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# DISPERSAL IN A HAPLO-DIPLOID RED ALGAL SPECIES : GENETIC STRUCTURE AND GENE FLOW IN *GRACILARIA GRACILIS*

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POPULATION GENETIC STRUCTURE
MATING SYSTEM
*F*-STATISTICS
ISOLATION BY DISTANCE
RED ALGAE

ABSTRACT. – Here we describe the genetic structure of ten *Gracilaria gracilis* populations from three different French regions. Using two microsatellite loci, we analyse within-population genetic structure and the variation of genetic differentiation among populations with respect to geographic distance. Our results show that populations are weakly structured and that, contrary to our predictions, the species is characterised by a low level of inbreeding. However, gene flow is restricted over short distances, demonstrating the phenomenon of isolation by distance. Nevertheless, at a local level (< 1 km), differentiation between populations does not depend solely on distance and the rising/ebbing tide may orient gene flow at this scale.

STRUCTURE GÉNÉTIQUE DES POPULATIONS
SYSTÈME DE REPRODUCTION
*F*-STATISTIQUES
ISOLATION PAR LA DISTANCE
ALGUES ROUGES

RÉSUMÉ. – Nous avons étudié la structure génétique de dix populations de *Gracilaria gracilis* réparties le long des côtes françaises. La structuration génétique intra-population d'une part, et la variation de la différenciation génétique entre populations en relation avec la distance géographique d'autre part, ont été analysées grâce à l'utilisation de deux marqueurs microsatellites. Nos résultats montrent que les populations sont faiblement structurées et que cette espèce, contrairement à nos prédictions, est caractérisée par un régime de reproduction faiblement consanguin. Quoiqu'il en soit, les flux géniques se produisent essentiellement entre populations proches géographiquement ce qui démontre qu'il existe un phénomène d'isolement par la distance. Néanmoins, sur une échelle locale (inférieure au kilomètre), la différenciation entre populations ne dépend pas seulement de la distance et il est probable que la marée puisse jouer un rôle dans l'orientation des flux géniques à cette échelle.

## INTRODUCTION

The study of gene dispersal is essential for understanding the maintenance and evolution of biodiversity within a species. The amplitude of gene flow, achieved by a species' dispersal vectors, is a parameter of great evolutionary interest since it will determine the level of genetic differentiation between populations and therefore the possibilities for local adaptation. A species' dispersal vectors are subject to the dispersal modes of their surrounding environment. The marine environment provides wide dispersal potential allowing homogenising of marine populations.

In the marine environment, data on genetic diversity, amounts of gene flow, and population genetic structure come mostly from animal species (Beaumont 1994; Palumbi 1994). In many of these species, it has been shown that gene flow and therefore dispersal can be limited in space (Palumbi 1994). Likewise, the few studies that have been conducted on the genetic structure of algal species demonstrated that the studied populations are significantly differentiated from one another (e.g. Innes 1984; Fujio *et al.* 1985; Sosa and Garcia-Reina 1992; Sosa and Garcia-Reina 1993; Lindstrom 1993; Lu and Williams 1994; Williams and Di Fiori; 1996). This among-population differentiation was attributed to prevalent asexual reproduction (Sosa and Garcia-Reina 1992; Lindstrom 1993; Sosa and Garcia-Reina 1993), gene flow barriers (Lu and Williams 1994), or to the facility of selfing (Williams and Di Fiori 1996).

