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# Concepts and issues of population genetics in seaweeds

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**Abstract:** The aim of this paper is to highlight the questions raised by a population genetics approach in order to discuss them in the context of seaweed populations studies. First, basic concepts of population genetics and their application in conservation biology are briefly reviewed. Second, we rapidly survey the methods for analysing the genetic structure of populations : these methods estimate parameters that characterise mating systems, quantify gene flow and evaluate genetic drift . To illustrate these points, examples taken from studies on algal populations are presented and discussed.

**Résumé** : L'objectif de cet article est de préciser les questions abordées en génétique des populations, et de les discuter dans le contexte des études de populations sur les macro-algues marines. Premièrement, les concepts de base de la génétique des populations sont brièvement rappelés ainsi que leurs applications possibles en biologie de la conservation. Deuxièmement, un aperçu rapide des différentes méthodes d'analyse de la structure des populations est présenté ainsi que les questions concernant l'estimation du système de reproduction, des flux géniques et de l'importance de la dérive génétique. Pour illustrer ces différents points, des exemples d'études de populations de macro-algues choisis dans la littérature sont discutés.

*Keywords* : Mating system, gene flow, dispersal, population structure, seaweed, genetic markers

## Introduction

Taxonomic identification and species delimitation have long been problematic in marine macroalgae due to a lack of clear morphological characters (Guiry, 1992; van Oppen et al., 1996). The use of DNA-molecular methods have allowed the resolution of many taxonomic ambiguities (Olsen, 1990). However, relatively few studies have addressed genetic relationships at the intra-specific level and those that have were, for the most part, concerned with large-scale biogeographical questions (van Oppen et al., 1996). Contrary to a macro-geographic approach, population genetics studies focus on the organization of genetic variability within and between populations of a species.

Population genetics-the study of progressive changes in the genetic composition of populations-emerged in the 1920's in an attempt by the mathematician R.A. Fisher and two biologists, J.B.S. Haldane and S. Wright, to conciliate Darwinian theory of evolution and Mendelian theory of the transmission of genes. The mathematical models of population genetics are based on allele (variants of a gene) frequencies within populations. The evolution of allele frequencies-and thus the changes in genetic composition-within and among populations are the basis of the microevolutionary processes of speciation and extinction.

Nonetheless, the lack of readily available genetic markers hindered the study of population genetics until the emergence of electrophoretic techniques in the late 1960's. For the first time, the analysis of the polymorphic electrophoretic variants of enzymes (allozymes) provided access to allele frequencies of many different natural populations. Today, the advances of molecular biology provide a choice of various polymorphic DNA genetic markers (Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD), microsatellites, etc.) that can be used for population studies.

The aim of this paper is to highlight the questions raised by a population genetics approach in order to discuss them in the context of seaweed population studies. First, concepts of population genetics are briefly reviewed. Second, using examples from the literature, we discuss the concept of genetic variability within and among seaweed populations.

## Basic concepts of population genetics

The basic unit in population genetics is the population, defined as a group of individuals which regularly exchange genes. In most cases, populations are composed of genetically distinct individuals and this genetic diversity can be quantified using allele frequencies (relative frequencies of the different variants of a gene occupying the same locus on a chromosome). Genetic diversity can therefore be measured at different spatial levels (e.g. within and among populations) and also at different points in time by using appropriate genetic markers (i.e. allozymes, DNA sequences or simple morphological traits). Population genetics theory predicts that only four different evolutionary forces induce changes in the allele frequencies of populations: (1) mutation, (2) natural selection, (3) migration and (4) genetic drift. These different forces are briefly reviewed in Box 1 but for more details see Hartl and Clark (1989).

## Dispersal *vs.* migration

Generally, in population genetics, dispersal[1] refers to the movement of genes *within* a population, while migration refers to gene flow *between* populations.

Within populations, the mating system and dispersal governs the movement of genes between individuals.

[1] Note that in population biology, dispersal refers to the general phenomenon of propagule displacements from one area to another regardless of scale.

**Box 1. The four evolutionary forces that induce changes in allele frequencies**

### Mutation

Mutation is the unique source of genetic novelty. Mutation introduces new alleles into populations and consequently increases genetic diversity. Most mutations are deleterious, some are neutral and very few are adaptive. The mutation rate is known to vary among DNA regions and among taxa (see for review, Drake et al., 1998). Genetic markers characterized by different mutation rates thus show various levels of polymorphism. However, markers typically used in population genetics generally show very low mutation rates (*i.e.* allozymes, $\mu = 10^{-6}$ to $10^{-7}$ new alleles per locus per generation; microsatellites, $\mu = 10^{-3}$ to $10^{-4}$). In most cases, these rates are negligible compared to the other three evolutionary forces.

### Selection

Natural selection promotes the differential transmission of particular alleles to the next generation. This is because individuals whose genotype increases their fitness (*i.e.* their capacity to survive and reproduce) are more likely to transmit their particular (advantageous) alleles on to the next generation. Therefore, while directional natural selection leads to adaptation it also results in genetic homogenization or reduced levels of polymorphism. There is abundant literature on the conditions for maintenance of polymorphism under natural selection (Dobzhansky, 1951; Dempster, 1955; Levene, 1953; Lewontin, 1974; Mitton & Grant, 1984). However, this paper focuses on the use of neutral markers to study the genetic structure of populations; therefore, this point will not be developed further.

### Migration

The coherence of a species depends on the amount of gene flow between populations. The migration rate is a measure of gene exchange among populations and also determines levels and patterns of genetic differentiation within a species. High levels of gene flow regularly introduce genotypes new to the population (but not novel to the species) —thereby homogenizing populations' genetic composition— but concomitantly decrease the possibility for local adaptation. Low levels of gene flow promote differentiation of allele frequencies among populations. Neutral genetic markers are useful tools for the estimation of genetic differentiation—disregarding selective pressures—among populations and, by inference, gene flow.

### Genetic drift

In each generation, only a finite fraction from the infinite number of gametes produced are sampled to form the individuals of the next generation. This sampling of gametes results in random fluctuation of gene frequencies, called genetic drift. The magnitude of these erratic changes in allele frequency depends on population size. Genetic drift increases with reduced population size and leads to loss or fixation of neutral alleles.

