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Genetic structure of wild European populations of the invasive Pacific oyster *Crassostrea gigas* due to aquaculture practices

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Abstract:

As a result of aquaculture activities, Pacific oysters *Crassostrea gigas* (Thunberg, 1793) have invaded European coasts. Using seven microsatellites, we found virtually no genetic differentiation between natural populations throughout the European range (from the south of the Wadden Sea (the Netherlands) to the south of France) and French cultivated oysters. The genetic homogeneity of Pacific oyster samples appears to be the result of repeated transfers from same seed stocks made for aquaculture and, to a lesser extent, widespread dispersal due to specific biological traits of this species. The only genetic differentiation of Sylt population in the north of the Wadden Sea (Germany) suggests a stronger, persistent impact of ongoing supply of new genetic material from hatchery production, corresponding to seeds selection made by breeders. All of our genetic data highlighted the importance of aquaculture practices on the genetic structure of the keystone invader *C. gigas* in Europe.

Introduction

Marine ecosystems have always been subject to changes in species composition due to natural migration and can be favored by climatic variations. For several decades, the impact of human activities on climate and transportation of marine organisms has modified the geographic distribution of species (Carlton 1996a). Besides species introductions resulting from ballast waters of boats, escapes or accidental introduction due to aquaculture production is an important source of alien species into coastal ecosystems (Carlton 1989). Fortunately, only 10% of introduced species are expected to become established and to spread in their new environments, and only a small fraction may furthermore induce changes to the recipient environment (Williamson and Fitter 1996). These species are described as invasive species (Lodge 1993) with rapid spreading and strong impact on their new habitats (Carlton 1996b; Williamson 1996; Davis and Thompson 2000; Reise et al. 2006).

The Pacific cupped oyster *Crassostrea gigas*, native from Japan and Korea and introduced massively for aquaculture interests into many parts of the world (Wolff and Reise 2002), is one of the 104 invasive marine species described in European waters (Goulletquer *et al.* 2002). Large numbers of individuals were introduced in many countries of the European coastal waters in order to offset decreasing production of the Portuguese oyster *C. angulata* and the European flat oyster *Ostrea edulis*, due to viral or parasitic diseases (Grizel and Heral 1991; Nehring 1999; Wolff and Reise 2002). *C gigas* was first introduced into France (Marennes-Oléron Bay) in 1966 (Grizel and Heral 1991), into the Netherlands (Oosterschelde estuary) in 1964 (Drinkwaard 1999), and into Germany (Wadden Sea, near the island of Sylt) in 1971 (Reise 1998; Diederich et al. 2005). Different geographical origins have been used for these transfers. In the Netherlands and in France, several hundred tons of adults were transferred from British Colombia (originated from Japan), followed by millions of juveniles from Japan (from 1964 to 1971, respectively) (Wolff and Reise 2002; Miossec and Goulletquer 2007; Smaal et al. 2009). In Germany, spat and larvae were first repeatedly imported from Scottish hatcheries with no apparent success (between 1971 and 1987) (Seamen 1985; Wehrmann et al. 2000). In a second phase, oysters were imported from British and Irish hatcheries since 1986 in the northern area of the German Wadden Sea (Reise 1998; Nehring 1999). Oysters in British Isles were imported from USA (West), Colombia Islands (West), Hong Kong, Israël, themselves initially supplied by Japan. In the following years, variable spatfalls were recorded leading to significant dispersal and increasing abundances (Nehring 2003; Diederich 2005; Wehrmann and Schmidt 2005; Schmidt et al. 2008).

Even though the reproductive success of imported oysters was expected to be limited in Northern Europe due to low water temperatures, this species gradually colonized new habitats, around oyster production areas. With the development of oyster farming, this species gradually invaded European coasts (Goulletquer *et al.* 2002). The Pacific oyster is now considered to be established in most European coasts: e.g. France, British Isles, the Netherlands (Drinkwaard 1998; Reise 1998) and in the Wadden Sea area of Denmark and Germany (Reise *et al.* 2005), and since 2007 in Sweden and in Norway (Wrange *et al.* 2009). In general the Pacific oyster has yet to establish permanent populations in northern areas, even if the coastal waters of Northern Europe were believed to be too cold and/or too limnic for the Pacific oysters to survive (Nehring 2006). Shortly after Pacific oyster farming started, natural spatfalls occurred, whereas the first oysters were recorded outside the culture plot only twenty years after (Reise 1998). Furthermore, the invasion and expansion of this species has been recorded in areas where no deliberate introductions were made, suggesting that this species has not yet reached its ecophysiological limits (Cardoso et al. 2007), so Pacific oysters may well continue to expand and modify invaded ecosystems (Troost 2010). The accelerated spread (worldwide and local) of the Pacific oyster might be facilitated by climate change (Diederich et al. 2005; Dutertre et al. 2010) as well as by the high phenotypic plasticity (Grizel and Heral 1991; Nehring 1999; Wolff and Reise 2002) and broad genetic diversity (Huvet et al. 2000) of this species. Genetic diversity of such invasive populations is an important factor that can help to trace their origin, affect their invasive potential through inbreeding or local adaptation. Throughout the invasive process, non indigenous species have to be well adapted to the new habitat (Lee 2002). However, high level of genetic diversity permits to the species a better potential to adapt to the new environment.

