Biological Invasions
September 2015, Volume 17, Issue 9, Pages 2581-2595
http://dx.doi.org/10.1007/s10530-015-0896-1
http://archimer.ifremer.fr/doc/00268/37973/

Invasion genetics of the Pacific oyster *Crassostrea gigas* in the British Isles inferred from microsatellite and mitochondrial markers

Lallias Delphine [1, *], Boudry Pierre [2], Batista Frederico [3], Beaumont Andy [1], King Jonathan W. [1], Turner John R. [1], Lapègue Sylvie [4]

[1] Centre for Applied Marine Sciences, School of Ocean Sciences, College of Natural Sciences, Bangor University, Menai Bridge, Anglesey LL59 5AB, UK
[2] IFREMER, RBE-PFOM, Centre Bretagne, ZI de la Pointe du Diable, F - 29280 Plouzané, France
[3] Estaçao Experimental de Moluscicultura de Tavira, Divisao de Qquicultura e Valorizaçao, Instituto Portugues do Mar e da Atmosfera, Av. 5 de Outubro s/n, 8700-305 Olhao, Porgual
[4] IFREMER, RBE-SG2M-LGPMM, Avenue de Mus de Loup, F-17310 La Tremblade, France

* Corresponding author : Delphine Lallias, email address : delphine.lallias@jouy.inra.fr

Abstract :

The Pacific oyster, *Crassostrea gigas*, native to northeast Asia, is one of the most important cultured shellfish species. In Europe, Pacific oysters first settled along the Atlantic coasts of France at the end of the 1960s but rapidly spread and are now widely established. Twenty-two sites in the United Kingdom (UK), Ireland, Denmark, France and Spain were sampled to assess genetic diversity and differentiation. Hatchery-propagated stocks from two hatcheries located in the UK also were included. Two main genetic clusters were identified from pairwise genetic differentiation indexes, Bayesian clustering methods or neighbour-joining analysis, based on 7 microsatellite loci: (1) a Northeast cluster (including feral samples from East England, Ireland and Denmark as well as UK hatchery stocks) and (2) a Southwest cluster (including samples from South Wales, South West England, France and Spain). The Southwest cluster had significantly higher allelic richness (A) and expected heterozygosity (H_e) (A: 45.68, H_e: 0.928) than in the Northeast (A: 26.58, H_e: 0.883); the two diverging by a small but significant F_{ST} value (F_{ST} = 0.017, 95 % CI 0.014–0.021). A 739-bp fragment of the major noncoding region of the mitochondrial genome was sequenced in 248 oysters from 12 of the studied samples in Europe and in 25 oysters from Miyagi prefecture (Japan). A total of 81 haplotypes were found. Haplotype frequency analyses identified the same two clusters observed using microsatellites. This study highlights how the number and size of introduction events, aquaculture practices, genetic bottlenecks followed by genetic drift and natural dispersal can act concurrently to shape the genetic diversity and structure of introduced populations.

Keywords : invasion genetics, Marine mollusc, Microsatellites, mtDNA, Pacific oyster, Crassostrea gigas

INTRODUCTION

Biological invasions are a major threat to coastal ecosystems and to global marine biodiversity. In addition to natural pathways of propagule dispersal (e.g. water currents), humans have caused an unprecedented redistribution of many taxa over recent centuries (Carlton 1989), by facilitating transport and introduction of species through a variety of activities such as shipping and aquaculture (Voisin et al. 2005; Lacoursière-Roussel et al. 2012). In order to successfully manage marine non-native species and to predict their potential range expansion, it is essential to identify or confirm the source locations, pathways and vectors (Grosholz 2002), along with the factors contributing to the success of an invasion.

Invasion genetics studies have been performed to determine the degree of population connectivity, the source of invasion and to assess the potential for spread of non-native species (Geller et al. 2010). Microsatellites (e.g. Astanei et al. 2005; Dupont et al. 2007; Rius et al. 2012) and mitochondrial DNA markers (e.g. Audzijonyte et al. 2008; Hayes et al. 2008; Kajita et al. 2012) have been widely used in such studies, including in combination (e.g. Neilson and Stepien 2011; Zhan et al. 2012).

The genetic diversity and differentiation of non-native populations are shaped by several factors, including those of the source populations and the demography of the invasion (Holland 2000). The number of individuals introduced, diversity and differentiation of source populations, and eventual selective processes determine the proportion of genetic diversity that is retained in the introduced range (Wilson et al. 2009). In particular, multiple introductions have been shown to facilitate the maintenance of high genetic diversity in introduced populations compared to source populations (Roman and Darling 2007; Dlugosch and Parker 2008; Gillis et al. 2009). Indeed, it has been shown that human-mediated dispersal may result in higher levels of within-population genetic diversity when compared to native populations (Voisin et al. 2005; Wilson et al. 2009).