The importance of random genetic drift depends on a single parameter: the effective size of a population ($N_e$). The effective population size of an actual population can be seen as a the number of genetically distinct individuals that effectively participate in the formation of the next generation. In general, $N_e$ is smaller than the census size, N. Different estimators of $N_e$ have been defined according to various criteria (*e.g.* number of reproducing individuals, variation of fitness among individuals, variation in allele frequency among generations; for review see Caballero, 1994 and Frankham, 1995).

Additionally, genetic drift may govern the fixation of selectively advantageous or deleterious genes. In isolated finite populations, the level of genetic diversity maintained is determined primarily by the joint action of natural selection and genetic drift. If a large population is reduced in size, genetic drift becomes the more important evolutionary force leading to a loss of neutral variation. An increasing proportion of variation becomes "selectively neutral" as natural selection becomes overwhelmed by stochastic events. In this case, the fixation of deleterious mutations may lead to inbreeding depression and to the extinction of a population. The loss of neutral variability is often used as a barometer of the total genetic variation of a species, a measurement often applied in conservation biology.

Limited dispersal produces a sub-structured population composed of patches of related individuals. At the between-population level, migration, or the rate of gene exchange, determines the force of drift. The relative importance of migration and drift are thus evaluated by studying the organization of genetic diversity within and between populations. Classically, gene flow is considered as a constraining element in evolution because it retards or even prevents genetic differentiation and, by extension, speciation. However, gene flow may also be considered as a "creative force" as restricted gene flow can actually enhance the potential for adaptive evolution (Slatkin, 1987). Population genetic studies allow us to determine if gene flow is great enough to counteract the possibility of local adaptation.

The effects of complex life histories with and without sexual reproduction and the characteristics of the propagules involved in dispersal and migration are the key factors that determine population structure and gene flow at different scales. Benthic seaweeds, with their variety of life histories, offer an opportunity to explore the relationship between life history traits and population genetic structure.

## Measuring population genetic structure

### *F-statistics: genetic structure within and among populations*

*F*-statistics are the most common statistics used to determine patterns of genetic structuring within and among sub-populations (Wright, 1931; 1951; see Box 2). Consider a population of diploid individuals arranged in different sub-populations; the basic idea is to compare the observed distribution of alleles or genotypes with the expected distribution that should be obtained if genes were shuffled at random within diploid individuals ($F_{IS}$) and among individuals ($F_{ST}$). $F_{IS}$ measures the within sub-population genetic structure by examining the correlation of the two alleles found within a single individual at a single locus, For this reason, $F_{IS}$ can be calculated for diploid individuals only. $F_{ST}$ measures the differentiation among subpopulations by estimating the variance of allele frequencies among sub-populations. Numerous software packages are available for the estimation of these parameters (Schnabel et al., 1998; Beerli, 1997; Luikart & England, 1999).

### *Mating system*

The allelic correlation $F_{IS}$, informs as to the mating system of the population in question. Indeed, random mating populations are characterized by the absence of allelic correlation while self-fertilization or bi-parental inbreeding results in significant positive correlation of alleles. Conversely, negative $F_{IS}$ values indicate that mating takes place between individuals that are more genetically dissimilar than random associations of gametes. Positive $F_{IS}$ values can also result from population subdividsion: if sampled individuals belong to different breeding groups, the estimated $F_{IS}$ values will be a combination of both the within-subpopulation (e.g. due to inbreeding) and among-subpopulation heterozygote deficiency; this is called the Wahlund effect. Multilocus genotypes can also be used in mating system analysis to estimate the proportion of self-fertilized progeny produced by an individual mother or the population (Ritland & Jain, 1981; Ritland, 1986).

**Box 2. F-statistics: statistical tools to describe the variance of allele frequencies within and among populations.**

The *F* parameters, introduced by Wright (1951, 1931) as "correlation coefficients between uniting gametes", are usually calculated as deficits of heterozygotes relative to the expectod frequency of heterozygotes at a given scale.

There are three *F*-statistics: $F_{IS}$ $F_{IT}$ and $F_{ST}$:

• $F_{IS}$ describes the mating system at the lowest spatial level of the population

$$F_{IS} = 1 - (H_o / H_e)$$

*where $H_o$ and $H_e$ are, respectively, the observed and expected (according to Hardy-Weinherg equilibrium) frequencies of heterozygotes at a locus (in panmitic population $F_{IS} = 0$ and for selfing species $F_{IS} = 1$).*

• $F_{ST}$ corresponds to the deficit of heterozygotes due to the subdivision of the total population into subpopulations (Walhund effect)

$$F_{ST} = 1 - (H_s / H_t)$$

*where $H_s$ and $H_t$, are, respectively, the observed und expected (according to Hardy-Weinberg equilibrium) frequencies of heterozygotes at a locus in total population*

The parameter $F_{ST}$ *is* used commonly as a measure of population subdivision, and provides a convenient approach for estimating between-population gene flow in models that assume selective neutrality. Under selective neutrality, population differentiation is the product of genetic drift and gene flow:

$$F_{ST} = 1 / (1+4\ N_e m) \text{ in diploids}$$

*where*
*$N_e$ = effective population size (genetic drift)*
*$m$ = migration rate (gene flow)*

• $F_{IT}$ corresponds to the overall deficit of heterozygotes, due to *both* population subdivision and mating system

When there is random mating within sub-populations $F_{IS} = 0$ and no genetic differentiation among sub-populations $F_{ST} = 0$ then $F_{IT} = 0$

A convenient way to estimate the *F* statistics has been developed by Weir & Cokerham (1984) based on the partitioning of *F* by analysis of variance.

### *Genetic differentiation between and within populations*

*Between populations*

*F*-statistics were developed by Wright (1931) in the framework of the island model. The island model represents an ideal population as one in which subpopulations exchange genes in a random fashion; in other words, as if migrating genes were drawn at random from a common pool of alleles (Box 3). At migration–drift equilibrium, $F_{ST}$, the genetic differentiation between populations is a function of the product of genetic drift and gene flow (Box 2).