Few are known about the genetic population structure and distinctness of the different invasions in European open waters. Recently, two origins of invasions in Wadden Sea were identified by mitochondrial analyses on naturalized populations from Denmark to the Netherlands (Moehler et al. 2011). In the northern part of this area, introductions for aquaculture in Sylt (Germany) conducted to a persistent impact on invasive populations. In the southern part, naturalized population was genetically closed to cultivated population form Oosterschelde estuary (the Netherlands) and also to naturalized populations from British Columbia (putative source in the Netherlands) (Moehler et al. 2011). However, nothing is known about the genetic population structure of invasive population in the other part of Europe and particularly along the Atlantic coasts. Such data is needed to clarify the origin of the invasive population and whether an invasion only happened once or on repeated occasions ((Roman and Darling 2007; Geller et al. 2010; Reusch et al. 2010)). Such multiple introductions are usually cryptic and

multiple invasions could only be revealed by genetic markers (e.g. invasion of green crab into West-Atlantic (Roman 2006)).

To test whether all Pacific oysters in Europe originated from one or more genetic stocks of the global oyster trade, we conducted an analysis of 7 microsatellite loci on European oyster's populations from north to south within the overall invaded area, from Wadden Sea in Germany to Arcachon Bay in France. To further evaluate the connectivity between aquaculture and invading populations (Voisin et al. 2005; Petersen et al. 2010), we also included specimens from one of two putative aquaculture sources in France (i.e. Arcachon Bay). Using this sampling scheme we can connect putative and known invasion routes with French aquaculture source and provide population genetic signatures of the most important invasion processes observed in the European coasts. The results offer implications for further research and management practice.

Materials and Methods

Study sites and sampling

Wild Pacific oysters were collected in open water during winter 2005-2006 from 10 sites in Europe close to oyster farms (<20km; Figure 1 and Table 1): one German (Sylt), three Dutch (Texel, Grevelingen and Oosterchelde) and six French populations (Arcouest, Squiffiec, Plougonvelin and Saint-Pierre Quiberon (St. P. Quiberon) in Brittany; Pornic and Arcachon Bay on the Atlantic coast). Oyster densities ranged between 100 to 1000 oysters per m^2 in all the populations (Reise et al. 2005; Lejart 2009; Nehring et al. 2009), except for the population Plougonvelin, where it ranged between 10 to 100 oysters per m^2 (Lejart 2009). Colonization by oysters started more than fifteen years ago in all of the studied locations except Plougonvelin, where it occurred in 2004 (one year before the sampling). Arcachon Bay is one of the two main sites for natural spatfall collection in France and seeds from this site are transferred annually throughout French oyster farming areas, including the French sites sampled in this study. Samples from this last site represented one-year-old seeds caught in the Bay of Arcachon. All the other samples corresponded to wild adults (10-17 cm) and therefore, contained a mixture of generations / age classes (Lartaud et al. 2010). About 48 oysters were collected at each site in 2005 or 2006, taking animals from the low tidal height zone (corresponding to 50 % emersion time), giving a total of 479 individuals. For each individual, gills or muscle were dissected and preserved in 90 % ethanol.

DNA extraction, PCR procedures and electrophoresis

Genomic DNA was extracted from 100 mg of tissue placed in extraction buffer (0.3 M Tris, pH 8, 0.02 M ethylene diamine tetra-acetic acid (EDTA), 0.1 M NaCl) with sodium dodecyl sulphate (SDS) and proteinase K at final concentrations of 0.6 % and 0.1 $mg.mL^{-1}$, respectively. After incubation at 55°C, the tissue was completely dissolved. NaCl was then added to give a final concentration of 1.3 M. After mixing, the samples were centrifuged at 3000 g at 20°C for 10 min. The supernatant was subjected to two successive phenol/chloroform/isoamyl alcohol (25: 24: 1) extractions. DNA was precipitated with absolute ethanol, recovered by 30 min centrifugation at 12 000 g and 4 °C, rinsed with 70 % ethanol, dried and resuspended in 1 mL of TE buffer (10 mM Tris, pH 8, 1 mM EDTA).

First, 12 *C. gigas* microsatellites published previously were tested (Magoulas et al. 1998; Li et al. 2003). Finally, seven polymorphic loci without null allele were retained for this study: *ucdCg-117*, *ucdCg-138*, *ucdCg-148*, *ucdCg-173*, *ucdCg-177*, *ucdCg-198* and *ucdCg-200* (Magoulas et al. 1998; Li et al. 2003).

