



Review article

How useful are the genetic markers in attempts to understand and manage marine biodiversity?

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Received 15 June 2001; accepted 8 November 2001

Abstract

The genetics of marine populations is a subject that has made little progress compared with the effort spent on the terrestrial environment. This is so despite “applied” aspects such as stock management, marine aquaculture, creation of reserves, conservation of the coastal zones, taxonomy, and protection of species. The crowded and dispersive marine environment, with its steep physical gradients, favours the existence of a planktonic larval stage for most species. The attendant high fecundity has important consequences for selection differentials and dispersal and therefore for the evolution of genetic structures. These features must be taken into account in order to understand the origin and maintenance of marine biodiversity and, in some cases, to manage it. In this article, after a definition of genetic diversity among other aspects of biodiversity, special features of the marine environment and processes governing genetic diversity are given together with the molecular tools required to study it. Then, an overview of the interesting scientific questions in marine biodiversity research is given concerning: Errorthe population structure as a function of dispersal systems and spatial constraints: gene flow and speciation in a dispersive environment, Errorthe phylogeography and historical biogeography of marine ecosystems; Errorthe functional and adaptive aspects of polymorphism: larval phase and genetic control of recruitment. Some uses of genetic diversity for assessment, conservation and protection purposes are also detailed. Organismal (specific) diversity does not enter the scope of the article. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Assessment; Conservation; Evolution; Infra-specific biodiversity; Management; Molecular ecology; Monitoring

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1. Introduction

The term biodiversity has multiple meanings depending on the biological scale to which it is applied (see Solbrig, 1991; Thorne-Miller and Catena, 1991; Norse, 1993; NRC, 1995; Heywood and Watson, 1995; Ormond et al., 1997). Most commonly, biological diversity refers to the full range of species on Earth, including single-celled organisms such as bacteria, viruses, and protista, as well as multicellular organisms such as plants, animals, and fungi. On a finer scale of organisation, biological diversity includes the genetic variation within species, both among geographically separated populations and among individuals within single populations. On a wider scale, biological diversity includes variations in the biological communities in which species live, the ecosystems in which communities exist, and the interactions among these levels.

Ecosystem diversity (at least in the sea) and genetic diversity are less readily understood by decision makers and the public, but are not less important than species diversity. The continued survival of species and natural communities require the preservation of biodiversity at all of these levels. This is included in the definition given in the text of the Convention on Biological Diversity (Rio de Janeiro, 1992): “Biological Diversity is the variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems” [Article 2] (ISCBD, 1994).

Given these various scales of biodiversity, the biological diversity of an area is conveniently described at three levels:

(1) Infra-specific (genetic) diversity is the variation within a population and among populations of a plant or animal species. The genetic makeup of a species is variable between populations of a species within its geographic range. Loss of a population results in a loss of genetic diversity for that species and a reduction of total biological diversity for the region. This unique genetic information cannot be reclaimed. This level of biodiversity is critical in order for a species to adapt to changing conditions and to continue to evolve in the most advantageous direction for that species.

(2) Organismal (species) diversity is the total number and abundance of plant and animal species in an area.

(3) The third level concerns the variety of natural communities or ecosystems within an area. These communities may be representative of or even endemic to the area. It is within these ecosystems that all life dwells.

Biodiversity also covers a complex set of relationships within and between these different levels of organisation, including human action, and their respective origins in space and time. All the components overlap (Fig. 1). A fourth level, the sea- (land-)scape diversity, integrates the type, condition, pattern, and connectivity of natural communities or ecosystems. Fragmentation of landscapes, loss of connections and loss of natural communities all result in a loss of biological diversity for a region. Humans and the results of their activities are integral parts of most landscapes.

Biodiversity is dynamic in its nature. Species and their populations are in continuous evolutionary change. The present-day diversity is the result of the combined effects of speciation and extinction. To understand biodiversity, it is thus necessary to investigate the

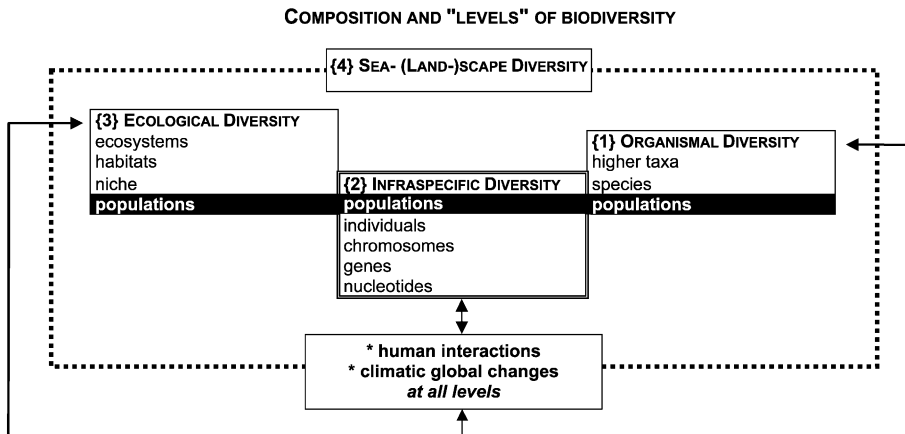


Fig. 1. Composition and levels of biodiversity. Biodiversity is conveniently described at three levels: infra-specific diversity, organismal diversity, and community or ecosystem diversity. A fourth level, the sea- (land-)scape diversity, takes into account global climatic changes and human activity impacts. It also integrates the type, condition, pattern, and connectivity of natural communities or ecosystems. (modified from Heywood and Watson, 1995).

underlying (genetic) processes involved. Genetics seeks to understand the heritable basis of variation and evolutionary change at all levels. This is represented in Fig. 1 by the fact that the population level reaches across all the components of biodiversity.

The potential of a species to respond to novel environments and to disturbances caused by human activities depends on the extent of diversity and the kind of diversity that is available. Genetic differences among individuals within a species provide the foundation for diversity among species and ultimately the foundation for the diversity among ecosystems. Genetic diversity (i.e. infra-specific diversity) determines the ecological and evolutionary potential of species.

2. Processes governing genetic evolution

Genetic diversity originates by mutation, but different processes determine the extent to which it is maintained within populations. Mutations go from a change in a single nucleotide to a modification of the karyotype. It is ultimately the source of all genetic variation, but it is unpredictable and, so far, an uncontrollable source of diversity. Mutations create new alleles whose evolutionary fates are governed by the three other forces that are selection, migration, and random drift (see Hartl and Clark, 1989; Weir, 1990; Avise, 1994; Slatkin et al., 1995; Thorpe and Smartt, 1995).