Nevertheless, other more realistic models exist such as the stepping stone (isolation by distance) and spatially explicit island models (Box 3). 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- $F_{ST}$)) (Rousset, 1997) between each pair of populations is plotted as a function of the natural logarithm of the geographic distance separating the two populations and the correlation is tested by a Mantel test (Mantel, 1967). A spatially explicit island model allows the exploration of the differentiation of populations in conjunction with their ecological correlates. For example, the populations of many algal species are found in divergent habitats, such as the subtidal and intertidal zones; this latter model allows us to determine if local adaptation occurs with respect to differences in the landscape.

*Within populations*

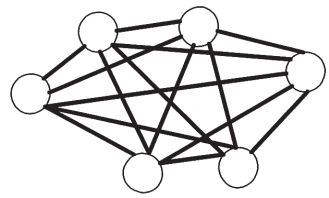
Finally, it is possible to study propagule dispersal within a population using spatial autocorrelation analysis. Spatial autocorrelation describes how the genotypic resemblance between individuals varies with respect to the distance separating individuals. If dispersal is restricted, even continuous populations consist of a mosaic of patches, or neighbourhoods. The area within which random mating occurs is referred to as the neighbourhood area (Wright, 1943; Crawford, 1984). The neighbourhood size, the genetically effective number of individuals that occupy the neighbourhood area, is analogous to $N_e$ because it measures the importance of genetic drift with respect to dispersal within a population. The neighbourhood size can be indirectly estimated using neutral genetic markers using spatial autocorrelation methods (Hardy & Vekemans, 1999; Rousset, 2000).

All the above-described models give indirect estimations of gene flow and dispersal. Alternative strategies—direct estimates of gene flow—can be explored, such as empirical observations of propagule movements (Levin & Kerster, 1974), paternity analyses (Sork et al., 1999), and assignment tests (Luikart & England, 1999). However, the last two methods require highly polymorphic genetic markers. Nonetheless, the value of obtaining both direct and indirect estimates has been strongly emphasized (Slatkin, 1987; Neigel, 1997).

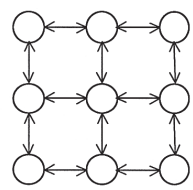
**Box 3. Gene flow models.**

***Substrucutred populations:*** *Individuals are grouped in sub-populations or demes.*

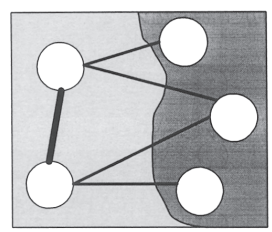
**Island model.** Sub-populations exchange genes with the same probability irrespective of the geographic distance that separates them, as if the genes were drawn at random from the whole population (Wright, 1931).



**Isolation by distance (stepping stone) model.** Genes are exchanged between neighbouring demes (Kimura & Weiss, 1964).



**Spatially explicit island model.** Gene flow depends not only on distance but also on the characteristics/quality of the landscape (indicated by the different shading) (Hanski & Simberloff, 1997). In this example, gene flow is great between populations found in the light-coloured landscape while populations found in the dark-coloured landscape exchange few or no genes among themselves. The latter populations, however, exchange a moderate amount of genes with the former.



## Population genetics of seaweeds

### *Genetic diversity in seaweeds*

Genetic diversity is typically evaluated by two indices: (1) the mean number of alleles per locus ($N_a$) and (2) the mean gene diversity or expected heterozygosity (Nei, 1978) $H_e$=

$1-\Sigma p_i^2$, where $p_i$ is the frequency of the $i^{th}$ allele at the considered locus. As discussed above, populations that have experienced a recent reduction of their effective population size (i.e. bottleneck) exhibit a correlative reduction in the number of alleles ($N_a$) and gene diversity ($H_e$) at polymorphic loci. The comparison of these two indices may provide a means to detect the occurrence of a recently bottlenecked population (see Cornuet & Luikart, 1996 and Luikart & Cornuet,1998) which has important implications in the field of conservation biology.

Most estimations of genetic diversity in seaweed populations are based on allozymes (for review, see Sosa & Lindstrom, 1999). These studies have often been disappointing because the allozyme systems employed generally showed low amounts of detectable variation (Table 1).

**Table 1.** Genetic diversity estimated in natural populations of seaweeds using allozymes (from Sosa & Lindstrom,1999)

**Tableau 1**. Diversité génétique estimée dans des populations naturelles de macroalgues marines en utilisant des allozymes (tiré de Sosa & Lindstrom, 1999)

| | Number of study species | Mean number of populations | Mean number of loci | $H_e$ | $N_a$ |
|---|---|---|---|---|---|
| Chlorophyta | 11 | 4.0 | 10.3 | 0.074 | 1.26 |
| Phaeophyceae | 7 | 3.8 | 9.2 | 0.054 | 1.00 |
| Rhodophyta | 25 | 4.1 | 13.4 | 0.085 | 1.44 |

$H_e$: expected heterozygosity or gene diversity (Nei, 1978); $N_a$: mean number of alleles per locus.

In fact, the usefulness of any genetic marker strongly depends on the degree of polymorphism at the examined locus. Comparative analysis of genetic diversity in plants and animals using different types of nuclear genetic markers including allozymes and microsatellite loci have been recently published (Table 2). Estimates of multilocus heterozygosity and the mean number of alleles per locus based on allozymes are always lower than for microsatellite loci (Table 2). Estoup et al. (1998) suggested that highly variable markers such as microsatellites may be better suited than allozymes to detect population differentiation, but biases may be introduced when using markers that show very high levels of polymorphism (Hedrick, 1999; Pannell & Charlesworth, 1999; Nagylaki, 1998). In addition, highly variable markers open new perspectives for population genetic studies based on the distribution of genotypes rather than allele frequencies because each individual may be explicitly identified by its multilocus genotype. Recently, the development of high polymorphic DNA markers in

