ICES CM 2002 / Theme session U: New Developments and Applications of Genetics in Fisheries Management and Aquaculture

Population genetics of the European flat oyster (*Ostrea edulis)*: from larvae to populations.

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ABSTRACT

PCR-based DNA markers enable the study of diversity and differentiation of marine organisms at different stages of their life cycle. Our study of European flat oyster (*Ostrea edulis*) populations has aimed to describe their spatial and temporal dynamics. First, we analyzed adults sampled over the natural range of the species. An Atlantic/Mediterranean differentiation pattern was observed. Similar patterns of variation were found using allozymes, microsatellites and mitochondrial DNA, all which support an isolation by distance model. High variability was observed in the range of diversity between populations using a mitochondrial marker, a reflection of small effective population sizes in some locations. A 10-fold quantitative difference was observed in *Fst* between the genomes, which may be due to an unbalanced sex ratio and/or differential reproductive success. Secondly, we initiated the study of reproductive dynamics at a finer scale. Several experiments were performed to document (1) the variance inallele frequencies during a natural settlement period, (2) the paternal contribution to fertilization by analyzing larvae sampled at the brooding stage within individual females, (3) the individual reproductive success within an experimental population. Our results are relevant in terms of management of the genetic diversity and the potential impact of hatchery propagation.

Keywords : Flat oyster, *Ostrea edulis*, population genetics, molecular markers, diversity, recruitment.

INTRODUCTION

Knowledge of the spatial and temporal distribution of genetic variability of a species is one of the essential step for proper management of this resource. This present distribution is the result of many biological (adult and gamete dispersal, mating…) and historical (demographic variation due to natural or anthropic pressures) factors that population genetics help to document. Genetic variation can be studied at different spatial and temporal scales: from individuals to the whole range of the species, and from yearly recruitment to the long term changes related to climatic variation.

The European flat oyster (*Ostrea edulis* L.) is a marine bivalve whose natural geographical distribution ranges along the European Atlantic coast from Norway to Morocco, in addition to the Mediterranean and Black Sea. It has furthermore been introduced into many other parts of the world (e.g. USA, Canada, Japan…) because of its aquacultural potential (Korringa 1976). The first genetic studies (Blanc et al. 1986;Jaziri et al. 1987; Johannesson et al. 1989; Le Pennec et al. 1985; Saavedra et al. 1987; Wilkins and Mathers 1973) showed low levels of intra-population polymorphism and inter-population differentiation. More comprehensive studies of allozyme differentiation over the whole natural range (Jaziri, 1990; Saavedra et al.,1993, 1995) concluded that, although overall differentiation was small, a significant divergence existed between Mediterranean and Atlantic populations. Jaziri (1990), working with a limited number of populations, observed lower variability in the Atlantic stocks and suggested that the Mediterranean Sea had been a refuge from which the Atlantic stock subsequently stemmed during Pleistocene glaciations. Saavedra et al. (1993; 1995) observed clines in allelic frequencies for some loci on either side of the Strait of Gibraltar. They suggested that this pattern resulted from secondary contact of the two stocks (Atlantic and Mediterranean) at Gibraltar after separation during the last glaciations.

To have a more precise view of the population genetics of this species, we analysed its geographical genetic structuring using microsatellite and mitochondrial (12S) markers, andcompared our results to previously published allozyme data. The results have led us to initiate studies at a finer scale in order to estimate the effective number of breeders and the temporal dynamics of recruitment. *O. edulis* is a viviparous species : after internal fertilisation and ten days of incubation, females liberate larvae which feed on plankton for a further ten days before fixation on substrate. Genetic variation of spat collected during a relatively short period was compered with those of adults. Finally, parentage analyses of larvae collected from brooding females were performed to estimate the number of males fertilising each female.

MATERIAL AND METHODS

Sampling

Adults : Fifteen populations of *Ostrea edulis* were sampled along the European Atlantic and Mediterranean coasts, and in the Black Sea (14 to 50 individuals per sample)(fig. 1). Populations were chosen that either had not been commercially exploited in the recent past, or were harvested from local stocks. When possible, we tried to avoid populations with a documented history of imports from foreign stocks (MacKenzie et al. 1997). We used the coding convention proposed by Saavedra et al. (1995): AN : Atlantic, Northern part of the study area; AS : Atlantic, Southern part of the study area; MW : Mediterranean, West; ME : Mediterranean, East; and BS for the Black Sea sample.

Juveniles: Collectors were deployed to collect "cohorts" of juveniles in two sites: Sète (MWb) and Quiberon (ASa). In Sète, the collectors were placed during two weeks in spring 1994 (Launey, 1998). In Brittany (ASa), 3 sets of collectors were successively deployed every two weeks and one set during the whole recruitment period in 2001.

Larvae: Oysters were sampled in Sète during summer 2001. Three brooding females (i.e. females presenting larvae in their paleal cavity) were found and their larvae sampled and preserved in ethanol.

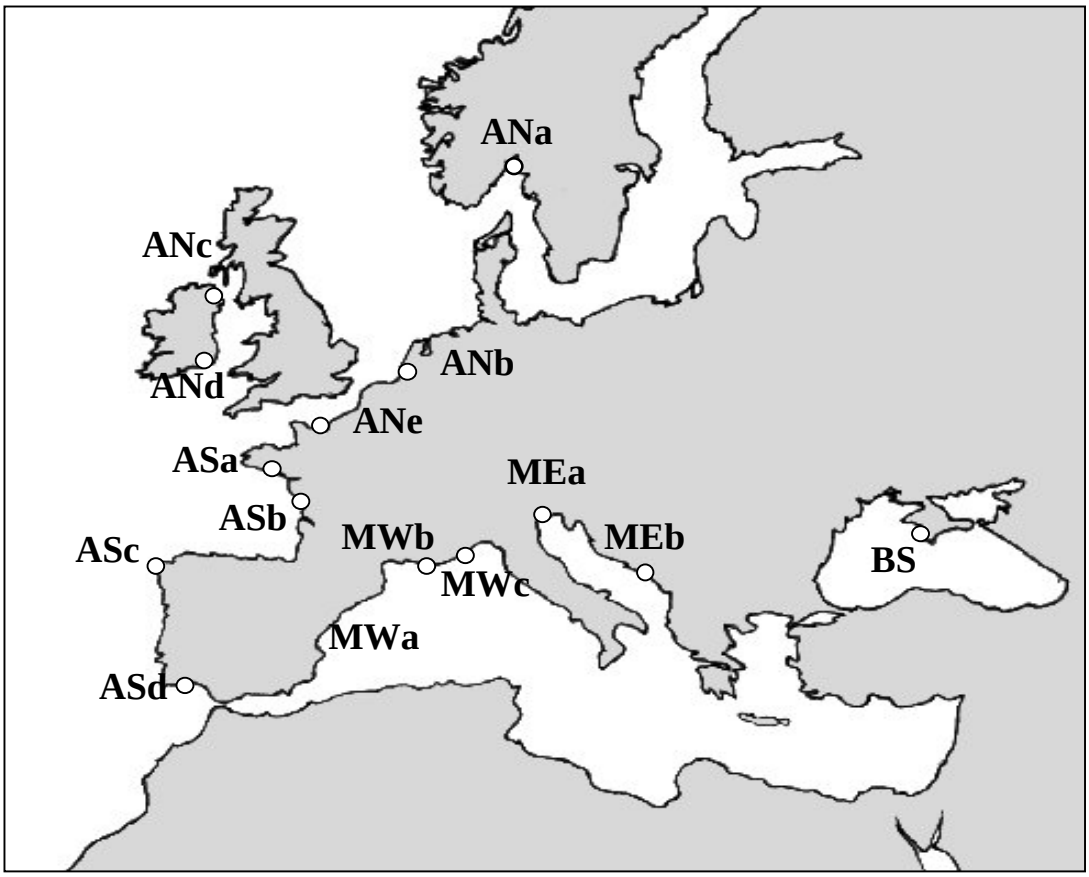

Figure 1: Location of the sampled populations.

Genotyping

Microsatellite markers: DNA was extracted with a rapid procedure using Chelex® (Biorad) adapted from Estoup et al. (1996). A small piece of gill tissue was heated at 55°C in 150 µl 5% Chelex, 15 µl TE (Tris EDTA), 10 µl proteinase K (Eurogentec, 10 mg/ml). Samples were then boiled for 10 minutes, centrifuged for 5 minutes (4000 rd/min), the supernatant was collected and kept frozen (-20°C) before use. PCR reactions were performed in a 10 µl reaction mix containing 2 µl template DNA, 1.5 µM $MgCl_2$, 75 µM each dNTP, 0.25 µM γ^{33}P-labelled forward primer, 0.4 µM reverse primer, 0.35 units of Goldstar Licensed Polymerase (Eurogentec) and 1X polymerase buffer (supplied by the manufacturer). Amplifications were processed as follow: pre-denaturation (94°C-2 minutes) followed by 30 cycles of denaturation-annealing-polymerisation (94°C-1 min, T_a-1 min, 72°C-1 min 15 s) and a final elongation step (72°C-5 min). T_a is the optimal annealing temperature for each pair of primers (Table 1). Amplification products were analysed on 7M Urea, 6% polyacrylamide gel using individuals of known genotype as size markers.

Table 1: Characteristics of the five microsatellite loci used in this study. T_a is the optimal annealing temperature.

