del 1%, - No se realizaron comparaciones

CHARACTERS	Natural populations	Artificial populations	Natural & artificial populations	Natural populations & Lenga	Artificial populations & Lenga
Total thallus length	3.14*	–	–	–	–
Main axis diameter	–	9.22**	2.19	–	–
Primary branch length	2.22	–	–	0.48	–
Secondary branch length	2.83*	1.06	0.84	0.95	0.80
Angle lateral branch/main axis	0.43	0.96	–	3.40*	1.63
No. proliferations/cm	4.29**	–	1.18	–	–
Cortical cell diameter	2.43	0.75	2.46	–	–
2nd cell row diameter	2.14	1.49	2.20	1.16	2.54*
3rd cell row diameter	1.94	1.76	2.14	4.20**	9.05**
4th cell row diameter	0.38	–	0.62	3.54*	2.02*
Medullary cell diameter	0.36	1.75	0.33	1.62	0.53

that the external morphology of the thalli growing from a mass of spores differs from that of thalli originated from a single spore. The former plants have several axes arising from a single basal disc. The branch size pattern was homogeneous and resulted in a bushy appearance. Plants originated from single spores developed only one main axis bearing branches of diverse length. In those areas where a significant number of reproductive plants coexist, simultaneous shedding of spores may increase the probability of spore coalescence during germination. Rocky intertidal areas are particularly well-suited for these events. Spore shedding in *Gracilaria* is stimulated by salinity changes which are common during a tidal-cycle, particularly in estuarine conditions (Santelices 1990), and packages of spores are released embedded in a mucilage stream in this and other species of *Gracilaria* (Oza 1975). Both conditions should contribute to the formation of basal discs from masses of spores that remain together sufficient time in order to germinate into a single holdfast. Spore coalescence could also explain observations of mixed phases in the same thalli (Prieto et al. 1991) of intertidal populations of *G. chilensis*.

The second and third hypotheses stated in this study regarding no significant dif-

ferences in the morphology between sterile and reproductive thalli and between phases of the life cycle were partially accepted according to the following results. A few trends appear in this study when comparing sterile versus reproductive thalli, especially in inner characters such as cell diameter since cells were larger in plants with visible reproductive structures. Differences among phases were also observed, but they included only a few characters and seemed to be only of local importance since they appeared in a single population. In either case, this intrapopulation variability does not interfere with the distinction among populations.

The last hypothesis was stated under the assumption that in populations originated by thallus fragmentation, sources of genetic recombination are far more restricted than in populations of plants originated from spores where recombination is possible through the process of syngamy and meiosis.

Populations originated from thalli fragments coincide in being also subtidal populations. Therefore, based solely on descriptive data, it is difficult to separate the effects of the environment from the effect of the origin of the plants in order to evaluate the contribution of each factor to the distinction of "artificial" versus "natural" populations. Conditions in the subtidal are