**Table 2.** Comparative analysis of allozyme and microsatellite markers

**Tableau 2.** Analyse comparée des marqueurs allozyme et microsatellite

| Taxa | Allozymes | | | Microsatellites | | | References |
|---|---|---|---|---|---|---|---|
| | $N_l$ | $H_e$ | $N_a$ | $N_l$ | $H_e$ | $N_a$ | |
| **Animals** | | | | | | | |
| Soay sheep | 5 | 0.078 | - | 6 | 0.509 | - | Bancroft et al., (1995) |
| Brown trout | 8 | 0.188 | 1.7 | 7 | 0.412 | 3.6 | Estoup et al., (1998) |
| Common toad | 6 | 0.313 | 2.2 | 1 | 0.683 | 6.0 | Scribner et al., (1994) |
| **Higher plants** | | | | | | | |
| White oak | 4 | 0.340 | 4.3 | 6 | 0.870 | 21.7 | Streiff et al., (1998) |
| Scots pine | 8 | 0.340 | - | 2 | 0.850 | - | Karhu et al., (1996) |

$N_l$: number of loci; $H_e$: expected heterozygosity or gene diversity (Nei, 1978); $N_a$: mean number of alleles per locus

seaweeds has opened a previously intractable area of intra-specific, population-level studies (e.g. multilocus fingerprinting, Coyer et al., 1995; Coyer et al., 1997; RAPDs, see for review van Oppen et al., 1996; microsatellite markers, Luo et al., 1999; Wattier et al., 1997; Billot et al., 1998 and polymorphic mitochondrial markers, Zuccarello et al., 1999). For example, in two seaweed species, a rhodophyte, *Gracilaria gracilis* and a kelp, *Laminaria digitata*, the microsatellite markers show substantial levels of genetic diversity compared to those found using allozymes (compare Tables 1 and 3). The application of such highly polymorphic markers is essential for accurate and realistic assessment of genetic diversity in seaweeds.

However, the importance of neutral genetic variation in the maintenance and evolution of populations is debated in the context of conservation biology. Indeed, the extinction of populations have demographic as well as genetic causes (Schemske et al., 1994).

## *Mating system in seaweeds*

### *Asexual reproduction*

Asexual reproduction reduces the effective population size and consequently, increases the effect of genetic drift, thereby reducing the intra-population genetic variation. However, the magnitude of this decrease in diversity depends in fact on the migration rate between populations. In their review, Sosa & Lindstrom (1999) suggested that the low level of polymorphism generally observed with allozymes in seaweeds can be attributed to the prevalence of

**Table 3.** Genetic diversity in two seaweed species using microsatellite markers.

**Tableau 3.** Diversité génétique chez deux espèces de macroalgues marines en utilisant des marqueurs microsatellites.

| Species | N | $N_1$ | $H_e$ | $N_a$ | Reference |
|---|---|---|---|---|---|
| *G. gracilis* | 50 | 7 | 0.494 | 3.637 | Valero, unpublished data |
| *L. digitata* | 35 | 7 | 0.533 | 5.297 | Billot, unpublished data |

N: number of populations; $N_1$: number of loci; $H_e$: expected heterozygosity or gene diversity (Nei, 1978); $N_a$: mean number of alleles per locus.

asexual versus sexual reproduction associated with restricted migration rates. Indeed, in many seaweed populations, the apparent absence of sexual organs (Edwards, 1973; Innes, 1984) suggests that asexual reproduction predominates. However, in natural populations, sexual reproductive structures are generally difficult to observe because the reproductive period is limited in time and sexual phases are often not well known (e.g. Kain (Jones) & Destombe, 1995; De Wreede & Klinger, 1988). Moreover, caution should be taken in interpreting reduced genetic diversity in seaweed populations. In addition to asexual reproduction, low genetic diversity can also be explained by external causes. In fact, genetic markers are frequently derived from the technical advances in various fields of biology (e.g. physiology, biochemistry, genetics and molecular biology). This is the case for allozyme markers which were first developed in model organisms (such as humans or *Drosophila*) in which most of the screened enzymes are involved in well-known biochemical pathways. Thus, it is possible that enzymatic systems showing high levels of polymorphism in animals or higher plants are less variable in seaweeds due to different (albeit unknown) physiological constraints.

Asexual reproduction (clonality) results in the multiplication of the same genotype in many copies within a population. Therefore, population genetic approaches are extremely useful for detecting asexual reproduction (see for discussion Ellstrand & Roose, 1987). First, clonality reduces the number of genotypes present in a population compared to the genotypic diversity expected under random mating (Stoddart & Taylor, 1988). Second, asexual reproduction is characterized by the absence of segregation of alleles; consequently, allele associations are fixed in either the homozygous or heterozygous state (depending on the locus), thereby generating significant positive and negative $F_{IS}$ values at different loci. Third, the lack of (meiotic) recombination among loci generates linkage disequilibrium by fixing associations of alleles at different loci. Paradoxically, only a few studies unequivocally demonstrate the occurrence of asexual reproduction with allozyme markers in seaweed populations using these criteria (e.g. in several *Caulerpa* species, Benzie et al., 1997; in *Enteromorpha linza,* Innes & Yarish, 1984; in *Lithothrix aspergillum,* (Pearson & Murray, 1997); in two *Gelidium* species, Sosa et al., 1998).

In contrast to allozymes, DNA fingerprinting methods allow the identification of each individual by its unique multilocus genotype due to the high variability at each locus and the large number of loci. This approach has been used in various clonal species to investigate the importance of asexual reproduction by analysing the number and distribution of genotypes among and within populations (Brookfield, 1992; Coffroth et al., 1992; Okamura et al., 1993; Alberte et al., 1994; Waycott, 1995; Piquot et al., 1996). Recently, DNA fingerprints have been successfully applied in natural populations of a kelp, *Postelsia palmaeformis* (Coyer et al., 1997), to detect genetic similarities among individuals.