Locus	Repeat motif	Size range of PCR product	T_a (°C)	Primer sequences[2]
*Oedu*H15	$(ATCT)_n$ [1]	165-225	50°C	H15-R TAA TGA TTT CGT TCG TTG AC
				H15-F TTT TGA CTC TGT GAT ATC GAC
*Oedu*J12	$(GT)_{14}$	216-272	50°C	J12-R TCG TCA CCT CCC TCT CAG AG
				J12-F GCT GTA TTT CCA TCA ATT CGA G
*Oedu*O9	$(GA)_{36}$	140-176	53°C	O9-R ACT TCA ATG TCT GTT CTA ATG G
				O9-F ATT CAA TTG ATT TTA GGT TGG
*Oedu*U2	$(AC)_{21}(AG)_7$	146-206	50°C	U2-R GAA AGA AAT GGA GGC AAT AAC
				U2-F ACC AAT GAA CAC AGA TCA CC
*Oedu*T5	$(CA)_{15}$	106-166	55°C	T5-R TAG TGA ATG GTC TTG CAT TCC
				T5-F2 CTT CGT TCT TGT ACG TAA GCG

[1]Full sequence of the repeat : $(ATCT)_4ATGT(ATCT)_2ATGTATCTATATATCTATGT(ATCT)_5A(TACC)_4AATTTTTCT(ATCT)_3$

[2]GenBank accession number : AF310009 – AF310015

Mitochondrial marker: The polymorphism of a 313bp fragment from the mitochondrial 12S rRNA gene was analysed. When universal 12S rRNA primers (Kocher *et al.*, 1989) were used on *O. edulis* genomic DNA, several fragments were amplified. Specific primers were subsequently designed on the basis of alignment of several sequences obtained with samples of this species. Sequences of new primers are: 12SOeduF: 5'-GAGCAGCTGCTTAAAACTCG-3', and 12SOeduR: 5'-GTTAATCTCCCTTTACTCCC-3'. Amplification was performed with 2.5mM $MgCl_2$, 200µM of each dNTP, 1µM of each primer, 0.7 U of TAQ polymerase and 10X PCR reaction buffer. PCR was carried out with an initial denaturation step at 95°C, followed by 30 cycles of amplification (denaturation at 95°C for 1 min, annealing at 54°C for 1 min and elongation at 72°C for 1 min) and a final elongation step at 72°C for 7 min. PCR products were screened for mutational differences by SSCP analysis (Single-Strand Conformation Polymorphism). The amplified fragments were denaturated at 95º during 7 minutes before being run by electrophoresis in a 10% 37.5:1 acrylamide /0.6X TBE gel, 0.4 cm tick at 30W (150mA, 200V), maintained at 5ºC during 14 hours. DNA single strand bands were observed by staining the gel with an ethidium bromide solution (10mg/l) for 20 minutes. At least two samples of each SSCP class were sequenced in order to verify that different SSCP variants were characterized by distinct DNA sequences. Sequencing reactions (35 cycles of 30" at 95ºC + 30" at 60ºC + 1' at 72ºC) were performed forward and reverse with ^{32}P radioactive primers on purified DNA PCR products. Sequence products were separated in a high resolution 6% 29:1 acylamid/0.5X TBE gel. Sequences were revealed by autoradiography of dried gels.

Data analysis

Microsatellite markers: For each population and locus, we calculated the number of alleles (Na), the observed heterozygosity (Ho), and the expected heterozygosity (He) according to Nei's unbiased estimate (Nei, 1978). One-tailed Mann-Whitney tests (Sokal and Rohlf, 1995) were used to determine whether the monolocus genic diversities are higher for the Mediterranean populations than for the Atlantic ones, as suggested by Jaziri (1990). Mean F_{is} (resp. F_{st}) were computed over loci and/or populations according to Weir & Cockerham's estimators, using GENETIX 4.0 (Belkhir et al. 1996-2001). Significance level were assessed

by permutation of the alleles (resp. multilocus genotypes) within populations (resp. across populations). Reynolds' genetic distance (Reynolds et al. 1983), was calculated using the PHYLIP 3.57 software package (Felsenstein 1989). The distance matrix was visualised as a Neighbor-Joining tree (Saitou and Nei 1997, using PHYLIP). Robustness of the nodes of the unrooted tree obtained was assessed by bootstrapping over loci. Correlation between geographical and genetic distances was estimated by a Mantel test using GENETIX. Geographical distances were measured along the coast lines; when different routes were possible, we opted for the most likely, according to the principal current flow in the area. Fst/(1-Fst) was used as a measure of genetic distance between each pair of population, as suggested by Rousset (1997). For allozyme data, pairwise Fst values were retro-calculated from pairwise Reynolds' genetic distance provided in the initial publication (Saavedra et al. 1993, C. Saavedra pers. comm., Launey et al., in press).

Both the number of alleles and the heterozygosity were used to compare the genetic variability of newly recruited spat to that observed in adults. A population at the mutation-drift equilibrium was set up following the sampling theory of Ewens in a way that our observed sample could originate. This was performed using a procedure programmed by K. Belhir and K. Dawson, (CNRS, Station Méditerranéenne de l'Environnement Littoral, Sète, France). The equality of the means for each parameter between samples was also tested (Sokal and Rohlf, 1995).

The number of male that fertilised the sampled brooding females was computed using PARENTAGE (Emery et al., 2001), taking into account the genotype of each female.

<u>*Mitochondrial marker*</u>: Nei's unbiased haplotypic diversity was estimated by the *Hnb* modified for haploid genome. 95% Confidence intervals for each population sample were obtained after 1000 bootstrap re-sampling of individuals as performed by Genetix 4.02. Sequences alignment was performed using the software CLUSTALW. Parsimony analysis was implemented with PHYLIP 3.57 using the program DNAPARS. Bootstrap analysis was performed using SEQBOOT (100 replicates) and CONSENSE programs from the Phylip package. Differentiation between populations was assessed using Wright's fixation index *Fst*, estimated by *Theta* (Weir and Cockerham, 1984) with GENETIX 4.02 software package. An analog of *Fst*, *Φst* (Excoffier et al, 1992),which takes into account divergence between haplotype sequences was estimated between pairs of populations using ARLEQUIN 1.0 software package. A hierarchical classification was constructed from Reynolds' genetic distances (D= -ln (1- *Fst*) ; Reynolds et al.,1983) between samples by using the Neighbor-joining method (Saitou and Nei, 1987) as implemented in the Phylip 3.57 software Package (Felsenstein, 1989). Correlation between genetic (as *Fst*/(1-*Fst*) and *Φst*/(1-*Φst*)) and geographic (as measured along the coast and accounting for principal current flow in each area) distance matrices was tested with a Mantel non-parametrical permutation test, as implemented in GENETIX 4.02.

RESULTS

Variability within adult populations

<u>*Microsatellite markers*</u>: Populations showed high levels of polymorphism (mean number of allele/locus/pop = 18.5 ± 4.5, mean He = 0.914 ± 0.018). Mediterranean populations (including the Black Sea sample) were more polymorphic than Atlantic populations (mean ± standard error : 20.6 ± 4.2 vs. 17.2 ± 4.2 alleles/locus/pop, $p<0.001$). Thirteen out of the 15

samples showed an overall heterozygote deficiency, as indicated by significantly positive values of the multilocus F_{is} . Heterozygote deficiencies were especially high for the OeduH15 locus since heterozygote deficiencies remained significant in only 4 samples when OeduH15 was discarded from the analysis.

Mitochondrial marker: SSCP Analyses of a 313bp 12S rRNA fragment revealed 14 haplotypes (named with capital letters). “A” and “B” haplotypes are present in all samples except in the Black Sea (BS), and show a frequency shift between Mediterranean and Atlantic seas: the “A” haplotype was the most common in the Atlantic ocean, whereas the “B” haplotype was more frequent within Mediterranean samples. Black sea (BS) and Norwegian (ANa) samples presented peculiar haplotypic compositions, with their principal haplotypes being “I” and “C” respectively.

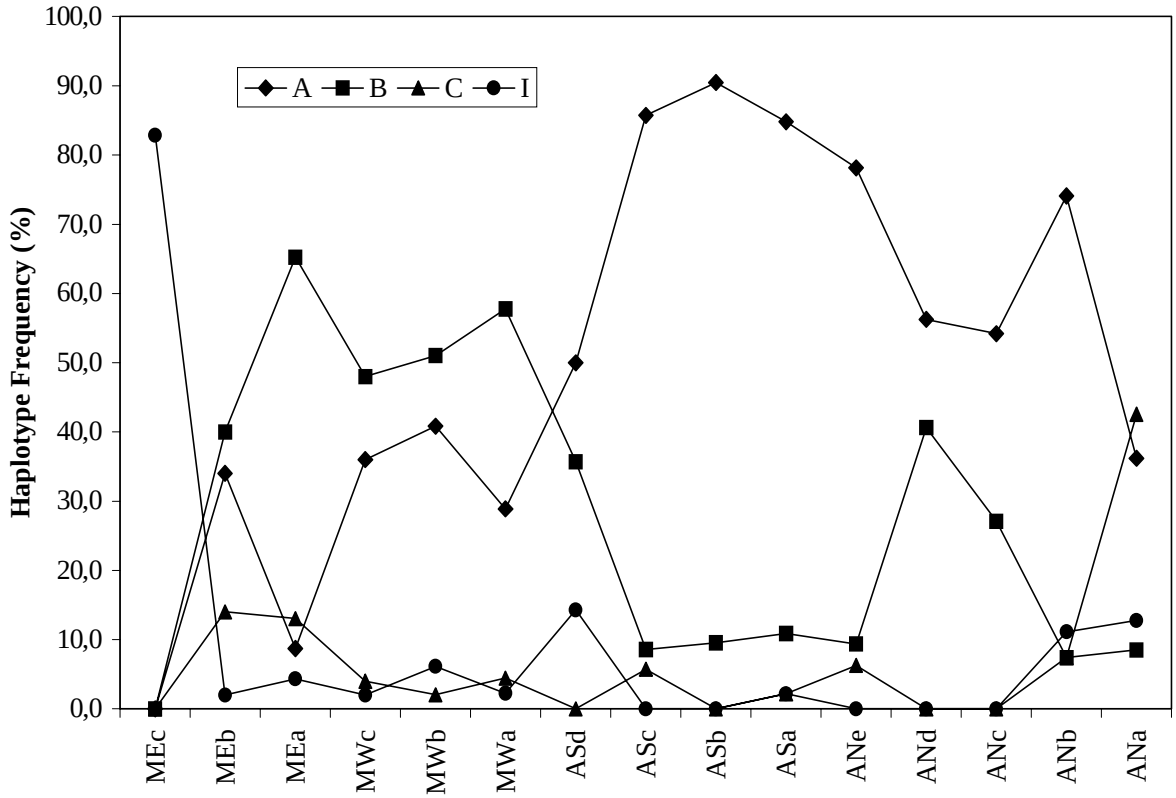

Figure 2. Frequencies of the more frequent haplotypes

The sequences of the haplotypes harboured 19 substitutions.The parsimony methodenabled the definition of three different groups of haplotypes : group I+C (including haplotypes I,N,M,C and E), group B (B and rare haplotypes F,J,H), and the less defined group A, with unclear roots (A and rare haplotypes L,S, G and D)(Fig. 3).