Multiplexed amplifications were performed using three sets of markers. Reactions were performed with 2 µL of Qiagen multiplex PCR master mix (Qiagen, Courtaboeuf, France), 0.2 µM each primer, 2 µL RNase-free water, 1 µL Q-solution and 50 ng DNA in a final volume of 10 µL, following the manufacturer's instructions. For genotyping, fluorescently-labelled PCR products were diluted 1/10 in deionized formamide, electrophoretically separated on an ABI3100 sequencer using the POP7 polymer and sized using the internal standard Rox500 (Applied Biosystems). GENEMAPPER v4.0 software (Applied Biosystems) was used to define size classes of alleles and to semi-automatically genotype all specimens in the complete dataset; these were finally verified visually one by one.

Data analysis

Allele frequencies and the observed, and expected heterozygosity values were calculated in GENETIX v 4.05.2 (Belkhir et al. 2004). FST and Single- and multilocus Fis (indicating heterozygote deficiency/excess) were estimated (Weir and Cockerham 1984) using GENETIX v 4.05.2. Deviation from the Hardy–Weinberg equilibrium (Fisher's exact test) and gametic disequilibrium (Fisher's exact test) among loci were tested using GENEPOP v4 (Raymond and Rousset 1995; Rousset 2008). Both tests were corrected for multiple simultaneous tests by calculating the q-value of each test which measures the minimum *false discovery rate*

(FDR) that is incurred when calling that test significant. The bootstrap method was chosen as recommended by Storey (2002) for a limited number of p-values. The q-values were calculated using the R package QVALUE (www.r-project.org/, Storey (2002)).

Null allele frequencies were calculated based on Brookfield (1996) using the program MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004). Allele frequencies were then used to calculate pairwise genetic distances D_C (Cavalli-Sforza and Edwards 1967) using GENETIX v4.05.2. The significance of the genetic distances was tested by 10 000 permutations of individuals between populations. Correlation of genetic over geographical distances (measured as the shortest distance between two locations along the coast line) for all pairs of populations were tested with the Mantel permutation procedure available in GENETIX v 4.04. To assess whether any indications for group structure could be observed, we performed a multidimensional scaling analysis (MDS) using the function Classical (metric) Multidimensional scaling (CMD Scale) in the R package stats, also known as principal coordinate analysis on the linearized pairwise genetic distances (Gower 1966).

The BOTTLENECK 1.2.02 program was used to investigate the presence of recent bottlenecks in wild oyster populations, according to Cornuet and Luikart (1996). When populations have experienced a recent reduction of their effective population size, allele numbers were reduced faster than the gene diversity or, in other words, gene diversity excess occurs. This program tests for departure from mutation-drift equilibrium, based on heterozygosity excess or deficiency under the infinite allele model (IAM), the stepwise mutation model (SMM) and the two-phase model (TPM). As recommended by Cornuet and Luikart (1996), the TPM model was used for our microsatellite data, with 80 % of SMM in TPM and variance set at 20 % as most microsatellite datasets fit the TPM better than the SMM or IAM (Di Rienzo et al. 1998). Significant bottlenecks were tested using the Wilcoxon signed rank test, calculated using 10 000 iterations. An Assignment test (Cornuet et al. 1999), implemented in GeneClass v2.0 software (Piry et al. 2004), was used to estimate the likelihood of an individual multilocus genotype being assigned to one of the 10 populations, using the self-assignment Bayesian option and leaving one out sub-option.

Results

The microsatellite variability of *C. gigas* appeared to be heterogeneous with the total number of alleles per locus ranging from 48 (*ucdCg*-200) to 105 (*ucdCg*-148) but with homogeneous observed heterozygosity per locus

(Table 2). For each locus, the genetic diversities observed within samples were of the same magnitude over the whole data set.

Genetic diversity within populations

Three microsatellites on the seven screened, exhibited substantial departures from Hardy-Weinberg equilibrium (HWE) for one location (*ucdCg*-138, *ucdCg*-177 and *ucdCg*-198), one for two locations (*ucdCg*-173) and one for three locations (*ucdCg*-200) (Table 2). The mean observed heterozygosity was similar in the five estuaries ($0.866 < Hobs < 0.942$). The multilocus statistics detected four significant deviation from HWE (Sylt, Oosterchelde, Plougonvelin and St. P. Quiberon). The software MICRO-CHECKER did not detect a significant departure from HWE linked to null alleles.

Genetic structure

Pairwise estimations of Fst (Weir & Cockerham's θ) showed a significant global multi-locus genetic differentiation mainly between Sylt and the other populations from the Netherlands and France, and between St. P. Quiberon *versus* both Arcouest and Arcachon (Table 3), confirmed by θ-values for locus cconsidered individually. Over the 100 pairs of populations considered, eleven pairs displayed a significant multi-locus genetic differentiation, confirmed after the correction for multiple tests.