*Selfing vs. outcrossing*

In contrast to Angiosperms where hermaphroditism is the most common breeding system (72% of species, Richards, 1997), most seaweed species are dioecious, i.e. male and female sexual organs occur on different individuals (Scagel et al., 1982). Thus, by constraint, most seaweed species are obligatory outcrossing organisms. However, in haplo-diploid species, mating between male and female gametophytes sired by the same parental tetrasporophytic individual is wholly analogous to selfing. Consequently, levels of inbreeding similar to those reported in hermaphroditic flowering plant species can be observed in dioecious haplo-diploid seaweed species when both spore *and* gamete dispersal are restricted. On the contrary, selfing cannot occur in dioecious diploid species. However, even in dioecious diploid species, biparental inbreeding (mating among close relatives) may be important if dispersal is limited. Consequently, the occurrence of dioecy does not automatically mean that inbreeding is negligible in natural populations. Here again, the population genetics approach has been proved to be very useful; mating systems have been elucidated in numerous plants and animals (see for review: Brown et al., 1990; Jarne & Charlesworth, 1993; Jarne, 1995; Barrett et al., 1997).

In seaweeds, two interesting allozyme studies were conducted on closely-related fucoid species, *Halidrys dioica* (Lu & Williams, 1994) and *Silvetia compressa,* as *Pelevetia fastigiata* (Williams & Di Fiori, 1996), exhibiting contrasting mating and dispersal systems. *H. dioica* is a dioecious species characterized by floating reproductive fronds and consequently potentially capable of long distance dispersal, while *S. compressa* is a bisexual (hermaphroditic) species characterized by low gamete and

zygote dispersal capabilities. Their data were partially consistent with their life history characteristics: *H. dioica* showed higher mean genetic diversity ($H_e$) and lower genetic differentiation (mean $F_{ST}$ value) on a small geographical scale (a few kilometers) than the hermaphroditic species *S. compressa*. However, the estimates of $F_{IS}$ were not very different between the two species; both showed high levels of heterozygote deficiency. Nevertheless, the number of available polymorphic loci (3 for *H. dioica* and 2 for *S. compressa*) was not sufficient to draw robust conclusions from the estimates obtained (e.g. $F_{ST}$ values varied from 0.023 to 0.942 depending on the locus studied in *S. compressa)*. Here again, the paucity of polymorphic markers seriously restricts such studies in seaweeds.

*Dispersal distance and neighbourhood area in seaweeds*

The time for which spores and gametes remain viable after release determines the dispersal ranges of benthic seaweeds. Seaweeds are generally considered as poor dispersers (see special issue of the British Phycological Journal, 1992, 27(3)). Indeed, spore survival is limited to a few days (Hoffmann, 1987) and their dispersal is generally limited to a few meters (*e.g.* between 1.5 and 40 m for some fucoid species; Santelices, 1990). However, little is known about gamete viability and dispersal. In the red seaweed *Gracilaria gracilis*, laboratory experiments showed that spermatia (male gametes) have, on average, a fertile life span of about five hours. Field experiments showed that fertilization occurred at a maximum of 80 m from a population (Destombe et al., 1990). In this species, using microsatellite markers, paternity analyses demonstrated that male gametes fertilized primarily the closest females (Engel et al., 1999): the majority of mating events (80%) occurred within a distance of less than 1 m. Nevertheless, a non-negligible proportion of zygotes (11 %) were attributed to males that were not present in the study population, revealing many more long-distance, inter-population matings than expected.

The most detailed studies of effective dispersal within seaweed populations were performed in the two previously-mentioned fucoid species, *Halidrys dioica* (Lu & Williams, 1994) and *Silvetia compressa* (Williams & Di Fiori, 1996). At the scale of a few meters, spatial autocorrelation analyses did not reveal any significant structure in dioecious species while, as expected, significant clusters of related genotypes occurred within distances of 2-3 m in *S. compressa* for which limited dispersal of zygotes was verified by Brawley & Johnson (1991). Moreover, in this latter species, Williams & Di Fiori (1996) obtained low direct estimates of effective neighbourhood area (2.3 $m^2$) and neighbourhood size (133 individuals). These direct estimates in addition to the significant fine-scale genetic structure consistently suggest that genetic drift strongly contributes to micro-geographic genetic differentiation in this species, promoting local adaptation at this scale.

*Genetic differentiation among seaweed populations*

In addition to spore and gamete dispersal, fragmentation of reproductive thalli can occasionally reinforce gene flow between populations. Fronds bearing reproductive structures break off, these fragments can be dispersed and release gametes and spores in a new site. Thus fragmentation, along with ship transportation constitute potential long-distance spore and gamete dispersal.

Estimates of genetic differentiation among seaweed populations ($F_{ST}$) vary widely according to species (for review see Sosa & Lindstrom, 1999). While allozyme studies generally suggested that genetic differentiation between populations increased with geographical distance, the regression of $F_{ST}$ on geographical distances was never specifically tested. However, in comparison with the island model, isolation by distance or stepping-stone models (Box 3) incorporate spatial information thereby allowing the hypotheses about relationships between the effective migration rate and spatial patterns of interpopulation connectivity to be tested. Recently, isolation-by-distance models were tested in the red algae *Gracilaria gracilis* (Engel et al., 1997; Valero, unpublished data) and in the kelp, *Laminaria digitata* (Billot, 1999). In these two species, more than 20 populations separated by 0.5 to 800 km were sampled in the same sites throughout the English Channel (coasts of Brittany, Normandy and Cornwall) and genotyped with microsatellite loci. Populations of study species exhibit different distribution patterns. *G. gracilis* is patchily-distributed while *L. digitata* has a continuous spatial distribution. Our results show that, while significant in both species, patterns of isolation by distance differ between the two species. First, as expected, genetic differentiation was weaker in the continuously-distributed brown seaweed species, *L. digitata*, than in the patchily-distributed red seaweed species, *G. gracilis*. Second, in *L. digitata,* landscape features appear to influence patterns of dispersal. In particular, the isolation-by-distance pattern was shaped by the occurrence of rocky shores, required for spore settlement, and by the prevailing long-term currents, which regulate migration. In the long-lived species, *G. gracilis*, the pattern of isolation-by-distance did not reflect current patterns. Furthermore, in *G. gracilis*, genetic differentiation was only poorly explained by the isolation–by-distance model at scales larger than hundreds of kilometres suggesting that other biological, ecological, and/or historical factors play a role in gene exchange between populations at this scale.