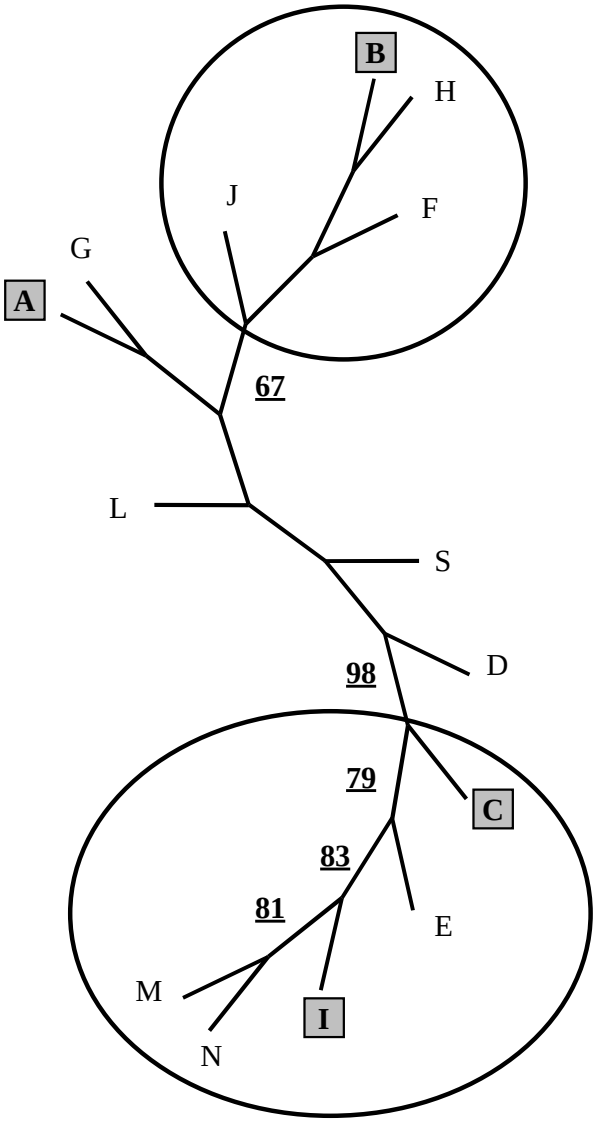

Figure 3. Consensus tree based on the mitochondrial 12S rRNA sequences (Bootstrap values are indicated on the branches).

Average mitochondrial haplotypic diversity was almost twice as low (*Hnb* = 0.49±0.17) as microsatellite genotypic diversity (*Hnb* = 0.91±0.02). The diversity observed for mitochondrial marker was much more variable (Fig.4). The lowest levels of variability were found in the Black Sea (BS) and on the South Atlantic coasts (for example ASb). These last samples showed a significantly lower *Hnb* than those found in Mediterranean samples and in some North-Atlantic samples. This was not true for the Portuguese sample, that shows a high diversity, despite its low sample size (ASd, n = 14 : *Hnb* = 0.6243).

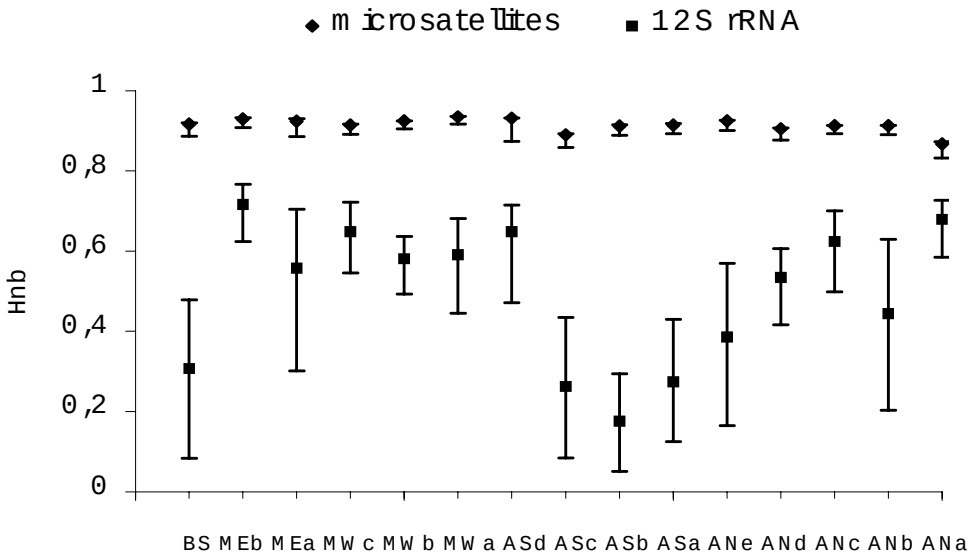

Figure 4. Nei's diversity calculated in the populations on the basis of the nuclear microsatellites data and the mitochondrial 12r RNA data.

Differentiation among populations

Microsatellite markers: The global multilocus estimate for F_{st} was 0.019. This estimate is significantly different from zero ($p<0.001$). The different loci did not contribute equally to the inter-population differentiation, monolocus F_{st}s ranging from 0.009 ($p<0.001$, OeduU2) to 0.030 ($p<0.001$, OeduH15). The overall pattern of genetic differentiation is summarised by the Neighbor-Joining tree (Figure 5) together with the allozymic tree obtained from Saavedra et al. 1995. The populations are clustered according to their geographical origin, with an Atlantic cluster, a western Mediterranean cluster (including the ASd population from Portugal) and an eastern Mediterranean cluster (including Black Sea). Apart from the northernmost population, Atlantic populations are not significantly differentiated from each other. The populations from the Adriatic and Black seas, however, are divergent from every other population. Note the intermediate position (between Atlantic and Mediterranean populations) of the samples from Thau (ASb), which can be linked to transplantation of Atlantic farmed stocks known to have occurred there (Goulletquer and Héral 1996).

Mitochondrial marker: Differentiation between populations obtained with the mitochondrial marker was estimated to be 0.224 ($p<0.001$). Although the mitochondrial marker shows differentiation levels ten fold greater than those found using microsatellites, both markers indicate very congruent population structure patterns, with significant differentiation between Norway and Black sea samples, and between Mediterranean and Atlantic samples. The central sample of Ria Formosa (ASd) only shows significant *F*st values with more external samples (ANa and MEb with microsatellites and BS with 12S rDNA). Similar patterns are also observed in comparing population genetic distance dendrograms obtained with each marker including the allozymes (Fig. 5).

Isolation by distance: Regression of $F_{st}/(1-F_{st})$ over coastal distances in km showed a positive correlation between genetic and geographical distances for allozyme, microsatellite, and

mitochondrial markers (Launey et al., in press) (fig. 6). The slope of regression was similar between allozymes and microsatellites when two of the allozyme loci (presumed to depart from neutrality) were removed from the analysis (Launey et al., in press). The correlation remained significant when the three eastern Mediterranean populations were omitted from the sample ($r = 0.39$, $p \leq 0.018$), indicating that the correlation between geographical and genetic distances is not an artefact caused solely by the strong differentiation of the eastern Mediterranean samples. Similarly, the correlation remains significant when we omit the locus showing the highest interpopulation differentiation, OeduH15 ($r = 0.362$, $p < 0.05$). However the slope of the regression for the mitochondrial marker was much higher than those of nuclear markers. This correlation is maintained even if the most differentiated external populations from Norway and the Black Sea are excluded.