The MDS analysis confirmed the isolation of the Northern population (Sylt) from other ones (Figure 2).The analysis of the distribution of the genetic variability between the Sylt and the group of nine others populations, performed with an AMOVA, explained 1.43 % of the total genetic variance in *C. gigas* ($F_{CT} = 0.014$, $p<0.01$), confirming the reduced but significant genetic structure over the data set. The mean allelic richness appeared significantly lower in the northern population of Sylt ($N_A = 28.7$) relatively to the other populations ($37.6 < N_A < 42.0$) (Table 2). No high levels of private alleles were observed in this population (data not shown). No significant linkage disequilibrium in the Sylt population or in the other ones was revealed in our study using Genepop v4 software. As expected from the low genetic structure among most locations, 18.5 % (range 1.5–36 %) of individuals were correctly assigned to their location of origin in the southern cluster and less than 20 % of each population was assigned to cultivated oysters from Arcachon Bay (Table 4). Only the Sylt specimens presented a relatively high correct assignment score (63 %). Finally, the test of Mantel revealed a lack of

correlation between geographical and genetic distances matrices (r = 0.37, *p>0.05*) either in all the datasets or in the southern group alone.

Founder effect

Under the TPM, only two of the three Dutch populations (Grevelingen and Texel) showed significant signs of having passed through a recent bottleneck (Wilcoxon signed rank test, P = 0.046). The more recent invasion observed in the Plougonvelin site did not result in a loss of genetic diversity in the samples in terms of allelic richness when compared with the putative French source population (Arcachon Bay) or the nearest site geographically (Squiffiec) (N_A = 37.6, 38.1 and 41.4, for these populations, respectively, Table 2).

Discussion

Specific significant departures from HWE

In this study, a low number of significant deficits of heterozygotes were observed for particular microsatellite loci. The problem of heterozygotes deficit for microsatellite loci was well-documented in bivalves including *C. gigas* (McGoldrick et al. 2000, Hedgecock et al. 2004, Yu & Li 2007), *Dreissena polymorpha* (Astanei *et al.* 2005), *Patinopecten yessoensis* (Li *et al.* 2007) and *Mizuhopecten yessoensis* (Sato *et al.* 2005). Heterozygote deficits would in such case result either from Wahlund effect due to the subdivision of local population into isolated and differentiated sub-populations (Castric *et al.* 2002) or to the recruitment of different cohorts of distinct origins (local genetic patchiness), or from, inbreeding through the mating of close relatives as demonstrated for fishes (Lenfant 2002). However, such biological explanations require a rather homogeneous effect across loci in the populations departing from panmixia, whereas scarce departure to HWE recorded here was heterogeneously distributed across loci. Departures to HWE are frequently linked to an artifact of the PCR amplification process that is, to the presence of null alleles. The software MICRO-CHECKER was tested on the oyster data set (in all the data set and in each sample) and did not detect a significant departure from HWE linked to null alleles. Furthermore, in this study, the oyster populations were considered to be at the Hardy-Weinberg equilibrium, thus reflecting the equilibrium between migration versus drift, classically observed for neutral markers.

Genetic differentiation of Sylt population

The genetic variability (multilocus allelic richness and heterozygosity) was identical in the oyster populations of the data set (France and the Netherlands) except in the population of Sylt (North of Wadden Sea), located in the northern part of *C. gigas* actual European repartition area. This population highlighted lower allelic richness (N_A = 28.7) compared to the other ones ($37.6<N_A<42.0$). Recently, population genetic of Sylt and close populations were directly related to aquaculture stocks of local oyster farms (Moehler et al. 2011) imported every year from British and Irish hatcheries since 1986 (Reise 1998; Nehring 1999). The spread of *C. gigas* around Sylt began 5 years after first introductions at the origin of a first invasion in this area (Reise 1998; Diederich 2005). Oyster seeds used for aquaculture on Sylt most likely originated from breeders on the British Isles. Reduction of genetic diversity due to the high variance of reproductive success of Pacific oysters, was well documented (Li and Hedgecock 1998; Boudry et al. 2002; Li et al. 2009). Crossing of divergent lines decreased inbreeding depression, which was common in Pacific oysters due to high genetic load (Launey and Hedgecock 2001). Breeders selected oysters for higher growth rates by outbreeding, to reduce genetic load of produced spat and increase yield (Hedgecock et al. 1995; Hedgecock and Davis 2007). Divergent lines selected are crossed together, leading to an artificial amplification of particular distantly related genotypes frequencies and a decrease of the global genetic diversity (Appleyard and Ward 2006). In this way, low level of allelic richness of naturalized population in Sylt revealed in this study by microsatellites may be the result of breeders stocks. Interestingly, this study detected highly significant genetic differentiation between Sylt and the other populations ($Fst>0.015$ with $p<0.001$). This level of genetic differentiation is converging with the first genetic differentiation detected by Moehler *et al*., (2011) for European populations of *C. gigas* sampled in 2008. The authors observed significant genetic differentiations at mitochondrial markers between oysters cultivated in Sylt associated with close wild populations and (1) invasive populations from the South of Germany to the Netherlands in one hand, and (2) naturalized oysters from British Columbia (stock origin on the British Isles (Syvret et al. 2008) on the other hand), indicating that the breeders must maintain a genetically distinct brood stock for spat production. Our study confirms the specific genetic signature of Sylt population, sampled in winter 2005-2006 and thus studied 20 years after the first introduction of *C. gigas* in this area.