Natural (or artificial) selection operates via differential survival and reproductive success of individual organisms. Those individuals with well-adapted phenotypes will pass a greater proportion of their genes on to the next generation. This means that in a population, only a fraction of individuals will produce progeny. Such a sampling of a finite

number of genes at each generation means that some may increase in frequency, others decrease and some may be lost. Moreover, genetic drift randomly alters gene frequencies. The importance of genetic drift as a source of genetic differentiation is inversely related to population size. It has its maximum effect in small populations. It is thus important to note that effective population sizes (N_e , a theoretical population where every individual has the same probability of contributing genes to the next generation) in marine organisms are the lowest that have been estimated to date (Frankham, 1995). At a steady state, equilibrium is reached between the creation of variants by mutation and the loss of others by genetic drift. As to the third force (migration), it produces gene flow because of the movement of individuals between established populations or because of the establishment of entire populations. Dispersal tends to homogenise gene frequencies and eliminates local differences. It occurs only during the larval phase in the sessile or benthic invertebrates with limited mobility. Gene frequencies may be altered through migration, if migrants entering the population have different genotypes and interbreed with residents. It has to be noted that even when organisms reproduce sexually, genetic loci are arranged on one or several chromosomes and sets of alleles tend to be inherited together. Recombination does not itself create genetic diversity at each locus, but it can create new combinations of alleles at different loci by mixing alleles of different individuals. It can therefore also be a source of diversity.

By definition, and except in the case of [inter-fertile] hybrids, different species do not exchange genes; therefore, they have accumulated and still accumulate differences. This implies that genetic variation can be analysed at various levels, from the individual (existence of polymorphism = inter-individual variation in a gene product), through populations (gene frequency differences), to species or higher taxa (accumulation of differences = building up divergence).

Current patterns of biodiversity are the result of past events and can sometimes provide important cues about the history of a species and details of its current population structure. The interest of genetic markers rests on the fact that the macromolecules making up the genome diverge under the effect of mutations. This generates variability (polymorphism) sorted in the course of time by the three forces previously described, which are genetic drift, selection and migration. These mutations are at the origin of two types of exploitable information: (1) they can accumulate in the same molecular strain; they thus constitute a measure of time since this strain split in two (“disputed” concept of the molecular clock, see Zuckerkandl and Pauling, 1965; Kimura, 1983; Futuyma, 1986; Avise, 1994; Hillis et al., 1996); (2) they are at the origin of the existence, at a given time, of several variants in the same population or individual and are at the origin of a molecular polymorphism analysable in terms of genetic structure.

3. The special features of marine genetic biodiversity

Dispersal is a central process in the dynamics and evolution of plants. This is mostly determined by the movements of seeds. Colonisation of new sites and/or extinction of local populations depend on seed dispersal ranges. Experimental studies on plants (trapping seeds at different distances from the source, recapture of marked and released propagules,

use of artificial analogues of dispersal propagules) have shown (1) that quantifying dispersal, especially over long distances, which has great relevance to population biology, is a difficult task (the maximum detected dispersal distance varying between species and experiments) and (2) that the majority of seeds disperse over quite short distances (cf. Ouborg et al., 1999). These facts suggest that the study of dispersal would greatly benefit from integration of both ecology and population genetic approaches (Silvertown, 1991). However, gene flow (see below) is not the same as dispersal. Gene flow refers to the movement of genes, which involves both seeds and pollen, whereas dispersal refers to seeds (and propagules) able to establish themselves (Ouborg et al., 1999). It requires also survival to reproduction and contribution of progeny to next generation. Its evaluation gives the resulting level of gene flow over the past generations, while a direct method gives the gene flow in the present year. In the case of free-spawning marine organisms, sperm limitation (dilution of gametes) may occur. However, recent surveys of naturally spawning populations indicate fairly high fertilization levels in many taxa (Bishop, 1998; Yund, 2000). Certainly, most of the processes maintaining biological diversity in marine ecosystems are similar to those in terrestrial ones. However, there are major differences in patterns of diversity largely as a result of the dispersive nature of marine larvae and to the wide distribution of organisms and of habitats. Larvae are not only passive dispersers but they are also able of sustained swimming abilities (Leis et al., 1996; Stobutzki and Bellwood, 1997). Moreover, late-stage of fish larvae are capable swimmers with considerable control over speed, depth and direction (Leis and Carson-Ewart, 1999, 2001). The marine environment has major characteristics of its own as to how genetic diversity is created and distributed (Strathmann, 1990; Briggs, 1994; Levin, 1994; May, 1994; Rapoport, 1994; Gray, 1997; Avise, 1998). Marine organisms are often less easily observable than terrestrial ones. Marine life space allows the existence of a planktonic phase in about 70% of the species, which is associated with very high fecundities, explosive reproductive potential and important dispersal and migratory capacities, and sometimes large population size. Planktonic larvae may spend several weeks, or even months, in the water column before metamorphosis and settling (Mileikovsky, 1968; Scheltema, 1971, 1986; Strathmann, 1978). Marine species generally show a higher genetic diversity than freshwater and terrestrial species. Ward et al. (1994) showed that average heterozygosity was similar in marine and freshwater species subpopulations, but was considerably less in terrestrial ones. There are some equivalents in other environments, but this remains marginal. The aerial milieu, for instance, is far less dense and viscous and it is trophically quasi-empty in contrast to most marine waters. Pressures on life history traits (e.g. those related to feeding and nutrition) occur during larval life. Seeds do not grow during dispersal. Marine species are often viewed as consisting of very widely distributed populations that are not strongly genetically structured. However, a large number of types of reproductive isolation such as differences in spawning time, mate recognition, environmental tolerance and gamete compatibility have been inferred in marine speciation events (Palumbi, 1994). Marine species represent a challenge to the allopatric speciation model that has been widely accepted for terrestrial organisms (at least for vertebrates). This challenge lies in the fact that many marine organisms develop through a planktonic larval stage that can be potentially widely dispersed by currents, able to cross any discernible barriers (Palumbi, 1992). However, in contrast to the geographical continuity of the World Ocean, marine ecosystems

may present zonations as complex and sometimes sharper (e.g. active hydrothermal vents) than on land, in the absence of recognisable barriers to dispersal (Neigel, 1997). These zonations often go along with marked gradients (temperature, salinity, light, trophic abundance. . .) developing in general over vast stretches.