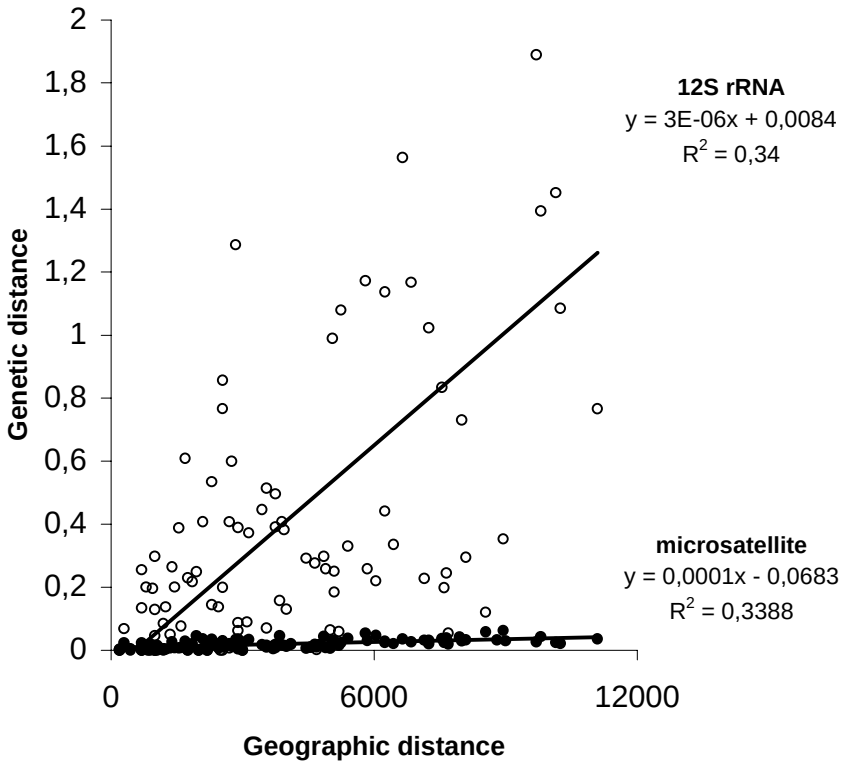

Figure 4. Regression of genetic distance versus geographic distance.

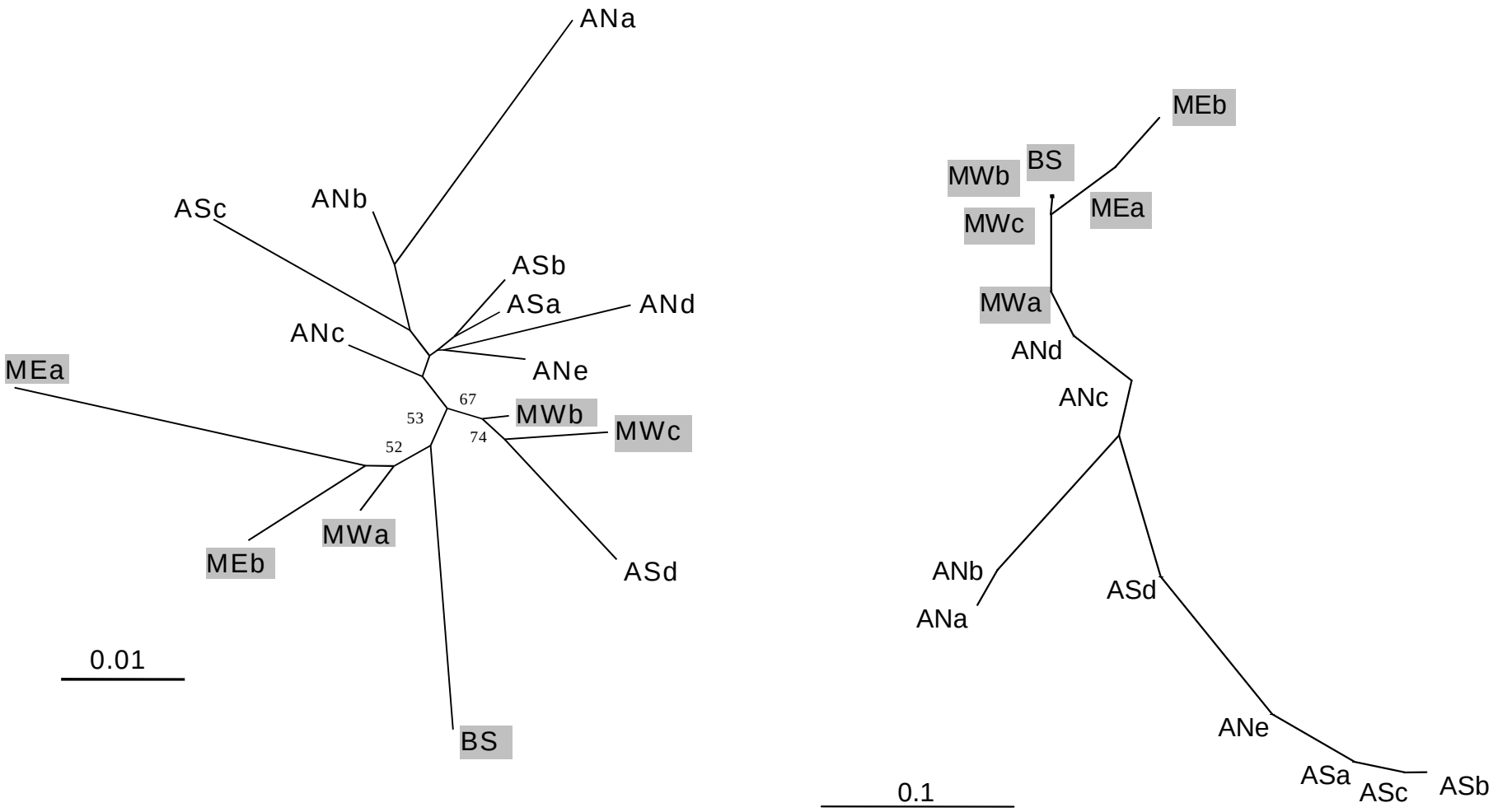

Figure 5: Nei's diversity calculated in the populations on the basis of the nuclear microsatellites data (left) and the mitochondrial 12r RNA data (right)

Genetic variability of spat

Mediterranean population: the individuals that settled during two weeks in spring 1994 showed a significantly lower variability that the local adult population. In this cohort of juveniles, the mean number of alleles per locus was 16.4 while it was 21.8 in adults (Launey *et al*., in prep). Based on the reduction in the number of alleles, the estimations of the number of parents that have contributed to this cohort estimates were less than 50 parents (Launey *et al*., in prep).

Brittany population: The variability of the four cohorts recruited in the Quiberon Bay in spring 2001 is presented in Table 2. When compared with the adult population and among them, no differences are observed in terms of heterozygosity, number of alleles and *Fst* values.

Table 2: Genetic variability observed in the adults and the four cohorts recruited in Quiberon bay.

	Adults	Cohort 1	Cohort 2	Cohort 3	Cohort 4
Nb. Alleles/locus	20.8	21.3	21.9	21.8	21.3
Hob	0.79	0.83	0.84	0.85	0.82
Hnb	0.88	0.90	0.90	0.90	0.90
Fis	0.125*	0.087*	0.081*	0.073*	0.096*

Parentage analyses of brooding females

Larvae of three brooding females, sampled in Sète during summer 2001, were analysed using four microsatellite markers. The number of alleles observed at these markers is presented in table 3. The estimated numbers of fertilising males for each female were low, ranging from 1 (in FM1) to 8 (in FM3).

Table 3: Genetic variability (number of alleles per locus) and estimated number of fertilising males observed in three brooding females sampled in Sète.

Female	Microsatellite marker				Number of
	*Oedu*T5	*Oedu*J1	*Oedu*H15	*Oedu*O9	fertilizing males
FM1	5	5	4	5	3 to 4
FM2	3	3	3	3	1
FM3	4	5	6	ND	6 to 8

DISCUSSION

We studied the genetic diversity in *Ostrea edulis* at different scales. First, the geographic differentiation was analysed using microsatellite and mitochondrial markers. These data were compared to the results already published using allozyme markers (Jaziri 1990; Saavedra *et al.* 1993, 1995). Results obtained by both type of markers are highly congruent, although their overall interpretation may differ from what has been proposed previously. Several interacting factors, such as relative male and female reproductive success and their variability, could

account for the distribution of the genetic variability in *Ostrea edulis* as revealed by nuclear and mitochondrial markers.

Intra-population variability

Nuclear markers: As expected, microsatellites reveal a much higher level of intra-population genetic variability than allozymes. All microsatellite loci are highly polymorphic, with a mean of 18.5 alleles per locus and per population, in contrast to 1.8 to 2.8 alleles per allozyme locus (Jaziri et al. 1987; Saavedra et al. 1995). Gene diversity for microsatellite loci is very high (He = 0.930) vs. 0.176 ± 0.052 for allozyme loci (Saavedra et al. 1995). These results confirm the high levels of polymorphism already observed for the flat oyster with other microsatellite markers (Naciri et al. 1995) and for the Pacific oyster *Crassostrea gigas*, with gene diversity higher than 0.8 (Magoulas et al. 1998; Huvet et al. 2000a,b). Similar levels of variability have been found in many other marine species (DeWoody and Avise, 2000). They are usually attributed to large effective population sizes, in turn related to the larger and more continuous nature of the marine environment.

Mitochondrial vs. nuclear markers:12S rRNA diversity was highly variable between populations. Although microsatellite diversities were shown to be significantly higher for the Mediterranean populations when compared to the Atlantic ones (Launey et al., in press), they appeared less variable than those observed with the mitochondrial marker. Since cytoplasmic markers have an increased sensitivity to drift due to a low effective population size (generated by their haploid nature and their maternal transmission), a population may loose its mtDNA variability during a bottleneck and still retain a significant fraction of its nuclear variability. Indeed, data on the genetic variability of spat recruited over a short period of time in Sète show that these juveniles were generated by a limited number of effective breeders. However, the dynamics of recruitment might differ between Brittany and the Mediterranean Sea (the variability of the recruited cohort being higher in Quiberon Bay than in Sète) and it is noticeable that the favorable period for reproduction is sometimes very short in the Atlantic (especially for the northernmost populations), and usually much longer in the Mediterranean. Nevertheless, our data suggest a higher variance in effective sizes in the Atlantic (and therefore a reduced variance N_e), than in the Mediterranean.
Another possible explanation deals with oyster parasites (*Marteilia refringens* and *Bonamia ostreae*) which have had a more critical effect on *O. edulis* stocks in the Atlantic than in the Mediterranean. Although some areas may have encountered recent population reductions because of the two parasitic epizooties, this may better be put forward to explain the lower variability observed along the South Atlantic coast (AS populations) rather than a severe bottleneck. Unfortunately, there is no precise data available about how these epizooties have affected natural population densities. We think however that this is rather incidental since, if the recruitment of natural spat from this species has been strongly reduced and the French production of flat oyster dropped by a 20x factor since the 1960s, it is still 2500 tonnes/year at present**.**

Inter-population differentiation

Nuclear markers: The genetic structure of *Ostrea edulis* populations has probably been influenced both by long-term evolutionary history and present and past human activities, which are not always easy to separate (for review see MacKenzie et al., 1997). Nevertheless, our data are consistent with a model of isolation by distance, which is apparent in both distance trees, where the populations are roughly ordered according to their geographic origin.