Genetic homogenization of wild oyster populations in Europe

Over the sample area, this study detected a genetic homogenization in Europe of Pacific oyster populations, running between the southern population from France and the northern population from the Netherlands as previously described from France to Spain using mitochondrial and nuclear markers (Huvet et al. 2004). This

relative genetic homogeneity across a rather large geographic scale could be attributed to specific *C. gigas* biological traits that permit a wide spread dispersal. An average market-sized female oyster can produce 50-100. 10^6 oocytes in a single spawning (Royer et al. 2008). The resulting pelagic larvae are planktotrophic, feeding on phytoplankton and growing over a period of 2 to 3 weeks depending on water temperatures (ranged to 20-26°C; (Rico-Villa et al. 2010)). When this period corresponds to 3 weeks, marine planktotrophic larvae could disperse with currents up to 10^2 or 10^3 km (Todd et al. 1998). In a review on the clinal patterns of genetic variation across species ranges, Hardie and Hutchings (2010) showed that most studies are consistent with the general perception that peripheral populations are less genetically variable than those inhabiting central areas. This decrease being particularly associated with stochastic processes (founder effects, genetic drift, isolation and/or low gene flow) occurring in the marine and freshwater environments (e.g. isolation-by-distance profile observed for the native European flat oyster *Ostrea edulis* (Launey et al. 2002)). In this study, the sampled sites are separated by more than 1800 km, but genetic distance between samples does not correlate with geographic location (i.e. no evidence of isolation by distance).Thus, relative genetic homogeneity among European samples could also reflect the effect of 'multiple introductions' of *C. gigas* caused by seed transfers from Arcachon Bay throughout Europe for aquaculture (Wolff and Reise 2002; Miossec and Goulletquer 2007). We suggest that the current maintenance of the genetic diversity for the oyster in Europe could be linked to the annually introductions of seeds throughout European aquaculture farms with same origins, with the particular exception of Sylt farms.

In many countries, oyster production is mainly based on the capturing of wild seeds. Marennes-Oleron Bay and Arcachon Bay are the two main seed production areas in France, the latter being the oldest. The 1-year-old seed caught in Arcachon Bay and examined in our study represents a single generation of juveniles, as does the seed transferred throughout France every year for aquaculture. Importantly, this sample representing only one cohort did not differ genetically from the adult European wild oysters representing a mix of generations, apart from the Sylt population. This result shows the high genetic diversity susceptible to be obtained in only one spawn and also the genetic homogeneity between one spawn and multiples generations. Nonetheless, mitochondrial analysis revealed that from Oosterchelde to closed Sylt neighborhoods (including Texel), naturalized and cultivated oysters were genetically close to naturalized oysters from British Columbia (Moehler et al. 2011). This corresponds to the second invasion in this area with oysters transported from farms in British Columbia (Canada) between 1964 and 1982 (Drinkwaard 1999; Smaal et al. 2009). In this study, cultivated oysters from the Bay of Arcachon resembled the naturalized populations from Texel to the south of France, *via* naturalized Dutch

populations. Furthermore, the oysters transferred to Dutch and French farms during the 1960s were imported from Japan, British Columbia and the US West coast based on wild-collected Miyagi oyster seed from Japan (Clark and Langmo 1979). These European transfers from French production areas were continued to sites throughout France and the Netherlands over several decades (Statistics Netherlands, CBS www.cbs.nl; Miossec and Goulletquer (2007)). Each year, seed transfer from seed production areas to on-growing sites may be considered as a potential introduction, contributing to gene flow between production sites. The recurrence of transfers is a critical component in the dynamics of invasiveness of the species. Because the origin of seed differs every year, depending on production costs at the two main French production areas, repeated transfers have maintained the overall genetic pool of the originally introduced stocks. This process has also been observed for other marine species like the kelp *Undaria pinnatifida*, which has a very limited dispersal potential compared to *C. gigas* (Voisin et al. 2007), or for terrestrial species (e.g. the shrub *Cytisus scoparius*; (Kang et al. 2007)). Taking into account the genetic homogeneity between oysters from Texel, Oosterchelde and British Columbia revealed by mitochondrial analysis (Moehler et al. 2011), the genetic homogeneity among European naturalized populations, including Texel, Oosterchelde and Arcachon Bay, revealed in this study by microsatellites may illustrate aquaculture practices with repeated transfers of seeds with large inoculum sizes. All of the oyster movements created opportunities for high levels of gene flow throughout Europe except in Sylt, where seed transfers were coming from breeders in British Isles.

Founder effect

One hypothesis to explain a decrease in genetic diversity in this introduced species during the invasive process could be instantaneous drift associated with the settlement of a small number of individuals, generating a so-called founder effect (Nei et al. 1975). This phenomenon has been observed in introduced species such as *Spartina anglica* and the Argentine ant *Linepithema humile*, using neutral markers (Tsutsui et al. 2000; Baumel et al. 2001; Tsutsui et al. 2001). However, the loss of genetic diversity through a founder effect may be limited in cases of recurrent introduction of relatively high numbers of individuals from the source populations (Barret and Husband 1990), as in the present case, and in highly fecund marine species in general (Voisin et al. 2005). Interestingly, a slight but significant bottleneck effect was observed in our study of wild populations in the Netherlands, showing lower N_A. Unlike in France, oyster translocations have been limited in the Netherlands since 2001 (Nehring 2006), which could reflect the beginning of genetic isolation of these populations at the

European scale. We can hypothesize that a founder effect could be measured in future decades in the Netherlands.