In general terms, marine species with long larval phases are thought to disperse further, have higher gene flow, larger geographic ranges, lower levels of genetic differentiation among populations, and higher levels of genetic variation within populations. Genetic studies of marine invertebrates have generally provided good support for this idea (Levinton and Koehn, 1976; Berger, 1983; Gyllensten, 1985; Waples, 1987; Palumbi and Wilson, 1990; MacMillan et al., 1992; Ward et al., 1994; Palumbi, 1995). However, various exceptions to this rule have been identified. Genetic pools of the majority of widely distributed species are rarely homogenous from one end to the other of their distribution area (Burton, 1983; Reeb and Avise, 1990; Watts et al., 1990; Karl and Avise, 1992; Hilbish, 1996; Borsa et al., 1997b; Neigel, 1997). This may be because of several nonrandom factors including strong selection through local adaptation (Hedgecock, 1986; Knowlton and Keller, 1986). Genetic partitioning in the marine environment, therefore, is thought to occur primarily through ecological and geographic limitations such as dispersal capability, niche partitioning or local adaptation (Hedgecock, 1986; Palumbi, 1994).

4. Molecular tools

By detecting genetic variations, genetic markers may provide useful information at different levels: population structure, levels of gene flow, phylogenetic relationships, patterns of historical biogeography and the analysis of parentage and relatedness. Different types of molecular polymorphism are accessible. We will view some of the most frequently used molecules and tools.

Biodiversity necessarily has a time component; therefore, the resolution of the molecular techniques used should match the time scale of interest. Knowledge at the genetic level has been limited mainly by the availability of tools and techniques for its study. New and sophisticated techniques are increasingly available (Avise, 1994; Beaumont, 1994; Skibinski, 1994; Bonhomme et al., 1995; Slatkin et al., 1995; Thorpe and Smartt, 1995; Burton, 1996; Ferraris and Palumbi, 1996; Hillis et al., 1996; Carvalho, 1998). Several waves of progress in molecular evolution studies have followed the introduction of new techniques. The most influential methods are given in Table 1.

During the last few years, the great strides of molecular biology virtually gave access to the entire genome, but at the price of relatively heavy techniques of molecular sorting and visualisation of polymorphisms. Their complexity and high cost limited their use to precisely targeted projects in population biology. Some years ago, polymerase chain reaction (PCR) induced a methodological revolution (Mulis and Faloona, 1987; Sakai et al., 1988; Erlich, 1989). The PCR technique is basically a primer extension reaction for amplifying specific nucleic acids *in vitro*. The use of a thermostable polymerase referred to as *Taq* (first isolated from the Yellowstone National Park hot springs bacteria *Thermus aquaticus*) allows a short stretch of DNA (usually fewer than 3000 bp) to be amplified to about a million fold so that one can determine its size, nucleotide sequence, etc. The

Table 1

Main steps in the use of molecular markers for population genetic purposes

Late 1960s	<ul style="list-style-type: none"> ● Protein electrophoresis Hubby and Lewontin (1966)
Late 1970s	<ul style="list-style-type: none"> ● DNA sequencing and mtDNA RFLP (Restriction Fragment Length Polymorphism) Maxam and Gilbert (1977), Sanger et al. (1977), Avise et al. (1979), Brown et al. (1979)
Late 1980s	<ul style="list-style-type: none"> ● DNA fingerprinting Jeffreys et al. (1985a,b, 1988), Burke (1989) ● PCR (Polymerase Chain Reaction)-mediated DNA sequencing and PCR-based RFLP approach/RAPD (Random Amplified Polymorphic DNA) Mulis and Faloona (1987), Saiki et al. (1988), Kocher et al. (1989), Weber and May (1989), Welsh and McClelland (1990), Williams et al. (1990) ● VNTR (Variable number of tandem repeats)—DNA mini- and microsatellites Jeffreys et al. (1985a,b); Nakamura et al. (1987); Litt and Luty (1989) ● Mutation detection techniques <ul style="list-style-type: none"> ○ TGGE (Temperature gradient gel electrophoresis) Rosenbaum and Riesner (1987), Riesner et al. (1991), Henco et al. (1994) ○ DGGE (Denaturing gradient gel electrophoresis) Myers et al. (1989, 1990) ○ SSCP (Single strand conformation polymorphism) Orita et al. (1989)
Late 1990s	<ul style="list-style-type: none"> ● AFLP (Amplified fragment length polymorphism) Vos et al. (1995) ● DALP (Direct amplification of length polymorphisms) Desmarais et al. (1998)

particular stretch of DNA to be amplified, called the target sequence, is identified by a specific pair of DNA primers, oligonucleotides usually about 20 nucleotides in length. The quantities of produced DNA are sufficient to be directly visualised on a gel by fluorescence after coloration with stains such as ethidium bromide. This DNA may also be digested by restriction enzymes or be sequenced. An advantage is that because of the large amounts of produced DNA, some PCR-based methods eliminate the need for radionucleotides and long exposure times to produce a scorable signal by autoradiography.

The main interest for population biology is that it is now possible to work with a very small initial amount of DNA (virtually, one cell is sufficient). The method is not necessarily destructive; the sample may be very tiny, preserved in ethanol or buffer, or dried. It is possible to work on museum samples (Higuchi et al., 1984, 1988; Ellengren, 1991) or even, under certain conditions, on fossils (Pääbo, 1989; Austin et al., 1997).

The main molecular markers used are given below.

Allozymes:

- Allozyme electrophoresis is used to identify different protein alleles by their rate of migration through a gel in an electric field. “New” alleles (consequence of mutation of the coding gene) can be detected. Allozymes are almost invariably codominant so that

heterozygotes are phenotypically different from homozygotes; consequently, the calculation of gene frequency is simple. These frequencies are then used to compare populations. Numerous individuals can be analysed at relatively low cost. However, the number of polymorphic loci (= gene positions within a chromosome) and alleles (= variants) per locus is often too low to characterise all genetic patterns or to assign parentage with confidence.

DNA sequencing:

- Sequencing: Nucleotide sequences of DNA or RNA offer the greatest genotype resolution as these methods survey the nucleotide themselves. Sequences can be obtained either by direct sequencing of genomic DNA using specific primers to amplify, via PCR, targeted regions of the genome, or by cloning such regions and sequencing from the cloned fragment. The most commonly used sequencing method is the Sanger dideoxy method (Sanger et al., 1977; Avise, 1994; Ferraris and Palumbi, 1996; Hillis et al., 1996; Page and Holmes, 1998). The Sanger method is the basis of most automated sequencing, which is presently the preferred method for sequencing.

- Nuclear genes: Single locus nuclear genes are particularly useful in detecting functional polymorphisms and population structure. Some nuclear genes have multiple copies in the genome. Ribosomal DNA repeats have been used extensively for systematic studies (Hillis and Dixon, 1991). They are easily assayed. Many coding gene regions are conserved but flanked by nonconserved spacer regions allowing for reliable amplification of the gene region over a broad taxonomic range. The spacers often show variation at the individual and population levels offering information on population structure and levels of gene flow.