As already shown by Borsa et al. (1997), previously published allozyme data (Saavedra et al. 1993; 1995) also show a positive correlation with geographical distance. Unlike the conclusions drawn by Saavedra et al. (1995), our results failed to show an obvious genetic discontinuity between Atlantic and Mediterranean flat oyster populations. Therefore, it does not seem that Saavedra's et al. conclusion, that the actual structuring of genetic diversity could be explained by a secondary contact between two ancient Atlantic and Mediterranean stocks, has very strong support based on our analysis, neither from the stand point of allozymes nor from the microsatellites.

Mitochondrial vs. nuclear markers: There are several studies that analyse population structure with both mtDNA and microsatellite makers. Some present consistent results between both markers (e.g. Tessier et al. 1997 in populations of *Salmo salar*). Others report quantitative but not qualitative differences. For example, Hansen *et al*. (1999) observe in whitefish populations (*Coregonus sp.*) mitochondrial *F*st 3 folds greater than microsatellite *F*st as well as differences on population trees. This is the same case in the present study, but mtDNA *F*st is ten fold greater than microsatellite *F*st. In addition to the intrinsic differences between mitochondrial and nuclear effective population sizes (Ne), there are other life-history elements that could reduce female effective population size and thus mitochondrial Ne. In the European flat oyster, sex-ratio within a reproductive season is male biased (attaining up to a 3:1 ratio). Furthermore, female gonad development is slower (Ledantec *et* Marteil, 1976) and more energy demanding. Moreover, *Bonamia ostreae* induce high mortality mainly within two- and three-year old adults (Culloty and Mulcahy, 1996). As *O. edulis* is a protandrous species, the proportion of individuals that achieve to reproduce as females in a population may be reduced by the action of this parasite.

Another factor affecting the mitochondrial effective size is the variance in female reproductive success. If males succeed in fertilizing several females, variability in oceanographic conditions should increase female variance in reproductive success (Hedgecock, 1994) more than male variance in reproductive success, thus reducing mitochondrial relative to nuclear Ne. Our data on the genetic variability of single-female progeny show that the studied females are fertilized by a limited number of males. More investigations are needed to directly demonstrate that each male fertilize several females, but our data tend to support this hypothesis.

CONCLUSION

The observed distribution of the genetic variability on the flat oyster *Ostrea edulis* results from many factors acting at different spatial and temporal scales. At the individual level, females can be fertilized by a limited (down to one) number of males. Comparisons between obtained data using nuclear and mitochondrial markers clearly suggest that female effective population sizes are smaller than male ones. This seems to be particularly the case on the Atlantic coast. At the within-population level, our data on spat genetic variability suggest that the dynamics of recruitment might also vary between the Atlantic Ocean and the Mediterranean Sea. At the species level, makers consistently show a clear pattern of isolation by distance. In any case, significant F_{st} values are found, even at a rather small scale. At the same scale, gene diversities were also quite variable, showing that populations with different diversities may coexist in close proximity. This points toward the fact that, despite the possibility of larval dispersal, local stocks may be quite independent dynamically and harbor varied instantaneous effective sizes likely to shape the gene diversity they contain. We think that human activities (overfishing, stock transfer…) are unlikely to have had a significant impact on genetic variability and population differentiation in this species. However, this may

change if the production of hatchery propagated spat in this species develops in the future. This is likely to efficiently contribute to the sustainability of the aquaculture of this species but it should be managed in such a way that it will not have a negative effect on the local genetic variability.

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Population genetics of the European flat oyster (*Ostrea edulis*): from larvae to populations

P. Boudry[1], S. Launey[1,2], E. Diaz Almela [1,2], Y. Naciri-Graven[1], C. Ledu[1], S. Mira[1,2], N. Taris[1,2], F. Bonhomme[2] and S. Lapègue[1].

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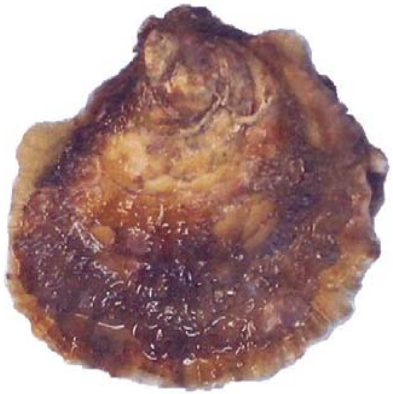
CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

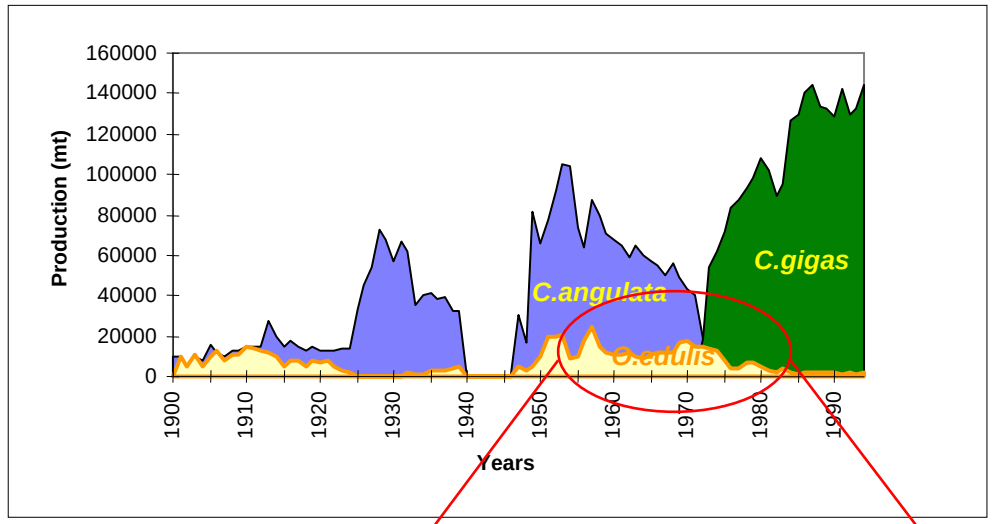
la science au service des hommes

Oyster production in Europe : three successively farmed species

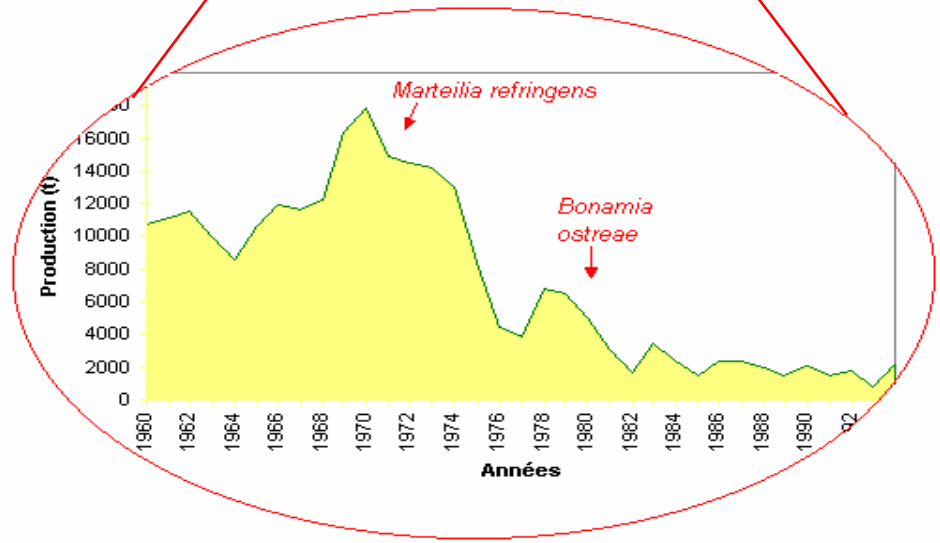
Ostrea edulis in 2001 :

World production = 6.400 t/year
French production = 2.500 t/year





Goulletquer and Héral, 19XX



Risks and benefits of hatchery propagated spat to enhance production

- **Breeding programs have been initiated to improve disease resistance** (Naciri-Graven et al., 1998; Culloty et al., 2001)

- **Hatchery populations usually have low *Ne*** (Hedgecock et al., 1992) **in *O. edulis* :**
  - ✓**Saavedra & Guerra (1996): *Ne* ≈ 4**
  - ✓**Launey et al. (2001): *Ne* = 3 to 20**

- **Today hatchery-based production is very limited**

- **Its development could have a <u>positive</u> impact in terms of aquaculture <u>but</u> might have a strong <u>negative</u> impact on genetic diversity of wild populations.**

O. edulis geographical range

(JAZIRI 1985)

O. edulis population genetics:

How is the genetic variability distributed...

… in space ?

- Within *vs* among populations ?
- Atlantic *vs* Mediterranean populations ?
- Isolation by distance ?

... among genomes ?

- Nuclear *vs* Cytoplasmic ?