In addition, no genetic differentiation was revealed in this study between the most recently established population (first settlement observed in 2004 in Plougonvelin), the oldest in this area (estimated before 1990 in Squiffiec) and aquaculture oyster from Arcachon Bay. Although allelic richness of oysters from Arcachon Bay (one single generation), and from Plougonvelin, presents lower levels of genetic diversity compared with the Squiffiec population, no bottleneck effect was measured in any of these two populations. This result indicates an establishment of a new population in only 2 years without a founder effect. Recent warm summers, which support the recruitment of the Pacific oyster, may have facilitated this process (Diederich et al. 2005) as observed in this area (average + 1°C of water temperature in winter, + 0.7 °C in summer; (Esnault 2005)).

In conclusion, our genetic data highlighted the importance of aquaculture practices on the genetics of the keystone invader *C. gigas* in Europe. The genetic homogeneity of Pacific oyster samples in Europe appears to be the result of repeated transfers from same stocks made for aquaculture and, to a lesser extent, widespread dispersal due to specific biological traits of this species. The only genetic differentiation of Sylt population may be due to seeds selection made by breeders and farmers, in agreement with previous mitochondrial analysis (Moehler et al. 2011). This demonstrates two aspects in which aquaculture practice can influence the characteristics of biological invasions by determining the starting material as well as providing continuous input into naturalized populations, thus resembling repeated invasions and admixture from genetically diverse sources (Kelly et al. 2006; Simon-Bouhet et al. 2006). Repeated genetic impact of aquaculture has been demonstrated for natural populations (McGinnity et al. 1997), but is actually scarce for invasive populations derived from aquaculture sources as demonstrated here for the case of Pacific oysters. Its genetic diversity and large dispersal potential predispose the Pacific oyster to be a successful invader by creating the possibility for selection of adapted individuals in each particular habitat. Such large-scale dispersal and homogenization would not prevent local selection of the juveniles every year but is likely negated by gene flow. In order to test this hypothesis and to ascertain the adaptive potential of this invasive species, future work needs to focus on the genetic structure of the Pacific oyster using genetic markers, which are presumably subjected to natural selection on spatial or temporal scales.

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Tables

Table 1 : Population details of C. gigas sampling.

Location	Sample	Co-ordinates	*n*
Germany	Sylt	N 54°55'; E 08°26'	48
The Netherlands	Texel	N 53°00'; W 04°47'	47
	Grevelingen	N 51°44'; E 03°59'	48
	Oosterchelde	N 51°33'; E 03°59'	48
France (Brittany)	Arcouest	N 48°47'; W 03°01'	48
	Plougonvelin	N 48°19'; W 04°43'	48
	Squiffiec	N 48°22'; W 04°22'	48
	St. P. Quiberon	N 47°31'; W 03°07'	48
France (Gironde)	Pornic	N 47°07'; W 02°06'	48
France (Loire-Atlantic)	Arcachon Bay	N 44°39'; W 01°10'	48

n number of individuals per site

Table 2 : Number of alleles (NA), observed (Hobs) and unbiased (Hexp) heterozygosities and FIS for each location and each locus, FIS was estimated according to Weir-Cockerham and was tested using the Markov chain method with 5000 iteration (*p < 0.05, **p < 0.01, ***p < 0.001). Bold print indicates FIS values that remained significant after Q-values correction.