- Mitochondrial DNA: The cytoplasmic mtDNA occurs in high copy numbers. It is inherited from the female parent so that each copy is identical. It offers insight into sex-biased population structure. mtDNA is highly variable and is now classically used in population biology. It has greatly contributed to the establishment of phylogeography (Avise et al., 1987). An increasing number of studies use coding regions of mtDNA (e.g. cytochrome *b*) or noncoding ones that are very variable and are implicated in the initialisation of replication and transcription of the mitochondrial DNA. “Universal” primers (Kocher et al., 1989) make possible their amplification by using PCR and their direct sequencing in an increasing number of phyla.

- Polymorphism induced by insertion (transposons): Genomes are under continual bombardment by DNA segments from a variety of sources. DNA segments have been transferred between genomes both between organisms (horizontal gene transfer) and between organelles within organisms (inter-organellar gene transfer). Mobile DNA elements are 2000 to 10000 bp sequences present in multiple copies per genome. They move from place to place within the genome, directly as DNA or via RNA intermediates—so-called retrotransposons (Finnegan, 1989).

Other methods of detecting DNA sequence variations:

- Restriction fragment length polymorphism (RFLP): Restriction endonucleases (Linn and Arber, 1968; Kessler, 1987; Avise, 1994) are highly specific enzymes that cleave DNA wherever a particular nucleotide sequence occurs (usually 4–6 bp). When the DNA is digested with such an enzyme, it is cut into fragments. Different individuals may produce a different number of restriction fragments that are separated by electrophoresis. Restriction

sites are gained or lost through mutation. The presence or absence of the restriction fragments of a given length are treated as character states. Two common methods for documenting variation in both single-copy nuclear DNA (scnDNA) and mitochondrial DNA (mtDNA) are RFLP analysis and DNA sequencing. RFLP on DNA fragments generated by PCR can be visualised directly. The obtained information concerns the variation of profiles of cleavage by restriction enzymes of the region of DNA and the variability of the sequence of DNA (different rates of evolution and/or heredity are measured). These techniques apply in population biology where many individuals can be analysed.

● Rapid mutation analysis (single strand conformation polymorphism (SSCP); denaturing gradient gel electrophoresis (DGGE); temperature gradient gel electrophoresis (TGGE); heteroduplex analysis (HA)): Rapid methods of detecting DNA sequence differences without having to sequence the fragments include various gel separation techniques, among which are DGGE (Myers et al., 1989, 1990) and SSCP (Orita et al., 1989). The DGGE method detects mutations (small deletions and insertions, point mutations) by separating PCR amplified DNA fragments, which differ from wild-type DNA in their melting behaviour, on a denaturing gradient gel. DGGE results in high probabilities of detection of DNA sequence differences, but requires special equipment to regulate temperature, and/or the pouring of gradient gels. For occasional use, these requirements can be prohibitive, and for large-scale screening, the accumulated costs are high. As to the alternative gel-based techniques, SSCP has the advantage that standard sequencing equipment can be used. SSCP relies on conformational intrastrand differences in DNA of different sequence. The mobility of single-strand nucleic acid molecules, electrophoresed under non-denaturing conditions, is determined by both their fragment length and their secondary structure, which is sequence dependent. A fragment may adopt several conformations for any given set of electrophoretic conditions and these are visualised as separate bands in the gel. Some or all of these bands may show a shift in a mutated sequence, a single base change being sufficient to alter secondary structure and hence mobility (see Potts, 1996). The basic idea of TGGE is to use heat as a source of energy to make the hydrogen bonds thermodynamically unstable. DNA or RNA fragments with point mutations will show a different melting behaviour (due to different melting temperature: T_m) and thus different conformation compared to wild type DNA. By applying a temperature gradient during the electrophoretic separation of DNA and RNA, fragments of identical length but different sequence can be separated. TGGE allows the differentiation of alleles by their different T_m . A typical application is a heteroduplex analysis. The DNA of a putative heteroallelic sample that contains both wild type *AA* and mutant *aa* is amplified by means of PCR. An additional denaturation and rehybridization step leads to four different kinds of DNA duplexes. Besides the homoduplexes *AA* and *aa*, two heteroduplexes *Aa* and *aA* are formed. Both of these heteroduplexes contain a mismatched base pair, which lowers the T_m of these DNA strands. When these samples are separated in a temperature gradient applied in the running direction, up to four different bands can be visualised. In contrast, the wild type or a homoallelic mutation will result in only one single band. Any mismatches in the heteroduplex DNA will cause it to have a different three-dimensional structure to the homoduplex DNA and therefore a lower mobility on polyacrylamide gels. The reduced mobility is proportional to the degree of divergence of the sequences (Delwart et al., 1993).

Polymorphism induced by size variation (number of copies):

- DNA fingerprinting: The use of multilocus probes makes it possible to reveal hypervariability within an individual's genome by developing a DNA fragment band pattern. These banding patterns are usually unique for each individual except identical twins. To be genetically interpretable, the number of bands produced has to be limited (Jeffreys et al., 1985b). Multilocus DNA fingerprinting has been the primary method to study mating systems (Jeffreys et al., 1985b; Burke, 1989). It is considered to permit the exclusion of offspring from putative parents and also to allow the assignment of excluded offspring to extra pair-parents (Burke et al., 1989). Single locus DNA fingerprinting is similar but uses single loci with variable number of tandem repeats (VNTR). This technique may be applied to both mini- and microsatellites. However, as for RAPD, due to the very large number of visualised loci, there are still difficulties in interpretation.

- Variable number of tandem repeats (VNTR): Relatively small tandemly repeated sequences have been found to be dispersed throughout the genome of all analysed eukaryotes. Some, referred to as *minisatellites*, have repeat lengths of several tens to a few hundred base pairs (Jeffreys et al., 1985a,b; Nakamura et al., 1987). *Microsatellites* are considered to have repeat lengths of one to some base pairs (Litt and Luty, 1989). There is a strong variability of the number of repetitions to such a given locus that corresponds to a relatively high mutation rate (errors of replication, unequal crossing over, polymerase slippage, gene conversion). Size variants are detectable by the position of the bands in a gel. This allows detection of a large numbers of alleles per locus.

- Random amplified polymorphic DNA (RAPD): Short arbitrary sequences of nucleotides can be used to amplify random fragments of DNA. This technique produces a large number of fragments, many of which are individual-specific (Welsh and McClelland, 1990; Williams et al., 1990). If inheritance is verified, RAPD patterns can be also used for population genetics (Williams et al., 1990). However, there is a possibility of dominance of RAPD markers that may hide genetic variation and the smaller fragments are usually not visualised. They can be ill suited for the characterisation of breeding systems or the calculation of genetic parameters, or phylogenetic inferences (Grosberg et al., 1996). Cluster analysis allows identification of parents while minimising the effects of artifactual fragments (Levitan and Grosberg, 1993).