Here we describe the genetic structure of *Gracilaria gracilis* populations. We analysed the variation of genetic differentiation among populations with respect to geographic distance to determine the scale of genetic divergence and the possibility for local adaptation. In parallel, within-

Table I. – Test for heterozygote deficiency ($F_{IS}$) at the two microsatellite loci.

| | | | locus Gv1CT | | | locus Gv2CT | | |
|---|---|---|---|---|---|---|---|---|
| **Region** | **Location** | **Pop** | **N** | **$F_{IS}$** | **P** | **N** | **$F_{IS}$** | **P** |
| Northern France | 1 | Aud H3 | 34 | +0.12 | ** | 36 | +0.25 | * |
| | 2 | Wim L1 | 20 | +0.63 | *** | 22 | 0.04 | ns |
| | | Wim H1 | 7 | +0.69 | *** | 10 | -0.13 | ns |
| | 3 | GN L1 | 117 | +0.44 | * | 125 | +0.07 | ** |
| | | GN H1 | 65 | +0.26 | ns | 66 | +0.08 | ns |
| Western France | 1 | Bat M1 | 14 | +0.35 | * | 27 | +0.27 | ** |
| | | Bat M2 | 42 | +0.29 | ** | 47 | +0.21 | *** |
| South Western France | 1 | Chau H1 | 35 | +0.56 | *** | 35 | +0.35 | *** |
| | | Chau H2 | 34 | +0.23 | ns | 35 | +0.12 | *** |
| | 2 | Sv H1 | 35 | +0.39 | * | 168 | -0.06 | ns |

N: number of diploid individuals
P: probability; ns: not significant, *<0.05, **<0.01, ***<0.001

population genetic structure, a parameter of the mating system, was explored and compared. We employed microsatellites, highly polymorphic, simple, codominant loci, as genetic markers in this study. Microsatellites, a relatively new class of DNA markers, are characterised by very short (1-5 bp) core motifs which are repeated in tandem; a variation in the number of repeats results in size variants at a particular locus. This type of marker has been developed for the first time in algae (the red alga *Gracilaria gracilis*) (Wattier *et al.* 1997); we have at our disposition two hypervariable loci.

## MATERIELS AND METHODS

### *Study organism*

*Graciliaria gracilis*, a benthic, intertidal red alga, is characterised by a haplo-diploid life cycle. The tetrasporophyte (diploid) and gametophyte (haploid) generations are isomorphic, independent, and perennial. Populations consist of diploid individuals, tetrasporophytes, and haploid individuals, male and female gametophytes. The tetrasporophytes produce, via meiosis, haploid spores that after release and germination become independent gametophytes. The gametes are formed by mitosis on the gametophytes; male gametes, which are not flagellated, are released and fertilise female gametes which are retained on the female (Fredericq and Hommersand 1989). The zygote, within the cystocarp, undergoes mitosis and thereby produces thousands of identical diploid spores (Hommersand and Fredericq 1990). These diploid spores are liberated by the thousands from the cystocarp some of which germinate and produce new diploid individuals. *G. gracilis*, like the majority of red macro-algae, has three types of passively dispersed propagules : haploid spores, diploid spores, and male gametes. Finally, asexual reproduction, the establishment of new individuals from thallus fragments, has never been observed in natural populations of *G. gracilis* in its native habitat (i.e. rocky intertidal zones) (Destombe *et al.* 1992).

### *Sampling*

Ten samples from natural populations (900 individuals; ca. 30 to 100 individuals per population) from three French regions (Southwestern France, Brittany and Northern France) were analysed using two microsatellite loci (Gv1CT and Gv2CT). This sampling scheme was hierarchical and stratified : each region consists of at least one location and each location is made up of one to two populations (Table I). This strategy allows for the assessment of the presence and degree of isolation by distance among populations.

DNA from algal material was extracted and genotypes at the two microsatellite loci were determined as described in Wattier *et al.* (1997).

### *Analyses*

*F*-statistics were used to determine both within and among population genetic structure (Wright 1951; Weir and Cockerham 1984). $F_{IS}$ measures the within population genetic structure by examining the (genetic) correlation of the two alleles found within a single individual compared with the expected heterozygosity (under Hardy-Weinberg conditions) of the population. For this reason, $F_{IS}$ can be calculated for diploid individuals only. Pairwise $F_{ST}$ measures the differentiation among two populations by comparing the variance of allele frequencies of each population with the allele frequencies of the two populations combined. This approach allowed us to consider haploid and diploid individuals simultaneously. The computations of $F_{IS}$ and $F_{ST}$ were implemented using the GENEPOP software package, version 3.0 and the probabilities reported are the results of exact tests (Raymond and Rousset 1995).