Population	Parameters	Locus							Multilocus
		ucdCg-117	*ucdCg*-138	*ucdCg*-148	*ucdCg*-173	*ucdCg*-177	*ucdCg*-198	*ucdCg*-200	
Sylt	N_A	32	24	36	34	32	21	22	28.71
	H_{obs}	0.938	0.958	0.938	0.958	0.917	0.917	0.708	0.866
	H_{exp}	0.944	0.928	0.954	0.938	0.913	0.919	0.923	0.940
	F_{IS}	0.018	−0.022	0.027	−0.012	0.006	0.013	**0.243*** **	**0.080*** **
Texel	N_A	37	36	49	43	42	30	26	37.57
	H_{obs}	1.000	0.950	0.978	1.000	0.957	0.915	0.913	0.915
	H_{exp}	0.966	0.954	0.974	0.968	0.965	0.938	0.919	0.966
	F_{IS}	−0.022	0.017	0.008	−0.021	0.019	0.036	0.017	0.053
Grevelingen	N_A	46	41	56	44	49	30	26	39.44
	H_{obs}	0.956	0.978	0.979	0.956	1.000	0.938	0.979	0.917
	H_{exp}	0.970	0.964	0.977	0.969	0.971	0.944	0.929	0.969
	F_{IS}	0.026	−0.003	0.009	0.026	−0.018	0.017	−0.043	0.054
Oosterchelde	N_A	48	36	51	44	43	30	25	39.57
	H_{obs}	0.957	0.933	0.956	0.935	1.000	0.826	0.870	0.874
	H_{exp}	0.975	0.962	0.972	0.969	0.966	0.937	0.925	0.969
	F_{IS}	0.029	0.041	0.030	0.046	−0.024	**0.129*** **	0.071	**0.099*** **
Arcouest	N_A	52	41	53	43	47	23	21	40.00
	H_{obs}	0.958	0.875	1.000	0.958	0.979	0.958	0.833	0.931
	H_{exp}	0.975	0.959	0.975	0.961	0.972	0.940	0.930	0.969
	F_{IS}	0.027	**0.098*** **	−0.015	0.014	0.003	−0.009	0.115*	0.040
Plougonvelin	N_A	42	39	51	47	38	22	24	37.57
	H_{obs}	0.978	0.911	0.933	0.886	0.909	1.000	0.917	0.900
	H_{exp}	0.963	0.959	0.974	0.967	0.963	0.903	0.913	0.959
	F_{IS}	−0.004	0.061	0.053*	**0.095*** **	0.067*	−0.098*	0.007	**0.063***
Squiffiec	N_A	43	38	57	46	50	30	26	41.43
	H_{obs}	0.917	0.958	1.000	0.958	0.917	0.958	0.875	0.938
	H_{exp}	0.962	0.959	0.978	0.970	0.971	0.946	0.926	0.968
	F_{IS}	0.058*	0.011	0.012	0.022	0.067	−0.002	0.066	0.032
St P. Quiberon	N_A	47	35	51	47	47	29	18	39.14
	H_{obs}	0.979	0.917	0.938	0.979	0.958	0.938	0.708	0.889
	H_{exp}	0.972	0.955	0.974	0.970	0.964	0.944	0.891	0.964
	F_{IS}	0.003	0.050	0.048*	0.001	0.017	0.018	**0.215*** **	**0.079*** **
Pornic	N_A	48	46	56	42	53	28	21	42.00
	H_{obs}	1.000	0.938	0.958	0.896	0.979	0.958	0.875	0.942
	H_{exp}	0.972	0.971	0.974	0.968	0.974	0.943	0.934	0.972
	F_{IS}	−0.018	0.045	0.028	**0.085*** **	0.005	−0.006	0.073	0.031
Arcachon Bay	N_A	49	42	51	41	41	25	18	38.14
	H_{obs}	0.979	0.958	0.979	0.938	0.896	0.917	0.792	0.921
	H_{exp}	0.972	0.958	0.970	0.966	0.965	0.926	0.921	0.964
	F_{IS}	0.003	0.011	0.001	0.040*	**0.082*** **	0.020	**0.151*** **	0.045
	Total N_A	84	86	105	81	94	53	48	38.38
	Mean H_{obs}	0.966	0.938	0.966	0.946	0.951	0.932	0.847	0.909
	Mean H_{exp}	0.967	0.957	0.972	0.965	0.962	0.934	0.921	0.964

Table 3 : Matrix of pairwise Fst values over all loci and statistical tests for microsatellite data (above main diagonal) and estimated geographic distances between locations (below diagonal). (*p < 0.05, **p < 0.01, ***p < 0.001). Bold print indicates Fst values that remained significant after Q-values correction.

	Sylt	Texel	Grevelingen	Oosterchelde	Arcouest	Plougonvelin	Squiffiec	St. P. Quiberon	Pornic	Arcachon
Sylt	–	**−0.017*** **	**0.017*** **	**0.016*** **	**0.015*** **	**0.017*** **	**0.016*** **	**0.020*** **	**0.017*** **	**0.019*** **
Texel	268	–	−0.002	0.002	0.002	0.000	0.001	0.002	0.000	0.002*
Grevelingen	476	209	–	−0.001	0.000	0.001	−0.001	0.000	−0.002	0.001
Oosterchelde	517	250	27	–	0.002*	0.002*	0.002	0.002	0.000	0.002*
Arcouest	1,046	845	606	604	–	0.002*	0.001	**0.003** **	0.000	0.001
Plougonvelin	1,218	1,017	778	776	172	–	0.001	0.001	0.002	0.002*
Squiffiec	1,243	1,042	803	801	197	25	–	0.001	−0.001	0.002*
St. P. Quiberon	1,405	1,203	964	963	358	186	211	–	0.001	**0.005** **
Pornic	1,479	1,278	1,038	1,037	433	261	286	91	–	0
Arcachon	1,721	1,520	1,281	1,279	675	503	528	351	295	–

Pairwise Θ-values were calculated according to Weir–Cokerham and were tested using 10,000 permutations (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

Bold print indicates *F*st values that remained significant after *Q* values correction

Table 4 : Matrix of self-assignment test percentages among the 10 European oyster samples, based on the Bayesian method.