Population genetic approaches provide valuable insights into the effects of gene flow, especially when landscape

information are incorporated. Indirect estimates such as $F_{ST}$ measure historical gene flow and are therefore not appropriate for the study of contemporary gene movement. On the other hand, molecular tools (i.e. microsatellites) could be used to estimate gene flow directly. For example, paternity analyses assess gamete immigration by identification of inter-population matings, i.e. the previously-mentioned paternity analysis in the seaweed *G. gracilis* (Engel et al., 1999). These ongoing estimates of gene flow can aid evolutionary and conservation biologists in predicting the genetic response of species to naturally occurring or human-mediated fragmented habitats.

## Conclusion

We have shown that population genetics approaches provide insights into our understanding of mating systems and dispersal capabilities of seaweed species. This information can be discussed in the context of the known reproductive biology of the considered species, confirming expectations based on observations or challenging long-standing dogmas. Moreover, estimates of the magnitude of gene movements within and between populations can be interpreted in the context of spatially explicit models. Indirect estimates of gene flow provide insights into the biogeography and ecotypic differentiation of species while direct estimates allow the investigation of observed landscape heterogeneity (e.g. due to recent anthropogenic disturbance) on contemporary gene flow. Although allozymes have been successfully used to examine genetic structure of many organisms, they are generally not ideal tools in seaweeds because of the low number of loci available and their low level of polymorphism. With the introduction of PCR-based techniques, a multitude of molecular tools have become available (for review see Harris, 1999). As recently advocated by Wolf & Morgan-Richards (1999), RAPDs or similar PCR-based techniques (AFLP, SSCP…) appear be the simplest and most efficient tools to answer the questions that could not be addressed using allozymes. In comparison, with the development of microsatellite markers, PCR-based techniques can be directly applied to any kind of organisms without extraordinary financial or technical investments.

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## References

**Alberte R.S., Suba G.K., Procaccini G., Zimmerman R.C. & Fain S.R. 1994.** Assessment of genetic diversity of seagrass populations using DNA fingerprinting: implications for population stability management. *Proceedings of the National Academy of Sciences USA,* **91:** 1049-1053.

**Bancroft D., Pemberton J.M. & King P. 1995.** Extensive protein and microsatellite variability in an isolated, cyclic ungulate population. *Heredity,* **74:** 326-336.

**Barrett S.C.H., Harder L.D. & Worley A.C. 1997.** The comparative biology of pollination and mating in flowering plants. In*: Plant Life Histories: Ecology, Phylogeny and Evolution* (Silvertown J., Franco M. & Harper J. ed), pp. 57-76. Cambridge University Press: Cambridge.

**Beerli P. 1997.** Statistical analyses of population genetic data. *Trends in Ecology and Evolution,* **12:** 488.

**Benzie J.A.H., Price I.R. & Ballment E. 1997.** Population genetics and taxonomy of *Caulerpa* (Chlorophyta) from the Great Barrier Reef, Australia. *Journal of Phycology,* **33:** 491-504.

**Billot C. 1999.** *Structuration génétique des populations de Laminaria digitata (Phaeophycées) en Manche: utilisation de marqueurs hypervariables*. Doctorat d'Université, I.N.A.P.G.: Paris. 222 pp.

**Billot C., Rousvoal S., Estoup A., Epplen J. T., Saumitou Laprade P., Valero M. & Kloareg B. 1998.** Isolation and characterisation of microsatellite markers in the nuclear genome of the brown alga *Laminaria digitata* (Phaeophyceae). *Molecular Ecology,* **7:** 1778-1780.

**Brawley S.H. & Johnson L.E. 1991.** Survival of fucoid embryos in the intertidal zone depends on developmental stage and microhabitat. *Journal of Phycology,* **29:** 528-533.

**Brookfield J.F.Y. 1992.** DNA fingerprinting in clonal organisms. *Molecular Ecology,* **1:** 21-26.

**Brown A.H.D., Clegg M.T., Kahler A.L. & Weir B.S. 1990.** *Plant Population Genetics, Breeding and Genetic Resources*. Sinauer inc. pub: Sunderland, Massachusetts.

**Caballero A. 1994.** Developments in the prediction of effective population size. *Heredity,* **73:** 657-679.

**Coffroth M.A., Lasker H.R., Diamond M.E., Bruenn J.A. & Bermingham E. 1992.** DNA fingerprints of a gorgonian coral: a method of detecting clonal structure in a vegetative species. *Marine Biology,* **114:** 317-325.

**Cornuet J.M. & Luikart G. 1996.** Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics,* **144:** 2000-2014.

**Coyer J.A., Olsen J.L. & Stam W.T. 1997.** Genetic variability and spatial separation in the sea palm kelp *Postelsia palmaeformis* (Phaeophyceae) as assessed with M13 fingerprints and RAPDs. *Journal of Phycology,* **33:** 561-568.

**Coyer A.C., Robertson D.L. & Alberte R.S. 1995.** Genetic variability and parentage in *Macrocystis pyrifera* (Phaoephyceae) using multi-locus DNA fingerprinting. *Journal of Phycology,* **31:** 819-823.

**Crawford T.J. 1984.** The estimation of neighbourhood parameters for plant populations. *Heredity,* **52:** 273-283.

**De Wreede R.E. & Klinger T. 1988.** Reproductive Strategies in Algae. In: *Plant Reproductive Ecology: Patterns and Strategies* (Lovett Doust J. & Lovett Doust L. ed), pp. 267-284. Oxford University Press: New York, USA.

**Dempster E.R. 1955.** Maintenance of genetic heterozygosity. In: *Cold Spring Harbor Symposia in Quantitative Biology* pp. 25-32.

**Destombe C., Godin J. & Remy J.M. 1990.** Viability and dissemination of spermatia of *Gracilaria verrucosa* (Gracilariales, Rhodophyta). *Hydrobiologia,* **204/205:** 219-223.

**Dobzhansky T. 1951.** *Genetics and the origin of species*. Columbia University Press: New York.

**Drake J.W., Charlesworth B., Charlesworth D. & Crow J. 1998.** Rates of spontaneous mutation. *Genetics,* **148:** 1667-1686.