The Pacific oyster, *Crassostrea gigas*, native to northeast Asia, has been introduced worldwide for the purpose of aquaculture and is among the most important cultured shellfish species in the world (FAO 2011). Naturalized oyster populations are well established in several European countries and can have important ecological impacts on coastal ecosystems (Troost 2010).

C. gigas has a complex history of introductions in Europe, which are relatively well documented (Figure 1). Significant quantities of juveniles were first imported from the native

range (i.e., Japan) to British Columbia between 1926 and 1932 (Quayle 1988; Miossec et al. 2009). Massive introductions (562 tons of adult oysters from British Columbia, then 5 billion seed from the Miyagi prefecture in Japan) occurred in France in the 1970s to sustain oyster production, following severe declines of the Portuguese oyster *Crassostrea angulata* (Grizel and Héral 1991). These rapidly became established in the wild. In the United Kingdom, several small introductions from British Columbia (50-76 adults at a time) were undertaken in the 1960s and 1970s and placed in quarantine facilities for hatchery propagation (Walne and Helm 1979; Utting and Spencer 1992; Spencer et al. 1994). Sporadic natural spatfalls (settlement and attachment of young oysters to the substrate) were first reported in Britain in 1990 (Spencer et al. 1994). In Ireland, *C. gigas* was first introduced in 1969 from the UK quarantined stocks (Shatkin et al. 1997). However, an open trade agreement in 1993 (Council Directive 91/67/EEC) meant that imports of seed to Ireland from France were permitted (Minchin et al. 1993). Since 1986, oyster seed produced in UK hatcheries were regularly imported into the northern area of the German Wadden Sea near and in the island of Sylt (Reise 1998). In Denmark, since the 1970s, large numbers were imported from the UK, The Netherlands and France (Nehring 2006). In 1999, natural dispersal from the German Wadden Sea was reported in the Danish Wadden Sea (Reise et al. 2005). In Northern Europe, *C. gigas* populations became established more recently and have been qualified as feral, since they commonly occur close to oyster farms (Troost 2010); they have been shown to be demographically independent and self-sustaining (Kochmann et al. 2012).

Invasion genetics of *C. gigas* have been studied in the Wadden Sea (Moehler et al. 2011), the South of France to the North of the Wadden Sea (Meistertzheim et al. 2013), and the South of France to Sweden (Rohfritsch et al. 2013). Two main genetic groups were identified, with a population break located in the Wadden Sea: (1) a high diversity southern group (including samples from France, The Netherlands and southern Wadden Sea, along with Japan (native range) and British Columbia (secondary source of introduction to various European countries) and (2) a low diversity northern group (including Germany, northern Wadden Sea, Denmark and Sweden). However, the spread of this species both into and from the British Isles, presumed to have been pivotal in the invasion of this species in Northern Europe, is unknown. In the British Isles, *C. gigas* has a patchy distribution, with some locally dense occurrences; however there are concerns that its range may expand further. A better understanding of population connectivity would help to predict such potential for further expansion.

The aims of the present study were to: i) assess the genetic diversity and differentiation of feral populations of *C. gigas* in the British Isles, since based on the history of introductions, the British Isles sites are expected to comprise a single genetically defined group; ii) compare patterns from microsatellite and mitochondrial DNA markers; iii) compare genetic diversity patterns with knowledge about the history of introductions and aquaculture practices.

METHODS

Sampling sites

Oysters were sampled between October 2009 and August 2011, in 22 sites (Table 1a, Table S1), in the UK, Ireland, France, Spain and Denmark (Figure 3b). These included 8 sites in East England (Kent: KB, KHB, KR; Essex: ESS, EWM, EBW, EB; the Wash: WGS), 4 in South West England (Devon: DS, DPS, DY; Cornwall: CF), 2 in South Wales (Milford Haven: MGP, MPP), 2 in Northern Ireland (Co Down: SLN, SLG), 2 in Ireland (Co Donegal: ILS; Co Galway: IGB), 2 in Spain (Galicia: GO, GC), 1 in Denmark (Nordjylland: DAN) and 1 in France (Brittany: FRB). Sites from France and Denmark were included to represent the higher diversity southern group and the lower diversity northern group, respectively, identified by Rohfritsch et al. (2013). The oysters were collected in a variety of habitats, including rocky shores, chalk reefs, flint boulders, groins, mussel beds, gravel, shingle, mud and muddy shingle. At Garron Pill (MGP, South Wales), we collected both loose oysters (presumed to be from a disused aquaculture facility) and oysters attached to the rock (Supplementary Figure S1). The number of oysters collected per site were 200 for ILS; 100 for KB, KR, KHB, SLN, SLG, WGS, MGP, DPS, DY, ESS, EBW, EB and DAN; 99 for FRB; 98 for EWM; 95 for IGS and DS; 75 for CF; 40 for GO; 38 for GC; 37 for MPP; totaling 2077 samples (Table 1a). Pieces of gill were preserved in 80% ethanol.