Population	Sylt	Texel	Grevelingen	Oosterchelde	Arcouest	Plougonvelin	Squiffiec	St. P. Quiberon	Pornic	Arcachon
Sylt	**63.2**	0.0	1.5	2.9	10.3	0.0	8.8	2.9	4.4	*5.9*
Texel	0.0	**10.6**	2.1	4.3	14.9	2.1	23.4	14.9	17.0	*10.6*
Grevelingen	0.0	5.9	**1.5**	7.4	13.2	2.9	14.7	2.9	8.8	*13.2*
Oosterchelde	0.0	6.3	6.3	**8.3**	8.3	10.4	20.8	20.8	14.6	*4.2*
Arcouest	2.0	3.0	6.0	3.0	**21.0**	2.0	24.0	9.0	13.0	*17.0*
Plougonvelin	0.0	2.1	8.3	4.2	10.4	**12.5**	20.8	20.8	10.4	*10.4*
Squiffiec	0.0	5.0	4.0	5.0	17.0	4.0	**26.0**	17.0	11.0	*11.0*
St. P. Quiberon	0.0	6.0	8.0	3.0	7.0	5.0	19.0	**36.0**	9.0	*7.0*
Pornic	0.0	3.5	1.2	1.2	11.6	2.3	31.4	11.6	**17.4**	*19.8*
Arcachon	0.0	5.0	2.0	3.0	13.0	2.0	25.0	7.0	10.0	***33.0***

Numbers in bold represent the percentage of oysters correctly assigned to their sample of origin

Numbers in italic represent oysters assigned to cultivated seeds from Arcachon Bay

Figures

Figure 1 : Geographical locations of the 10 European C. gigas populations.

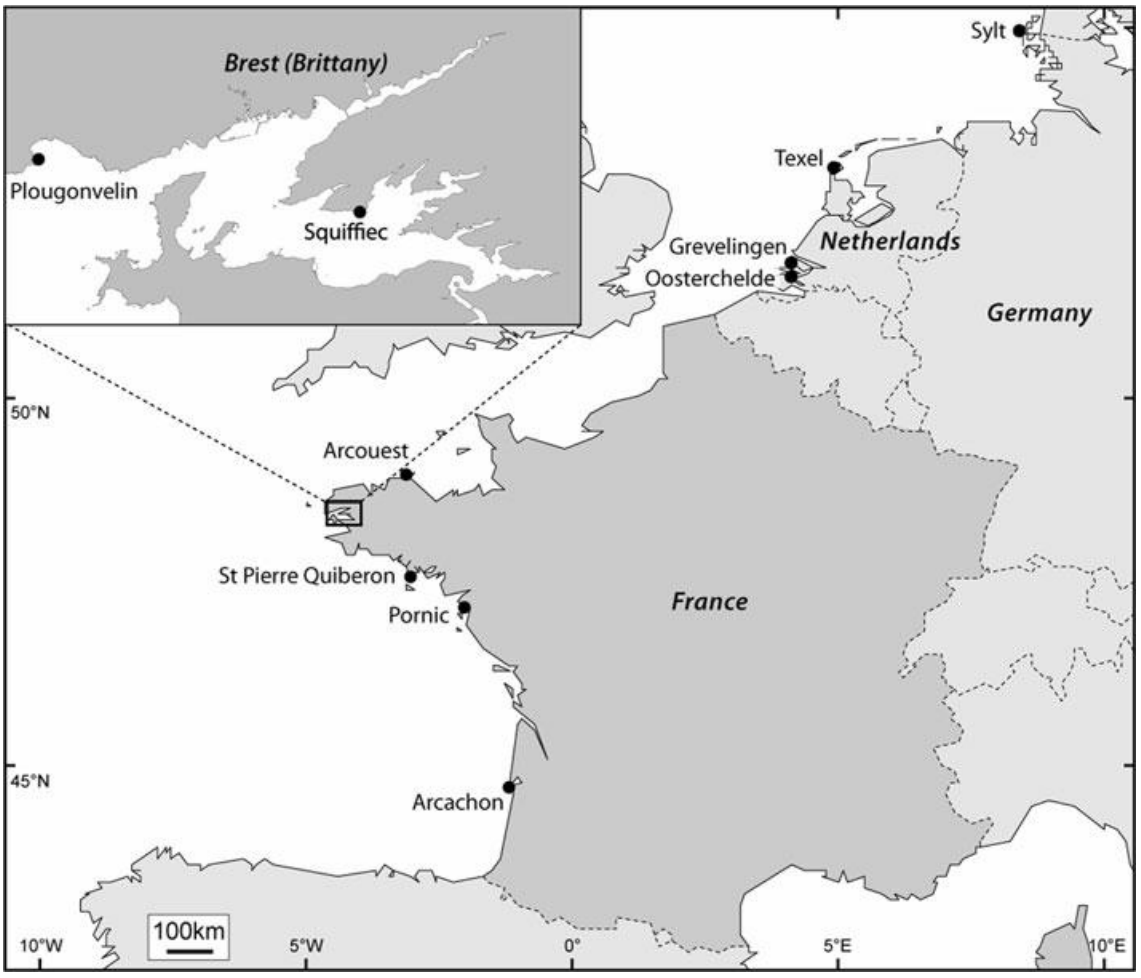

Figure 2 : Multidimensional scaling (MDS) representation microsatellites on linearized pairwise genetic distances *Dc*.