- Amplified fragment length polymorphism (AFLP): This approach overcomes some of the disadvantages of many of these earlier techniques as it involves both the restriction digestion and PCR, and thus is rapidly becoming very popular among genome scientists (Vos et al., 1995). The primary reason for rapid acceptance of AFLP technology is its ability to detect a large number of polymorphic DNA markers rapidly and in a reproducible manner. In fact, AFLP involves the detection of “presence” or “absence” of restriction fragments rather than differences in their lengths.

- Direct amplification of length polymorphisms (DALP) (Desmarais et al., 1998): This approach uses an arbitrarily primed PCR (AP-PCR) to produce genomic fingerprints and to enable sequencing of DNA polymorphisms in virtually any species. Oligonucleotide pairs were designed to produce a specific multi-banded pattern, and all the fragments thus generated can be directly sequenced with the same two universal M13 sequencing primers. This strategy combines the advantages of a high-resolution fingerprint technique and the possibility of characterising the polymorphisms.

There are important differences in characteristics among marker types (Fig. 2). Moreover, there are also species-specific differences in polymorphism and inheritance of markers. Most molecular markers represent variation in noncoding DNA regions. It is generally assumed that variation expressed by these markers is essentially selectively neutral. Markers linked to QTL (quantitative trait loci—Falconer and MacKay, 1996) or microsatellites may not be neutral. Molecular markers differ in the amount of variability they display. The general picture is that allozymes are less variable than RAPD or RFLP markers, which are less variable than microsatellites or minisatellite fingerprints (Fig. 2). Markers can be classified as either codominant (banding pattern of homozygotes can be distinguished from the patterns of heterozygotes) or dominant (banding patterns of heterozygotes are identical to banding patterns of one of the homozygotes). Codominant markers (allozymes, microsatellites, most RFLP) allow estimation of allele frequencies in populations. Despite the fact that they are codominant, the typically large number of bands generally resulting in minisatellite fingerprints makes it very difficult to estimate allele frequencies. Dominant markers (RAPD, AFLP) allow estimation of genotypes but not of allele frequencies. Markers differ in their mode of inheritance. Nuclear DNA is bi-parentally inherited. Mitochondrial DNA (and chloroplast DNA) is often uni-parentally inherited. The latter is not subjected to recombination during the process of sexual reproduction.

Molecular techniques are more expensive than most ecological or morphological approaches to the study of genetic or species diversity. Nucleotide sequencing tends to be one of the most expensive methods of surveying genetic diversity. The tradeoffs

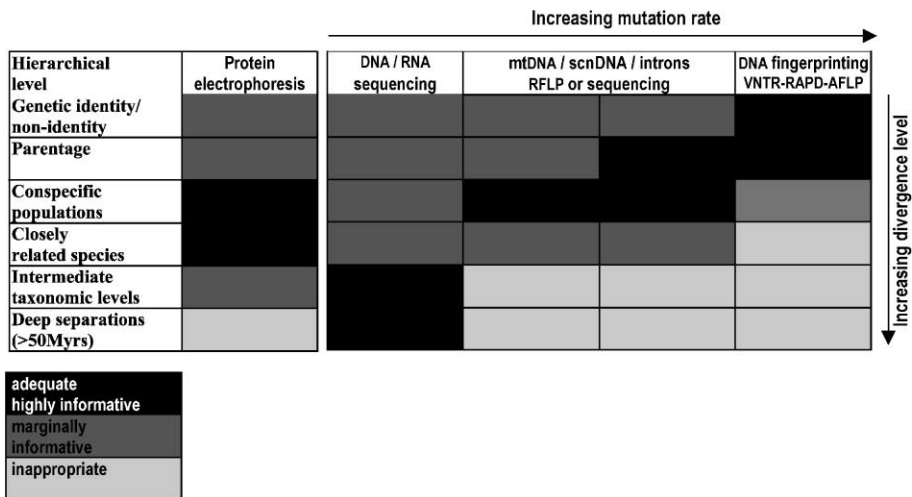


Fig. 2. Choice of molecular markers available to determine parentage depending on the level of evolutionary divergence (cf. Avise, 1994; Thorpe and Smartt, 1995; Hillis et al., 1996; Ferraris and Palumbi, 1996) [AFLP: amplified fragment length polymorphism; mtDNA: mitochondrial DNA; RAPD: random amplified polymorphic DNAs; RFLP: restriction fragment length polymorphism; scnDNA: single copy nuclear DNA; VNTR: variable number of tandem repeats].

between cost, amount of genetic material surveyed and desired resolution all depend upon the questions being addressed. The use of some techniques may be appropriate but are unlikely to be cost effective (Avice, 1994; Thorpe and Smartt, 1995; Hillis et al., 1996). Fig. 2 gives the levels of evolutionary divergence at which various molecular genetic methods normally provide informative phylogenetic markers.

4.1. Analysis and interpretation of molecular data

This aspect will not be developed here. Details of the analytical methods of population genetics and phylogenetics that can be applied to molecular data can be found in Futuyma (1986), Felsenstein (1988), Hartl and Clark (1989), Weir (1990), Avice (1994), Slatkin et al. (1995), Ferraris and Palumbi (1996), Hillis et al. (1996), Page and Holmes (1998), and Swofford (1999).

Another type of problem to face is understanding the relations between molecular evolution and the principles governing evolution at the phenotypic level. An indication of the potential evolutionary response of a character to environmental changes is given by the concept of heritability, which is a measure of the genetic diversity affecting a quantitative character, morphological, physiological or behavioural [= characters affected by numerous loci] (Falconer and MacKay, 1996; Hartl and Clark, 1989). Breeding studies make it possible to make inferences about the genetic basis of quantitative characters. It is still difficult to establish relationships between variability at the genetic and phenotypic levels. However, such studies may provide means of predicting the distributions of phenotypes among offspring from the parental phenotypes. The chance for success in identifying QTL is much improved by selecting Mendelian markers that may have a functional relation to the trait (candidate genes). Such approaches will underline the regions of the genome going through strong selective constraints, either for themselves, or in interaction with others, in particular in the situations where the populations are subjected to genetic tensions such as adaptive crises, bottlenecks or hybrid zones.

Owing to the tremendous increase in the mass of data that can be gathered with molecular tools, and to the high information content of hypervariable loci allelic frequency vectors, the whole field of data processing is changing rapidly. Probabilistic methods are now becoming possible by modern desktop computer capabilities that allow the study of the statistical significance of complex parameters for which no theoretical distribution is analytically available. Computing intensive methods such as Maximum Likelihood inference and other multidimensional techniques are developing fast, they provide modern tools for the numerical study of genetic biodiversity.