These pairwise $F_{ST}$ values were used to determine if gene flow is restricted geographically. Under the hypothesis of isolation by distance, a positive correlation is expected among $F_{ST}$ and geographical distances (Slatkin 1993). To test this hypothesis, the genetic distance ($F_{ST}/(1-F_{ST})$) between each pair of populations was plotted as a function of the natural logarithm of the geographic distance separating the two populations and a regression analysis was carried out (Rousset 1997).

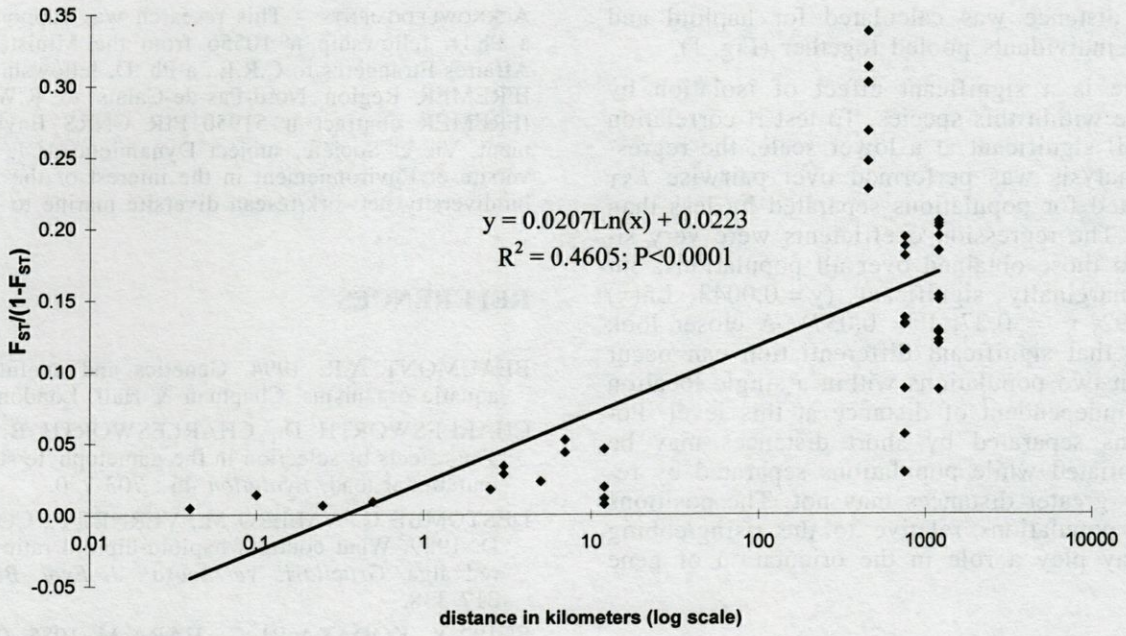


Fig. 1. – Genetic distance ($F_{ST}/(1-F_{ST})$) with respect to geographic distance. Pairwise $F_{ST}$ values are plotted according to the separation distance between each of the ten populations studied.

## RESULTS AND DISCUSSION

### *Population genetic structure*

In order to determine if the study populations have an endogamous breeding system, the heterozygote deficiency ($F_{IS}$) was estimated within each population (Table I). Endogamous systems are characterised by high $F_{IS}$ values ($0.8 < F_{IS} < 1.0$) (Lande and Schemske 1985).