**Edwards P. 1973.** Life history studies of selected British *Ceramium* species. *Journal of Phycology,* **9:** 181-184.

**Ellstrand N.C. & Roose M.K. 1987.** Patterns of genotypic diversity in clonal plant species. *American Journal of Botany,* **74:** 123-131.

**Engel C., Wattier R., Destombe C. & Valero M. 1997.** Dispersal in a haplo-diploid red algal species: Genetic structure and gene flow in *Gracilaria gracilis*. *Vie et Milieu,* **47:** 333-337.

**Engel C., Wattier R., Destombe C. & Valero M. 1999.** Performance of non-motile male gametes in the sea: analysis of paternity and fertilization success in a natural population of a red seaweed, *Gracilaria gracilis*. *Proceedings of the Royal Society of London, Series B Biological Sciences,* **266:** 1879-1886.

**Estoup A., Rousset F., Michalakis Y., Cornuet J., Adriamanga M. & Guyomard R. 1998.** Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). *Molecular Ecology,* **7:** 339-354.

**Frankham R. 1995.** Effective population size / adult population size ratios in wildlife: a review. *Genetical Research (Cambridge),* **66:** 95-107.

**Guiry M. D. 1992.** Species concepts in marine red algae. In: *Progress in Phycological Research* (Round F.E. & Chapman D.J. ed), pp. 251-278. Biopress Ltd.

**Hanski I. & Simberloff D.1997.** The metapopulation approach, its history, conceptual domain, and application to conservation. In: *Metapopulation Biology: Ecology, Genetics and Evolution,* (Hanski, I. & Gilpin M.E. ed), pp. 5-26. Academic Press, San Diego.

**Hardy O.J. & Vekemans X. 1999.** Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetics models. *Heredity,* **83:** 145-154.

**Harris S. A. 1999.** RAPDs in systematics - a useful methodology? In: *Molecular systematics and plant evolution* (Hollingsworth P. M., Bateman R. M. & Gornall R. J. ed), pp. 211-228. Taylor & Francis: London.

**Hartl D.L. & Clark A.G. 1989.** *Principles of population genetics*. Sinauer, Sunderland Massachusetts: 682 pp.

**Hedrick P.W. 1999.** Highly variable loci and their interpretation in evolution and conservation. *Evolution,* **53:** 313-318.

**Hoffmann A. J. 1987.** The arrival of seaweed propagules at the shore: a review. *Botanica Marina,* **30:** 151-165.

**Innes D.J. 1984.** Genetic differentiation among populations of marine algae. *Helgolander Meeresunters,* **38:** 401-417.

**Innes D.J. & Yarish C. 1984.** Genetic evidence for the occurence of asexual reproduction in populations of *Enteromorpha linza* (L.) J. Ag. (Chlorophyta, Ulvales) from Long Island Sound. *Phycologia,* **23:** 311-320.

**Jarne P. 1995.** Mating system, bottlenecks and genetic polymorphism in hermaphroditic animals. *Genetical Research (Cambridge),* **65:** 193-207.

**Jarne P. & Charlesworth D. 1993.** The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annual Review of Ecology and Systematics,* **24:** 441-466.

**Kain (Jones) J.M. & Destombe C. 1995.** A review of the life history, reproduction and phenology of *Gracilaria*. *Journal of Applied Phycology,* **7:** 269-281.

**Karhu A., Hurme P., Karjalainen M., Karvonen P., Kärkkäinen K., Neale D. & Savolainen O. 1996.** Do molecular markers reflect patterns of differentiation in adaptive traits in conifers? *Theoretical and Applied Genetics,* **93:** 215-221.

**Kimura M. & Weiss G.H. 1964.** The stepping-stone model of population structure and the decrease of genetic correlation with distance. *Genetics,* **49:** 561-576.

**Levene H. 1953.** Genetic equilibrium when more than one ecological niche is available. *American Naturalist,* **87:** 311-313.

**Levin D.A. & Kerster H.W. 1974.** Gene flow in seed plants. *Evolutionary Biology,* **7:** 139-220.

**Lewontin R. C. 1974.** *The genetic basis of evolutionary change*. Columbia University Press: New York.

**Lu T.T. & Williams S.L. 1994.** Genetic diversity and genetic structure in the brown alga *Halidrys dioica* (Fucales: Cystoseiraceae) in southern California. *Marine Biology,* **121:** 363-371.

**Luikart G. & Cornuet J.M. 1998.** Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology,* **12:** 228-237.

**Luikart G. & England P. R. 1999.** Statistical analysis of microsatellite DNA data. *Trends in Ecology and Evolution,* **14:** 253-256.

**Luo H., Mörchen M., Engel C., Destombe C., Epplen J.T., Epplen C., Saumitou-Laprade P. & Valero M. 1999.** Characterisation of microsatellite markers in the red alga *Gracilaria gracilis*. *Molecular Ecology,* **8:** 700-702.

**Mantel N. 1967.** The detection of disease clustering and a generalized regression approach. *Cancer Research,* **27:** 209-220.

**Mitton J.B. & Grant M.C. 1984.** Associations among protein heterozygosity, growth rate and developmental homeostasis. *Annual Review of Ecology and Systematics,* **15:** 479-499.

**Nagylaki T. 1998.** Fixation indices in subdivided populations. *Genetics,* **148:** 1325-1332.

**Nei M. 1978.** Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics,* **89:** 583-590.

**Neigel J.E. 1997.** A comparison of alternative strategies for estimating gene flow from genetic markers. *Annual Review of Ecology and Systematics,* **28:** 105-128.

**Okamura B., Jones C.S. & Noble L.R. 1993.** Randomly amplified polymorphic DNA analysis of clonal population structure and geographic variation in a freshwater bryozoan. *Proceedings of the Royal Society of London,* **253:** 147-154.

**Olsen J.L. 1990.** Nucleic acids in algal systematics. *Journal of Phycology,* **26:** 209-214.

**Pannell J.R. & Charlesworth B. 1999.** Neutral genetic diversity in a metapopulation with recurrent local extinction and recolonization. *Evolution,* **53:** 664-676.