5. Examples of uses of genetic markers for infraspecific biodiversity assessment

5.1. Population structure as a function of dispersal systems and spatial constraints: gene flow and speciation in a dispersive environment

In the ocean, geographic heterogeneity of gene pools has often been overlooked, because larval dispersal and marine currents would a priori ensure a thorough mixing of

genes. However, reality is often somewhat different, with levels of differentiation much higher than one would have thought (see above). It therefore becomes important to study for a growing number of cases the relationship between hydrology, larval behaviour and differential adaptation to environmental conditions.

A certain fraction of this differentiation, as may be evidenced by genetic studies, probably corresponds to differential adaptation to environmental variations (Avisé, 1994). For the mollusc *Ruditapes philippinarum*, it has been possible to relate the polymorphism of a lysosomal enzyme (the leucine aminopeptidase 1) to the activity rate of different allozymes. Individuals carrying the *Lap*⁹⁵ allele show stronger catalytic activity for this enzyme and are thus more able to resist hyperosmotic shock. The frequency of the *Lap*⁹⁵ allele varies from 42% for the Bailleron site (a high-salinity site in the Gulf of Morbihan, South Brittany), to 25.2% for the Kédreán site (a variable-salinity site in the Gulf of Morbihan) to 14.7% for the Lanveur site (a variable-salinity site in Brest Harbour). The results obtained for the *Lap* locus among *R. philippinarum* indicate the predominance of abiotic factors (such as salinity) rather than geographical distance where the genetic structure of populations is concerned (Moraga, personal communication).

Among the cupped oyster *Crossostrea gigas*, the selective action of agricultural pesticides (atrazine and isoproturon) has been brought to light by experiments in the controlled environment of the laboratory. These pesticides cause differential mortality rates in the same way as other micropollutants in the marine environment. The findings permitted the identification of two groups of individuals, one “sensitive” and the other “resistant”, each of which is characterised by the presence of specific genotypes for individual loci (Moraga and Tanguy, 1997).

5.2. Phylogeography and historical biogeography of marine ecosystems

Another part of genetic differentiation simply reflects the fact that genes are not instantaneously reshuffled throughout the species range, and that there are some biological or physical obstacles to panmixia. Not many case studies have provided a detailed analysis of these two types of factors. However, it would be particularly interesting to census the zones of strong genetic gradients, such as may be the case in a straight like Gibraltar (cf. Borsa et al., 1997b).

Such places may be used as natural laboratories to understand the factors explaining the maintenance of genetic differentiation in a dispersive milieu. Studies of these zones of transition could thus constitute a focal topic likely to gather ecologists and geneticists in the same effort. The faunal relationships of the Atlantic and the Mediterranean have been affected by tectonic change in the Miocene, by climate change in the Pleistocene and by hydrological discontinuity related to, e.g. glaciations. A population genetics approach to the biogeography of the Mediterranean marine fauna, and in particular to description of the level of recent or present-day exchanges with the Atlantic, is useful. The level of genetic differences between populations was estimated from published and unpublished data on 16 species from the northeastern Atlantic and the Mediterranean by Borsa et al. (1997b). For a majority of species, either tropical or subtropical or boreal fishes, or coastal invertebrates, it revealed a moderate to strong genetic cline between each side of the Gibraltar strait area. Such a pattern was also observed in a proportion of species between each side of the Sicilo-

Tunisian strait. In a limited number of cases, the geographic patterns of population genetic structure, however, conformed with the expectations of an isolation-by-distance model. The molecular phylogeographic approach in a boreal species, the flounder *Platichthys flesus*, led to the inference of historical movements of colonisation and geographic isolation of the populations (Borsa et al., 1997a). A similar approach used in a cosmopolitan species (the grey mullet *Mugil cephalus*) and a tropical species (the Spanish sardine *Sardinella aurita*) revealed close genetic relationships between Mediterranean and west-tropical Atlantic populations (Crosetti et al., 1994; Chikhi et al., 1997, 1998).

Phylogeographical analysis, by Garcia de Leon et al. (1995, 1997) shows that sea bass (*Dicentrarchus labrax*) populations in the Atlantic and in the western and eastern Mediterranean are clearly differentiated between the two basins (the Strait of Gibraltar area appearing as a distinct geographical barrier on a line between Almeria and Oran). Genetic diversity, here revealed by microsatellite DNA markers, is created and blended on a spatial scale much smaller than might have been expected, given the species' capacity for migration and larval dispersal. Nevertheless, exchanges between lagoonal and marine populations are sufficient for the latter to remain undifferentiated as far as neutral markers are concerned, whereas certain genes subject to selection could have their frequencies modified because of the differential mortality rates that occur during juvenile life in brackish waters (Allegrucci et al., 1994, 1997).

Another example is the molecular phylogeography of *Mytilus* based on a new marker gene. The mussels of the genus *Mytilus* are flagship species for population biology and the ecology of marine organisms (Levinton and Koehn, 1976; Raymond et al., 1997). The current systematics of the complex *Mytilus edulis* recognises three species described primarily on the basis of allozymic frequencies, and characterised by a remarkable infra-specific homogeneity. It is difficult to match the current worldwide distribution of the populations of *Mytilus* to a coherent biogeographic scenario. Research in progress tries to elucidate the phylogeographic relations of *Mytilus* using hypervariable nuclear markers. The results recently obtained starting from the data on the intron length polymorphism of the actin gene locus mac-1 tend to call in question certain conclusions based on allozymic data (Ohresser et al., 1997; Daguin and Borsa, 1999). Thus, *Mytilus* sp. of the Antarctic Ocean (Kerguelen Islands, Tasmania) present affinities with *M. edulis*, not with *M. galloprovincialis*. It could have colonised the southern hemisphere starting from the trans-equatorial migration of a proto-*edulis* population. The recent introduction of *M. galloprovincialis* into the northwestern Pacific is confirmed: indeed, *Mytilus* stocks of Korea present close affinities with the populations of *M. galloprovincialis* of the Adriatic. The data of mac-1 also make it possible to look further into the analysis of the geographical distribution of *M. edulis*, *M. galloprovincialis* and their hybrids along the European coasts. The gradient of introgression between the two species does not seem to follow any geographical logic, which is contrary to a traditional clinal model. It is a zone of genetic mosaics.

At a greater observation scale, one should consider as a priority the reconstitution of the historical aspects of the building up of extant biodiversity in the world ocean. Genes' geography and molecular phylogenies have shown their remarkable performance in this matter on the continental domain. These biogeographical reconstructions allow a better understanding of the impact on genetic diversity of the palaeoclimatical changes

frequently recorded during the various glacial episodes. They allow also to address numerous questions such as the impact of larval dispersal or the origin of vicariance in a dispersive environment.