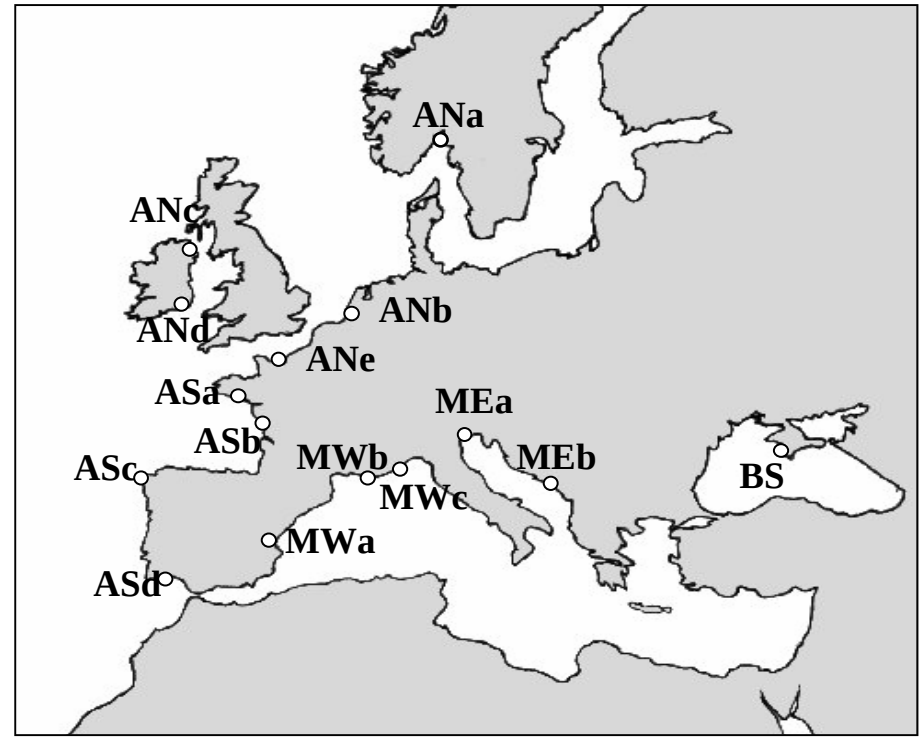
… over time and/or generations ?

- Adults *vs* juveniles
- Females *vs* larvae ?

Spatial distribution ?

Sampling:

15 populations sampled
14 to 50 individuals per location



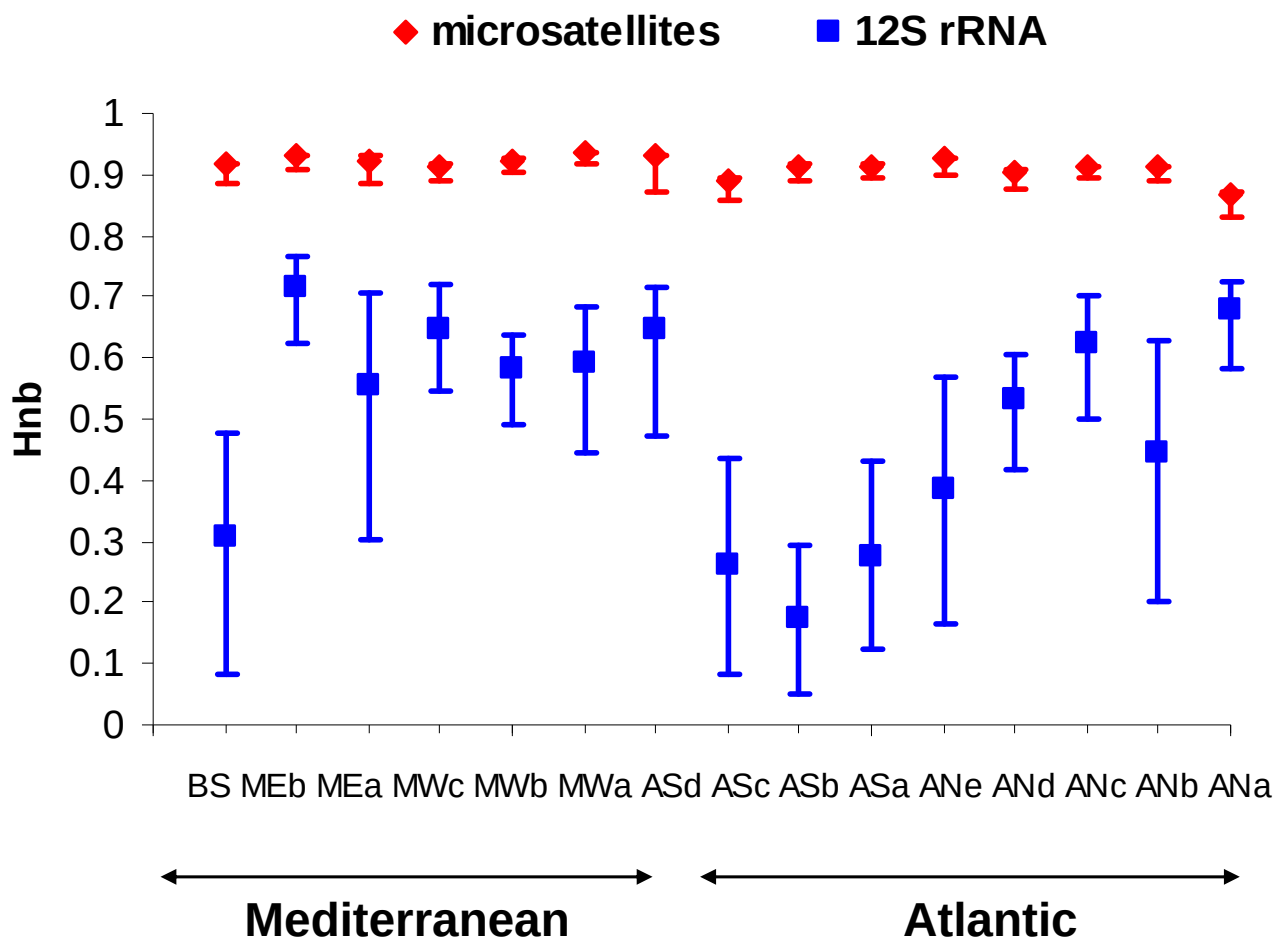
Markers:

- Allozyme loci (Saavedra *et al.*, 1993, 1995)
- microsatellite loci (Launey *et al*., in press)
- 12S rRNA SSCP (Diaz *et al*. in prep)

Within population diversity :

Microsatellites: allele/locus/pop = 18.5 ± 4.5
mean He = 0.914 ± 0.018

12S rRNA: 14 SSCP haplotypes

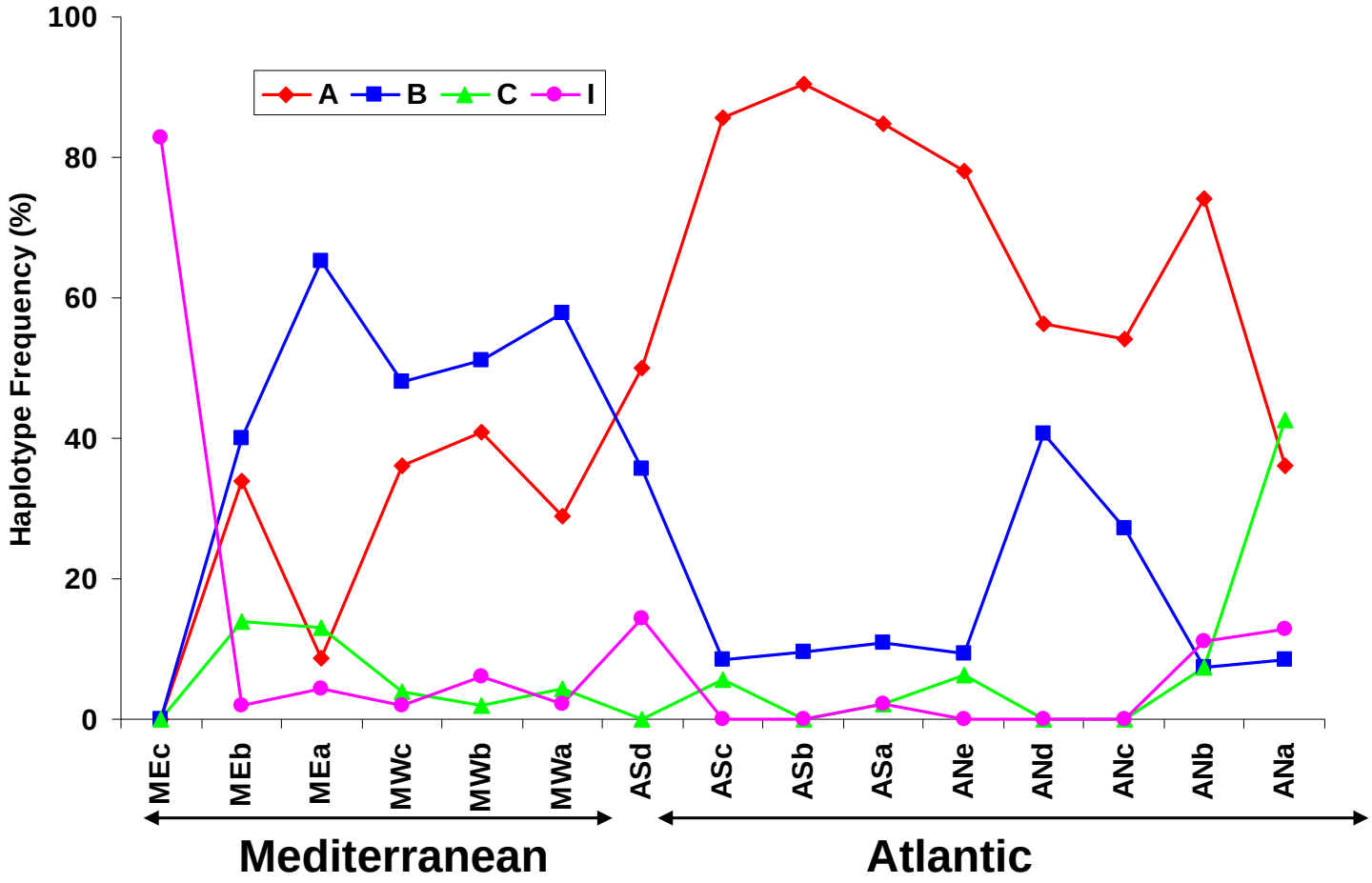


Among population differentiation :