**Pearson E.A. & Murray S.N. 1997.** Patterns of reproduction, genetic diversity, and genetic differentiation in California populations of the geniculate coralline alga *Lithotrix aspergillum* (Rhodophyta). *Journal of Phycology,* **33:** 753-763.

**Piquot Y., Saumitou-Laprade P., Petit D., Vernet P. & Epplen J.T. 1996.** Genotypic diversity revealed by allozymes and oligonucleotide DNA fingerprinting in French populations of the macrophyte *Sparganium erectum*. *Molecular Ecology,* **5:** 251-258.

**Richards A.J. 1997.** *Plant breeding systems*. Second edition. Chapman & Hall: London, UK.

**Ritland K. 1986.** Joint maximum likelihood estimation of genetic and mating structure using open-pollinated progenies. *Biometrics,* **42:** 25-43.

**Ritland K. & Jain S. 1981.** A model for the estimation of outcrossing rate and gene frequencies using *n* independent loci. *Heredity,* **47:** 35-52.

**Rousset F. 1997.** Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics,* **145:** 1219-1228.

**Rousset F. 2000.** Genetic differentiation between individuals. *Journal of Evolutionary Biology,* **13:** 58-62.

**Santelices B. 1990.** Patterns of reproduction, dispersal and recruitment in seaweeds. *Oceanography and Marine Biology an Annual Review,* **28:** 177-276.

**Scagel R.F., Bandoni R.J., Maze J.R., Rouse G.E., Schofield W.B. & Stein J.R. 1982.** *Non vascular plants. An evolutionary survey*. Wadsworth Publishing company: Belmont, California. 586 pp.

**Schemske D.W., Husband B.C., Ruckelshaus M.H., Goodwillie C., Parker I. M. & Bishop J.G. 1994.** Evaluating approaches to the conservation of rare and endangered plants. *Ecology,* **75:** 584-606.

**Schnabel A., Beerli P., Estoup A. & Hillis D. 1998.**A guide to software packages for data analysis in molecular ecology. In: *Advances in molecular ecology* (Carvalho G.R. ed), pp. 291-303. IOS Press: 291-303 pp.

**Scribner K.T., Arntzen J.W. & Burke T. 1994.** Comparative analysis of intra- and interpopulation genetic diversity in *Bufo bufo,* using allozyme, single locus microsatellite, minisatellite and multilocus minisatellite data. *Molecular Biology and Evolution,* **11:** 737-748.

**Slatkin M. 1987.** Gene flow and the geographic structure of natural populations. *Science,* **236:** 787-792.

**Slatkin M. 1993.** Isolation by distance in equilibrium and non-equilibrium populations. *Evolution,* **47:** 264-279.

**Sork V.L., Nason J., Campbell D.R. & Fernandez J.F. 1999.** Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology and Evolution,* **14:** 219-223.

**Sosa P.A. & Lindstrom S.C. 1999.** Isozymes in macroalgae (seaweeds): genetic differentiation, genetic variability and systematic applications. *European Journal of Phycology,* **34:** 427-442.

**Sosa P.A., Valero M., Batista F. & Gonzalez-Perez M.A. 1998.** Genetic variation and genetic structure of natural populations of *Gelidium* species: A re-evaluation of results. *Journal of Applied Phycology,* **10:** 279-284.

**Stoddart J.A. & Taylor J.F. 1988.** Genotypic diversity: estimation and prediction in samples. *Genetics,* **118:** 705-711.

**Streiff R., Labbe T., Bacilieri R., Steinkellner H., Glössl J. & Kremer A. 1998.** Within-population genetic structure in *Quercus robur* L. and *Quercus petrea* (Matt.) Liebl. assessed with isozymes and microsatellites. *Molecular Ecology,* **7:** 317-328.

**van Oppen M.J.H., Klerk H., de Graaf M., Stam W.T. & Olsen J.L. 1996.** Assessing the limits of random amplified polymorphic DNAs (RAPDs) in seaweed biogeography. *Journal of Phycology,* **32:** 433-444.

**van Oppen M.J.H., Klerk H., Olsen J. & Stam W.T. 1996.** Hidden diversity in marine algae: some examples of genetic variation below the species level. *Journal of the Marine Biological Association U.K.,* **76:** 239-242.

**Wattier R., Dallas J.F., Destombe C., Saumitou-Laprade P. & Valero M. 1997.** Single locus microsatellites in gracilariales (Rhodophyta): high level of genetic variability within *Gracilaria gracilis* and conservation in related species. *Journal of Phycology,* **33:** 868-880.

**Waycott M. 1995.** Assessment of genetic variation and clonality in the seagrass *Posidonia australis* using RAPD and allozyme analysis. *Marine Ecology Progress Series,* **116:** 289-295.

**Weir B.S. & Cockerham C.C. 1984.** Estimation *F*-statistics for the analysis of population structure. *Evolution,* **38:** 1358-1370.

**Williams S.L. & Di Fiori R.E. 1996.** Genetic diversity and structure in *Pelvetia fastigiata* (Phaeophyta: Fucales): does a small effective neighborhood size explain fine-scale genetic structure? *Marine Biology,* **126:** 371-382.

**Wolf K. & Morgan-Richards M. 1999.** The use of RAPD data in the analysis of population genetic structure: case studies of *Alkanna* (Boraginaceae) and *Plantago* (Plantaginaceae). In: *Molecular systematics and plant evolution* (Hollingsworth P. M., Bateman R.M. & Gornall R.J. ed), pp. 51-73. Taylor & Francis: London.

**Wright S**. 1931. Evolution in mendelian populations. *Genetics,* **16:** 97-169.

**Wright S.** 1943. Isolation by distance. *Genetics,* **28:** 114-138.

**Wright S.** 1951. The genetical structure of populations. *Annals of Eugenics,* **15:** 323-354.

**Zuccarello G. C., Burger G., West J. A. & King R. J. 1999.** A mitochondrial marker for red algal intraspecific relationships. *Molecular Ecology,* **8:** 1443-1447.