First, the results are not consistent among the two microsatellite loci studied. For nine out of ten populations, the $F_{IS}$ values were greater at the Gv1CT locus than at the Gv2CT locus; at Gv1CT, values range from 0.12 to 0.69 while values for Gv2CT range from – 0.13 to 0.32. This discrepancy between the two loci may be due to the disparity of their polymorphism level : more than 50 alleles are observed at the Gv1CT locus and ca. 10 alleles at Gv2CT. Consequently, the results observed at Gv1CT may be biased by the three following factors : (1) the mutation level may be too high so that the assumption of identity by descent is no longer valid; (2) the polymorphism level may be underestimated because the sampling effort was not large enough at this locus; and (3) the range of allele size variation is very large at this locus and therefore the number of heterozygotes may be underestimated due to selective pressures against (or experimental bias in the detection of) heterozygotes with two alleles of two very different sizes. This last hypothesis seems to be the most likely as the experimental bias in the detection of heterozygotes was demonstrated by Wattier *et al.* (submitted). Finally, for these reasons, the Gv1CT locus will not be used to study genetic differentiation among populations. However, this locus will be very useful for fingerprinting individuals and, in particular, for paternity analyses (Engel *et al.* in prep).

Second, theories on haplo-diploid life cycles predict that isomorphic haplo-diploid species suffer little from inbreeding depression since the same genes are probably expressed in both haploid and diploid phases and deleterious alleles can be purged from the genome during the haploid phase (Charlesworth and Charlesworth 1992; Richerd *et al.* 1993 ; Otto and Marks 1996). Haplo-diploid species can therefore "afford" to be inbred and possibly even favour this type of breeding system. However, even if we consider the Gv1CT locus, the $F_{IS}$ values obtained are not indicative of an endogamous breeding system. Moreover, two observations support the prevalence of sexual reproduction : there were many unique genotypes; and no differences were observed in allele frequencies in the haploid and diploid phases.

### *Gene flow*

Using the data obtained with the Gv2CT locus, pairwise $F_{ST}$ values were calculated for the ten populations; a genetic distance matrix was thereby constructed. The correlation of $F_{ST}$ and geogra-

phical distance was calculated for haploid and diploid individuals pooled together (Fig. 1).

There is a significant effect of isolation by distance within this species. To test if correlation was still significant at a lower scale, the regression analysis was performed over pairwise $F_{ST}$ calculated for populations separated by less than 12 km. The regression coefficients were very similar to those obtained over all populations, but only marginally significant ($y = 0.0043\ Ln(x) + 0.0192$; $r^2 = 0.27$; $P = 0.057$). A closer look reveals that significant differentiation can occur between two populations within a single location but is independent of distance at this level. Populations separated by short distances may be differentiated while populations separated by relatively greater distances may not. The positions of the populations relative to the rising/ebbing tide may play a role in the orientation of gene flow.

## CONCLUSION

First, populations of *G. gracilis* do not seem to be inbred and have a predominantly sexual reproduction strategy. These results are in contradiction with the model that predicts an inbred mating system for populations that pass through a prolonged haploid phase as in, for example, the haplo-diploid cycle (Otto and Marks 1996).

Second, gene flow is determined by geographical distance on a large scale in populations of *G. gracilis*; that is, gene flow is restricted over short distances and local adaptation is possible between populations separated by a few hundred meters to several kilometres. A high level of genetic differentiation occurs among regions separated by 500 to 1 200 km and a slight but significant differentiation occurs among locations separated by 2.5 to 12 km. This suggests that differentiation respects a spatial scale in this species and that populations are exchanging genes, at most, on a small scale. However, at the local level, that is, comparing populations within a location, the level of genetic differentiation is no longer explained solely by geographic distances. In fact, we strongly suspect that gene flow is oriented from high populations to low ones; the ebbing tide concentrates dispersal vectors in the small rivulets that connect high populations to low ones at low tide while dispersal vectors are more likely to be greatly diluted in the large water body that moves up the beach at rising tides. Nevertheless, a recent look at gene flow via male gametes suggested that gene flow, while relatively restricted to less than a meter within a population, is noticeable from low to high populations (Engel *et al.* in prep).

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