In the Antarctic and sub-Antarctic zones, the majority of the benthic species of marine invertebrates are brooding species, in all the phyla. The assumptions, which consider brooding as an adaptation to the polar conditions, are unsatisfactory (Clark, 1992; Pearse, 1994; Féral et al., 1994; Poulin and Féral, 1994). The problem of the prevalence of brood protection in Antarctica actually comprises two quite different questions: What is the adaptive significance of brooding? and which are the factors responsible for the evolutionary success of brood protection in Antarctica? It was shown that brood protection has effects on isolation and genetic differentiation within a species of sea urchin in Kerguelen (*Abatus cordatus*). Genetic differentiation was estimated to be on the order of a 10-km distance, according to a stepping stone model of dispersal between small, dense and isolated populations (Poulin and Féral, 1995, 1998). A model based on the difference of the speciation/extinction relationships between brooding species vs. species with a pelagic phase has been proposed to explain the prevalence of the brood protecting species in the Southern Ocean and their great present diversity (Poulin and Féral, 1996, 1997).

Here again, studies in the oceanic realm lag somewhat behind, and it is striking to see that the biogeographical zones most widely spread at the planet's surface, such as the Indo-Pacific, is still almost completely unknown as to the history of their biodiversity and the phylogeography of the species which inhabit them (Avice, 1994; Palumbi, 1996). The same applies to wide area of the World Ocean whose relationships are poorly understood (Antarctic fauna, temperate faunas, Atlantic/Pacific relations or Mediterranean/Atlantic through the quaternary fluctuations in particular). Of great interest are the opposite effects of two "mega-experiments" (in terms of inter-oceanic canals): (1) the first relates to the closing of the isthmus of Panama and the impact on the biodiversity of the two resulting tropical provinces and (2) the other, much more recent, is related to the opening of the Suez Canal and to the migrations known as *lessepsian migrations* towards the Mediterranean.

5.3. Functional and adaptive aspects of polymorphism: larval phase and genetic control of recruitment

As mentioned before, every bit of genetic differentiation occurring between species or between populations has first appeared within a population as inter-individual polymorphism. The extant biodiversity is thus first sieved by genetic drift and selection inside a population before it can exist on larger scales. Therefore, it is at this more basal level, where coding information and diversity are generated, that one should focus attention first. Actually, this is where evolutionary novelties are tested against each other by their relative contribution to individual fitness, and this is the intraspecific biodiversity, which conditions the survival of a species in the face of pathogens or environmental changes. One of the sensitive steps during which this selective sieve occurs is recruitment. In marine species, recruitment undergoes temporal fluctuations, which are the target of many studies made by ecologists or population dynamicists to model the abundance of a resource. Independently of these fluctuations of trophic origin, there is growing evidence for the

occurrence of detectable genetic differences from one recruited cohort to the other, especially in species with very high fecundity. It therefore becomes important to know if this corresponds to the differential success of young recruits, or if it reflects a reproduction fragmented in small patches of genitors.

One may also observe fluctuations of genotypic frequencies in time or in response to certain pathogens. These fluctuations, such as those reflected for instance by the heterozygosity/fitness relationship in many invertebrate species, are the signature of differential mortalities. One touches here the very significance of biodiversity in terms of adaptation, and it is possible that some species utilise these genetically determined differential mortalities, made possible by the very high fecundities associated with a free larval stage, as a mode of instant local adaptation. Significant progress in our understanding of the underlying mechanisms may result from the fine-grained monitoring of polymorphism now allowed by modern molecular techniques.

Heterozygote deficiencies have been often encountered in natural bivalve populations. Multilocus heterozygosity (MLH) for allozymes has been positively correlated with fitness-related traits in juveniles or adults, such as growth or survival (Gaffney, 1994) while nuclear RFLP loci failed to produce significant correlation (Zouros and Pogson, 1994). The option of working with PCR on larvae only a few hours old has opened up various avenues promising new developments in the genetics of early development and recruitment. Bierne et al. (1998) studied the descent of consanguineous crossings (full-sibs) of the oyster *Ostrea edulis* using four microsatellite loci. The distribution in classes (shell length quartile) of MLH shows no early heterozygote deficiencies at the youngest stage and a highly significant heterozygote excess for the bigger size classes. A strong selection was operating during the larval stage (differential mortalities). Under the hypothesis that microsatellites loci are neutral, these results suggest that these markers cosegregate with fitness-associated genes (FAGs [cf. QTL, see above]). Thus, a high genetic load has been evidenced for *O. edulis*, a fact which it would be tempting to relate with the overdominance (direct or associative?) that has been abundantly demonstrated in bivalve populations (David et al., 1995; David, 1998). Moreover, this study also highlighted a growth/heterozygosity relationship between the beginning and the end of the larval growth. This makes possible to definitively refute the assumption of alternative selection between larval and adult stages, which had been put forward to explain the deficits in heterozygotes frequently observed in bivalves.

It is not possible to predict the response of natural populations to a major change in the environment. A species may respond in a variety of ways that do not require genetic evolution. This may be considered as another field using molecular markers in relation to the understanding of functional aspects of biodiversity and permitting the comprehension of phenotypic plasticity. One may consider it as a particular character that has its own genetic basis and has an adaptive and evolutionary significance. The goal will be to quantify plasticity and to draw response curves in a gradient of environments for different genotypes within a population. It will also lead to understanding how evolutionary strategies of species favour either plasticity or genetic variability.

Variability in neutral sequences might not be sufficient to represent evolutionary potential of a species. Thus, both neutral and selected variability should be taken into

account. Some of the questions to consider are: Do specific genes of plasticity exist? Does a relation between phenotypic variability and characters linked to fitness exist (survival, reproductive potential)? What are the physiological and developmental mechanisms that produce these phenotypes? What are the roles of environmental factors? How to interpret and model plasticity? These problems do not only concern marine biodiversity. However, large-sized marine populations of species with very high fecundity are well suited for such approaches.

6. Genetic markers, management and conservation issues

6.1. Assessment of biodiversity

The first step to assess biodiversity is to identify species. In that field, molecular techniques, including also cytogenetics (Thiriou-Quévieux, 1994) and flow cytometry (Partensky et al., 1997), provide a suite of methods for quantifying the phylogenetic relatedness between species and higher taxa, recognising intraspecific genetic variability, defining species limits, identifying and quantifying difficult-to-identify species such as microbes (see Burton, 1996).