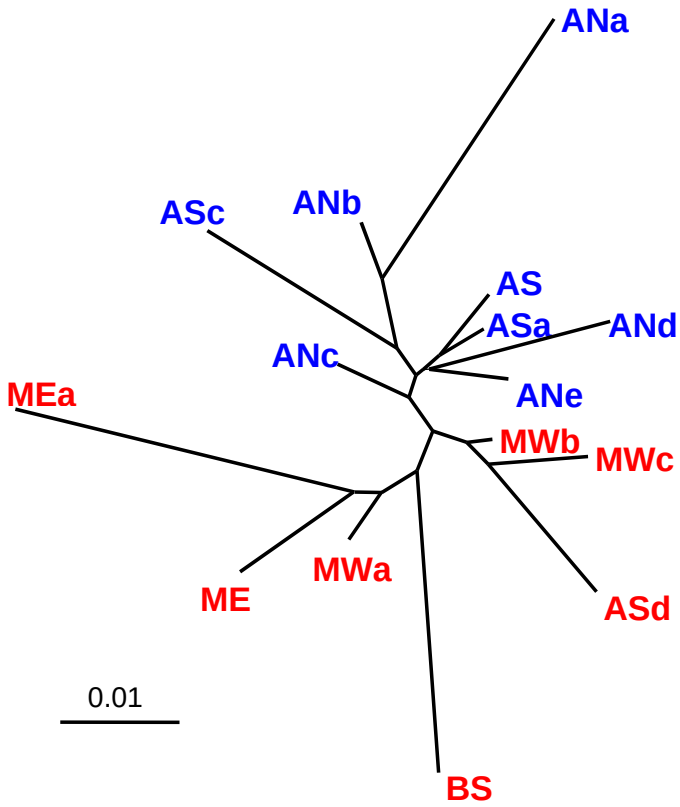
Microsatellites : *Fst* = 0.019***

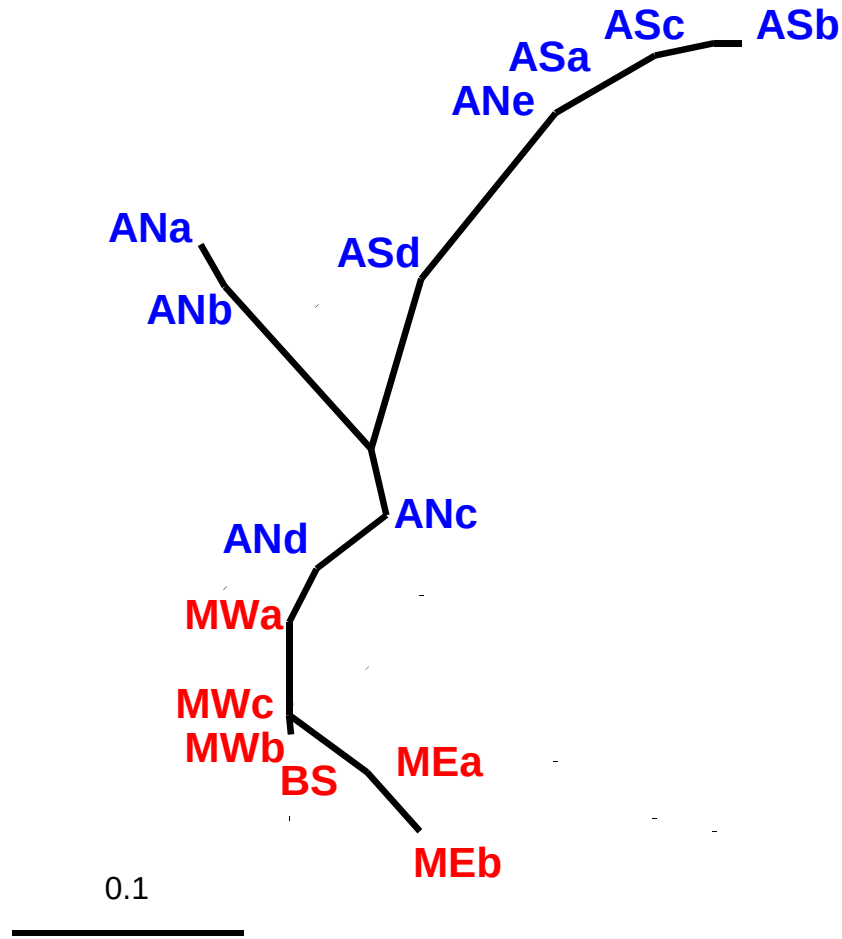
12S rRNA : *Fst* = 0.224***

10 X



Among population differentiation :