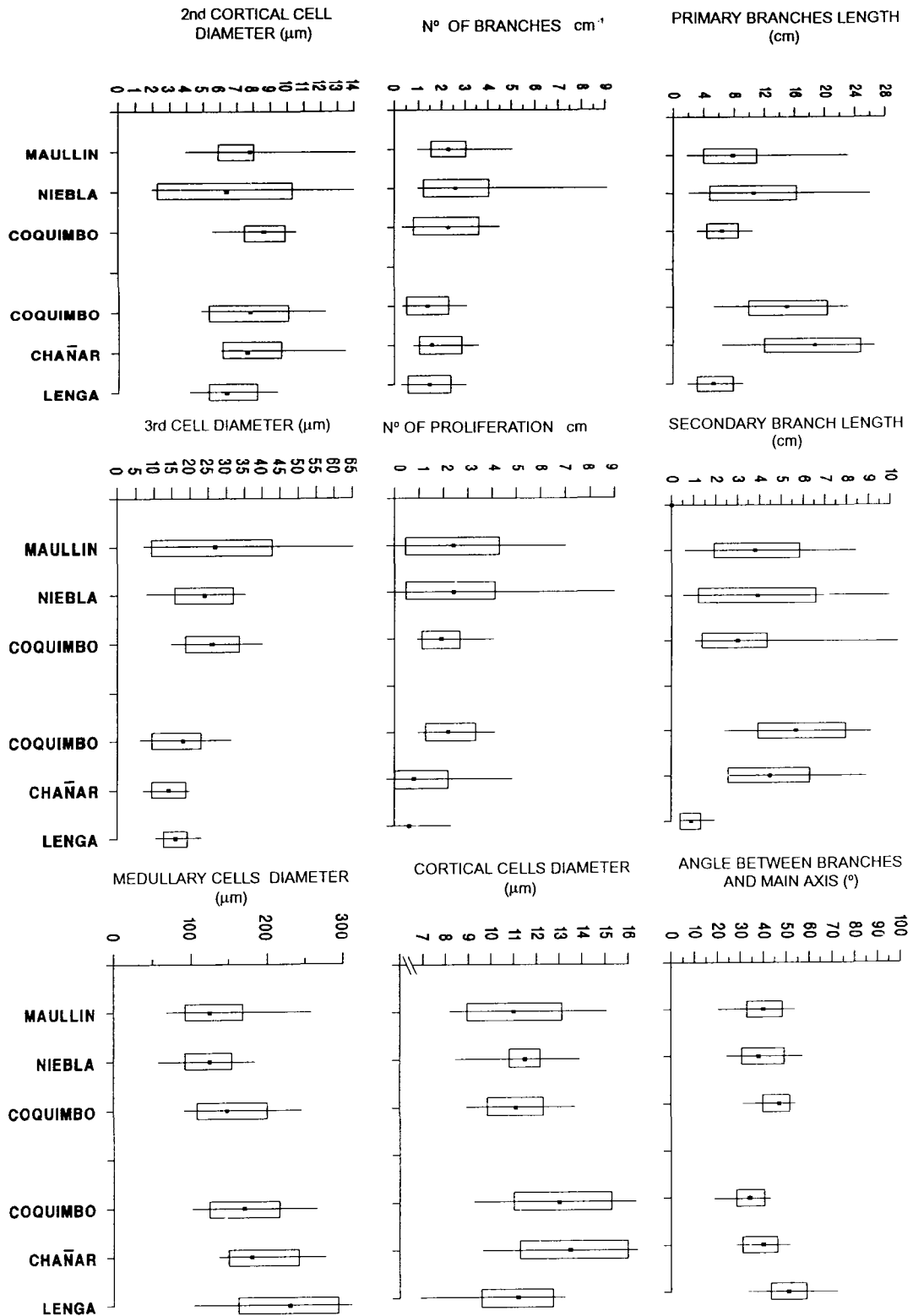


Fig. 4: Variation in some of the morphological characters evaluated in different populations of *Gracilaria chilensis*. Total ranges are expressed by the entire length of the bars, standard deviations are expressed by the portion of the bars enclosed in a rectangle.

Variación en algunos de los caracteres morfológicos evaluados en las distintas poblaciones de *Gracilaria chilensis*. Los rangos totales están expresados por la longitud completa de las barras, las desviaciones estándar están expresadas por la porción de las barras encerradas en rectángulos.

generally considered more stable than in the intertidal and, as a consequence, we should expect morphological characters in *Gracilaria* to be more uniform in subtidal populations. This was confirmed in three of the external characters for "natural" populations where the number of juvenile branches, the length of secondary branches and the total thallus size were more variable in some of the stands. Artificial populations, on the other hand, showed no significant differences in the variability of their characters except for thallus diameter. Therefore the last hypothesis should be accepted, nevertheless an interesting outcome of this comparison is the higher variability of several characters in plants from Lenga. This result was unexpected since Lenga clearly differed from the rest of the localities suggesting that, despite its variability, this population is fairly well-differentiated.

An attempt to detect discontinuities in the variation patterns of several vegetative characters in six populations of *Gracilaria* was made in eastern Canada (Chapman et al. 1977). Only the degree of flattening, separated the studied populations into two groups. The other morphological traits resulted too variable, seasonally as well as geographically, to separate these populations. In Chile a single study had been previously conducted to assess whether morphological variability within populations of *Gracilaria* exceeds that detected among populations. Twenty-one characters, including the morphology of reproductive structures (sporangial diameter, thickness of the pericarp, etc.) did not show significant differences among localities or groups of localities (León 1990). However, the author observed that plants from Lenga differed in six characters from the rest of the sites. Unfortunately León (1990) did not include information about growth habits and merged sporophytes from some localities with gametophytes from others, preventing the possibility of comparisons between phases.

At this point we ignore whether the differences we detected between populations could be reverted if transplants were made. We suspect changes would occur but with a certain delay in time and depending on manipulation. Massive cultures in Coquimbo

started with fine and profusely branched thalli from Maullín, changing into long and thick poorly-branched plants after several months and frequent harvest according to observations of the fishermen involved in its culture (pers. com. from the local fishermen of Bahía La Herradura, Coquimbo). Lenga appears to represent a different situation, where some genetic changes have occurred through time. In fact the RFLP analysis of organellar DNA from different morphotypes of this population resulted in differences presented by a green strain from the normally reddish-colored individuals (González et al. 1994²), indicating genetic variability within the population. Growth rate differences obtained in laboratory cultured clones of *Gracilaria chilensis* (Santelices & Varela 1993) either have no incidence upon the final morphology of the plants or, field plants analyzed have already undergone a selection process and we are measuring the morphological variants that survived.

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