Molecular tools led to the recent discovery of an unsuspected reservoir of diversity: the eukaryotic photosynthetic picoplankton (see Partensky et al., 1997). In the ocean, a very large proportion of photosynthetic biomass takes the form of microalgae below 3 μm in size (picoeukaryotes). Picoeukaryotes obtained from cultures have their representatives in most algal classes, but the diversity of natural stocks is not properly appreciated. By combining conventional observation methods, such as electron microscopy, with tools of molecular biology (gene sequencing), it is possible to unambiguously identify species that are notoriously difficult to study because of their minuteness and fragility. This strategy has made it possible, in samples taken from the Pacific Ocean and the Mediterranean, to discover new species, including a new class of algae related to the Diatoma, the Bolidophyceae, whose distribution is being studied with the help of molecular probes. Furthermore, research on the genetic diversity of natural samples from the oceans shows that species obtained from cultures makes up only a small percentage of in situ diversity (Guillou et al., 1999). Recently, powerful molecular methods have been brought to identify often microscopic larvae of marine invertebrates (Olson et al., 1991; André et al., 1999; Comtet et al., 2000).

A complication in making any assessment of genetic biodiversity arises with the existence of (unsuspected) cryptic species. This seems to be a particular problem in certain taxa of marine invertebrates where the adult lacks useful morphological characters. These invertebrates are sessile or have low mobility and the larval stage is of short duration and low dispersal capability. Their apparent distribution is often circumglobal. However, genetic examination often reveals that ostensible cosmopolitan species include a number of distinct and sometimes not even closely related cryptic species. Sibling species continue to be discovered even in extremely well studied or economically important genera such as the annelid *Capitella* or the bivalve *Mytilus* (Knowlton, 1993; Daguin, 2000).

6.2. Monitoring marine biodiversity: invasive species and aquaculture escapees

These aspects are linked to the previous series of examples, but they are also the consequences of human activities. Marine invasions may involve large, conspicuous, and easily identified species. However, most invasions are cryptic because of the incompleteness of marine systematics. Invading species may not be properly identified, may be undescribed, or erroneously redescribed as native species or may resemble native species. Most invasions are caused by the passive transport of nonnative species. This is the case of plankton in the ballast water of ships. Much of it, especially meroplankton, cannot be identified at the species level. Aquariology has had the same kind of impact. The tools of molecular systematics are well suited to approach these problems by identifying potential invaders, correlating invading adults and larvae, pointing out evolutionary change in invaders, reconstructing the history of invasion or detecting cryptic invasions (Geller, 1996).

The increasing development of marine aquaculture will make it necessary to evaluate the genetic impact of the escapees entering the natural environment (Féral, 2000). Accidental genetic pollution may happen due to the transport of reproductively active adults or of juveniles of large-scale cultivated species such as, in Europe, sea bass, sea bream, turbot, oysters or mussels. The techniques used to study the genetic structure of a species and its phylogeography are obviously well suited to also identify such impacts, without drawing conclusions about negative or positive effects. Freshwater fishes are of most concern as escaped transgenic species, which are organisms into which heterologous DNA has been artificially and stably integrated into their genomes (Chen et al., 1996). They will also be easily identified in the natural environment, using molecular techniques.

6.3. Long-term and short term-planning/gene conservation and molecular ecology

Contribution of molecular genetics to conservation has been stressed during the last decade (e.g. Avise, 1989, 1994; Vrijenhoek, 1989; Woodruff, 1989; Solbrig, 1991; Moritz, 1994; Burton, 1996). However, one must underline that the molecular methods are generally inefficient as a force for rebuilding depleted populations on a time scale of interest to humans. They are of little relevance to immediate conservation needs and are of lower priority than demographic analysis (Lande, 1988; see also Moritz, 1994).

In fact, different time scales have to be considered. Long-term planning involves phylogenetic information (gene conservation). Evolutionarily significant units (ESU) (Ryder, 1986) are a set of populations with a distinct evolutionary (long term) history. This fits well with the goal of recognising and maintaining genetic biodiversity (Moritz, 1994). Phylogeographic structure may provide significant help to identify ESUs and assess conservation value (= conservation priority of taxa or areas from an evolutionary point of view). On a different time scale, genetic markers may be a tool for ecologists to define the appropriate geographic scale for monitoring and management, to identify the origin of individuals (a challenge at sea, i.e. a dispersive milieu containing indirect developing species [larval stage] and migratory species [imaginal stage]) and to detect changes in population size and connectedness. One of the best examples of application is given by fisheries, which a long time ago recognised that species consist of a number of different

stocks (= management units (MU)) that respond independently to harvesting and management. In contrast to ESU, MU are best defined by significant divergence in allele frequencies, regardless of the phylogeny of alleles. Allele frequencies respond more rapidly to population isolation than to phylogeographic patterns and permit the definition of genetic tags (Ovenden, 1990; Dizon et al., 1992; Moritz, 1994). Molecular approaches to stock assessment permit the comparison of information provided by inherited vs. acquired markers (such as physical tags). They also permit the distinction between evolutionarily deep vs. shallow population genetic structures (Awise, 1994).

6.4. Heterozygosity and demography

Awise (1994) summarised this topic: “Most discussions of genetics in conservation biology concern terrestrial environments. They have centred on the topic of heterozygosity or related measures of the within-population component of genetic variation. Indeed, heterozygosity is exceptionally low in many rare or endangered species, presumably because of genetic drift and inbreeding accompanying severe population reductions. However, in most cases, causal links between heterozygosity and fitness have proven difficult to establish firmly”. As previously underlined, some people think that behavioural and demographic concerns should take priority over heterozygosity issues in management programs for endangered species (Lande, 1988).

The habitat of marine species, especially coastal ones, is rarely continuous. The structural and qualitative variations of the environment (space-time heterogeneity of the environment) impose a heterogeneous spatial distribution, whose expanse is accentuated under the effect of anthropic action. Populations of a species are subdivided in more or less homogenous sub-assemblies whose functioning and pressures of selection may differ. In natural environments, small population size increases the rate of loss of genetic diversity, which can lead to a reduction in fitness. When closely related individuals interbreed, their offspring are often less fit than the parents (inbreeding depression). The converse situation is heterosis whereby offspring of unrelated parents tend to be more fit than those of related parents (hybrid vigour). This leads to a genetic structuring (neutral diversity vs. selected diversity, consanguinity and demographic consequences) AND a demographic structuring (viability), suggesting that in reality, both concerns are important to consider along with the evolutionary functioning (e.g. coevolution of dispersion with other life history traits such as fecundity or longevity).

Acknowledgements

I am very grateful to Anne Chenuil, François Bonhomme and Sigurd von Boletzky for reading the manuscript and for helpful discussions. Thanks are due to S.v. Boletzky who also kindly helped to improve the English text, and to the anonymous referees for valuable comments. Most of the case studies mentioned in this review were partly financed by the *Réseau Diversité Marine* (French National Program on Biological Diversity-PNDB/Marine Diversity Network) and by IFRTP (French Polar Institute).
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