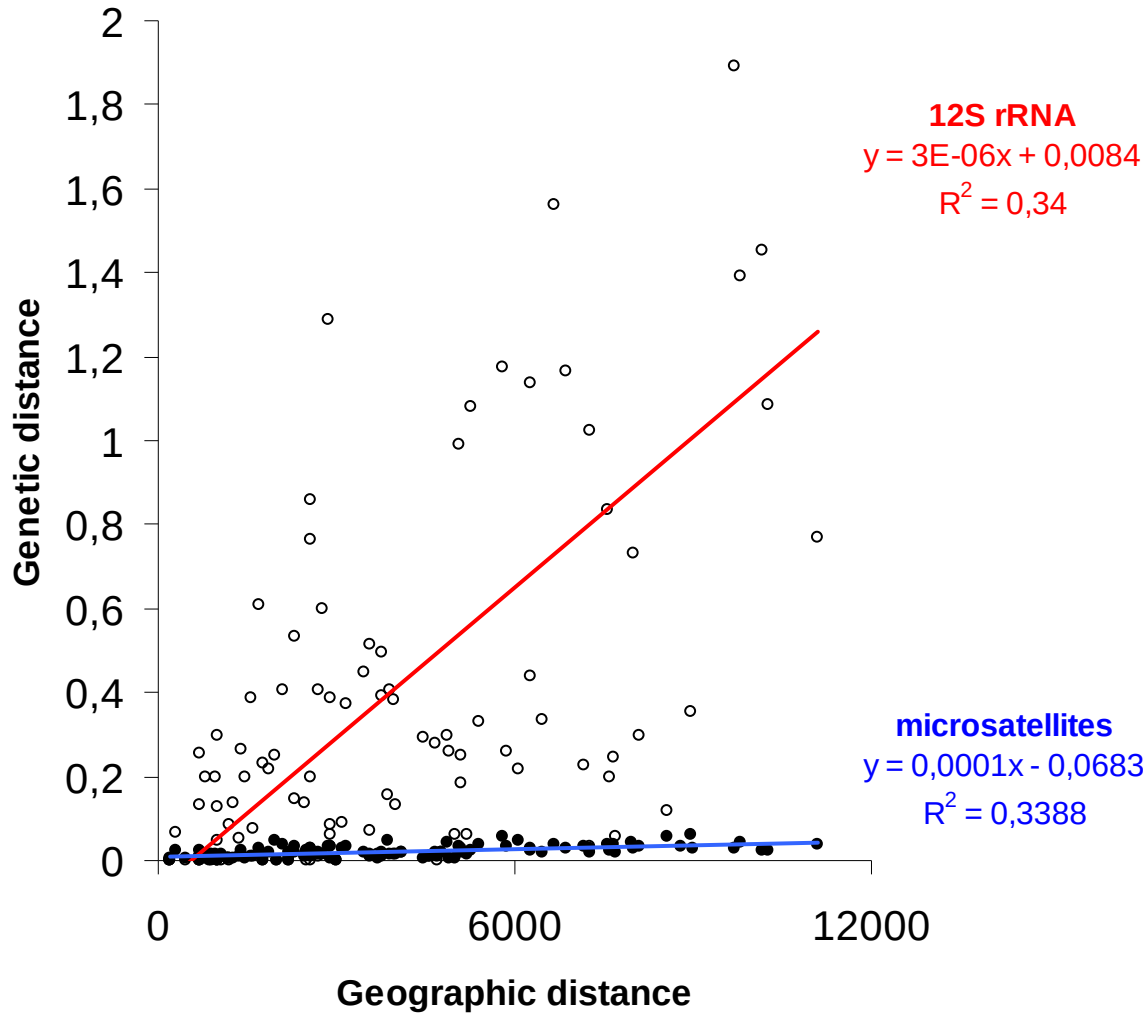




Microsatellites

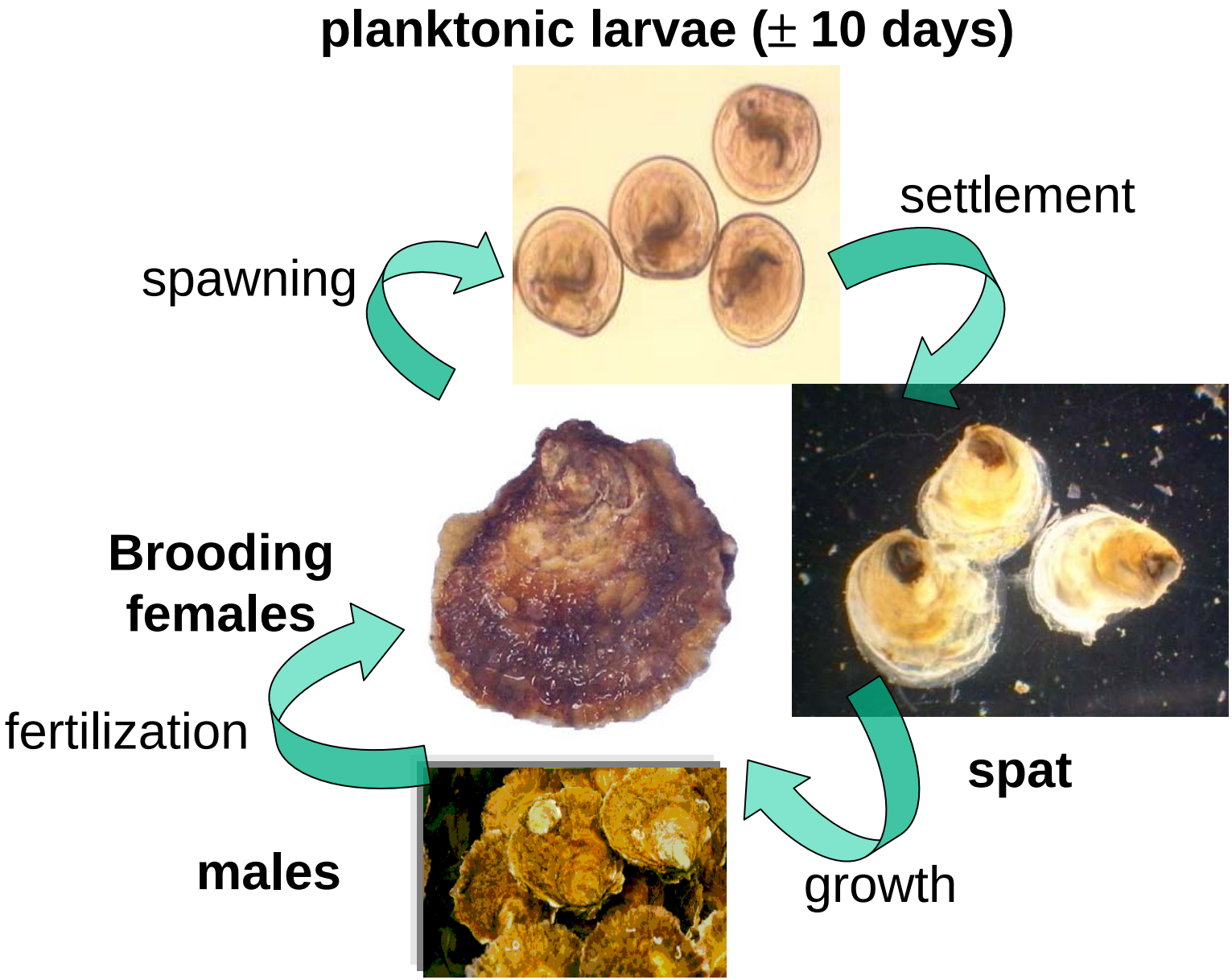
12S rRNA

Isolation by distance :



Higher variance in reproductive success in the female than in the male ?

Reproductive cycle

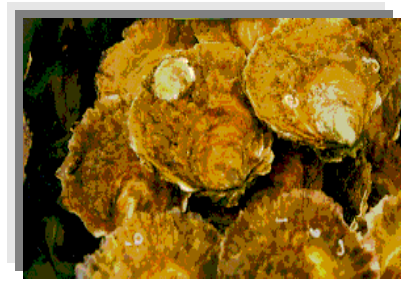


Variability over generation: how many males / female ?

E

??

Γ



Brooding female	Nb alleles / locus T5	J12	H15	O9	U2	Number of fathers
MED1	5	5	4	5		3 - 4
MED22	3	3	3	3		1
MED25	4	5	6	--		6 - 8
ATL1		18	10		18	10
ATL3		5	6		7	7 - 8
ATL5		7	5		9	4 - 7
ATL7		5	4		4	2

(PARENTAGE)

Mean number of males / female = 5.2

Variability over generations : *Ne* = ?

??

E

± 5

Γ

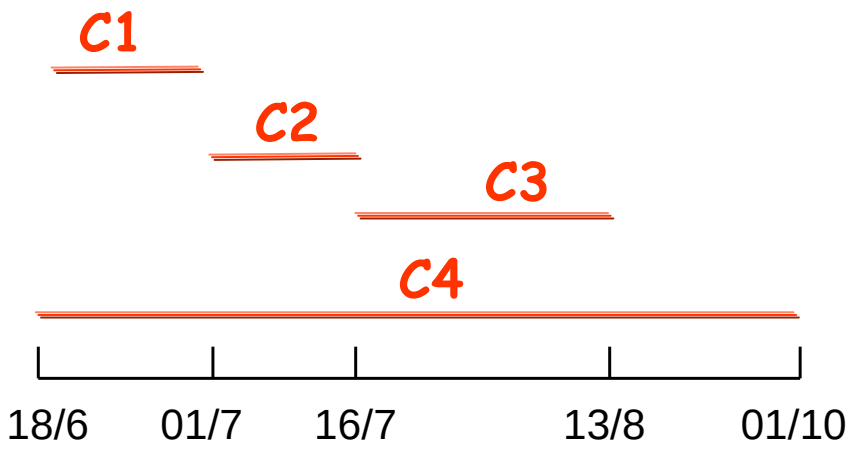
- **1994 : one cohort recruited over 15 days in Sète**

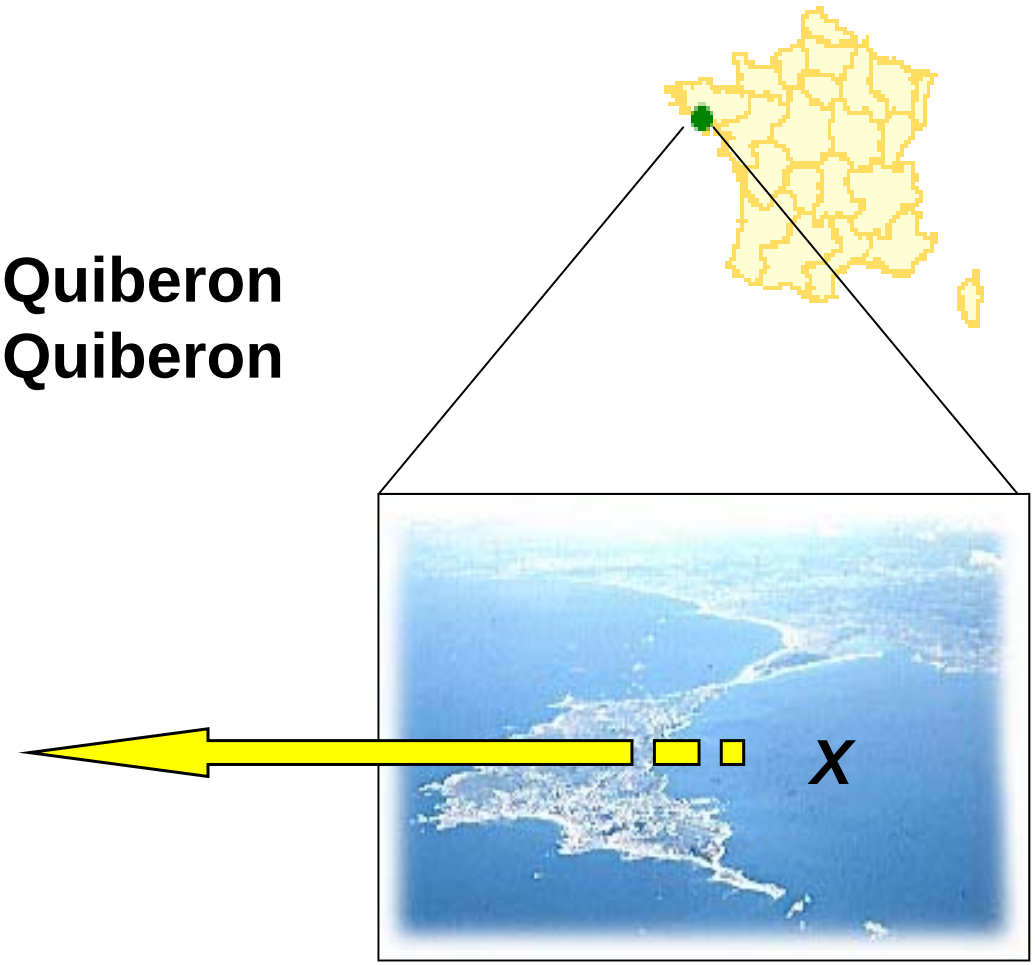
alleles / locus : spat (16.4) ≠ adults (21.8)

Ne = 16 [11,23] **(Launey *et al*., in prep)**

Variability over generations: *Ne* = ?

1995 : 49 adults collected in Quiberon
2001 : 68 adults collected in Quiberon
+ 4 cohorts :





Variability over generations: *Ne* = ?

- **alleles / locus : spat = adults**
- **adults /spat : *Ne* = 135** [44, -924]
- **adults 1994 / adults 2001 : Ne = 207** [60,-503]

Conclusions :

- **Wild populations are strongly geographically structured**
- **Effective population sizes might be low**
- **The impact of hatchery propagation could be strong**

Recommendations :

- **Special attention should be given to the genetic management of hatchery-based populations.**
- **Research on triploidy (sterile oysters, not currently handled) should be supported**