Three hatchery-propagated stocks also were analyzed (Table 1a) from 2 companies (Hatchery A: HA (year 2008); Hatchery B: HB1 (year 2005) and HB2 (year 2006)). Fifty oysters were analyzed for HA; 46 for HB1 and 49 for HB2.

DNA extraction and microsatellite genotyping

DNA was extracted from gill tissue with chloroform/isoamyl alcohol, followed by absolute ethanol and sodium acetate (3M pH 4.8) precipitation. The DNA pellets were resuspended in 100 µl ultrapure water.

161 Eight EST-SSRs (CGE007, CGE009, AMY, Cgsili46, Cgsili44, Cgsili39, Cgsili50 and
162 Cgsili4) were amplified in three multiplex PCRs, following Li et al. (2010). Three
163 anonymous microsatellites (Cg108, Cg49: Magoulas et al. 1998; L10: Huvet et al. 2000a)
164 were amplified in a multiplex PCR reaction, following the same protocol, but with an
165 annealing temperature of 55°C and a final concentration of 0.15 µM of each primer. PCR
166 products were loaded in an ABI 3130*xl* Genetic Analyzer (Applied Biosystems) with
167 GeneScan™ 500 ROX™ size standard. Fragment lengths were determined using
168 GeneMapper v.4.0 (Applied Biosystems).
169
170 mtDNA amplification and sequencing
171 A 739-bp fragment of the Major Noncoding Region (MNR) of the mitochondrial genome was
172 amplified from18-25 randomly picked individuals per sample, from 12 of the 25 samples
173 analyzed, namely: ILS, SLG, EWM, KB, DS, DY, DAN, FRB, GO, HA, HB1 and HB2,
174 representing the major regions plus hatchery stocks. Additionally, 25 individuals from JM
175 (Miyagi Prefecture, Japan) were also analyzed. Since this population previously was
176 genotyped for microsatellite markers (Rohfritsch et al. 2013) only its mtDNA was analyzed
177 here. MNR sequence data from Moehler et al. (2011), including British Columbia (secondary
178 source for the introductions in the UK) and the European Wadden Sea (location of the genetic
179 break), were also analyzed. Primers developed by Aranishi and Okimoto(2005) were used in
180 25-µl PCR reactions containing 2 µl of 1:10 diluted DNA, 0.4 µM of each primer and 1X
181 PCR Master Mix with 1.5 mM $MgCl_2$ (Thermo Scientific) and PCR amplification protocol of
182 Moehler et al. (2011). Successful amplification was checked by electrophoresis on 2%
183 agarose gels. PCR products were purified using Exo-TSAP (Promega) and sent to Macrogen
184 Europe (Netherlands) for direct sequencing with the forward primer. Twenty-three singletons
185 (sequences occurring once in the dataset) were re-amplified and re-sequenced to control for
186 possible amplification or sequencing errors.
187
188 Microsatellite data analysis
189 Genotypic linkage disequilibrium was tested using the Markov chain exact probabilities from
190 GENEPOP version 4.2 (Raymond and Rousset 1995; Rousset 2008), for each pair of markers
191 per sample. Deviations from the Hardy Weinberg equilibrium (F_{IS}: estimator of fixation
192 index) were evaluated using FSTAT v.2.9.3.2 (Goudet 2002) software for both loci and
193 samples. Significance of any departure of F_{IS} values from 0 was assessed by randomizing

194 alleles within samples, using 5,500 randomizations. Bonferroni corrections were applied to
195 account for multiple comparisons.
196 Genetic diversity was assessed by computing number of alleles (N_a), allele
197 frequencies, allelic richness (A) (El Mousadik and Petit 1996), observed (H_o) and unbiased
198 expected (H_e) heterozygosities (Nei 1978) using FSTAT. The number of private alleles
199 (unique to a single site) was calculated with GenAlEx v.6.41 (Peakall and Smouse 2006).
200 Genetic differentiation was assessed using different methods. Firstly, genetic
201 differentiation was estimated using three methods. Theta (θ) (Weir and Cockerham 1984) was
202 calculated with FSTAT, while G_{st_est} (Nei and Chesser 1983) and D_{est} (Jost 2008) were
203 assessed using DEMEtics package (Gerlach et al. 2010). Pairwise genetic differentiation was
204 plotted as heat maps using Pheatmap package (Kolde 2012). Secondly, neighbor-joining
205 dendrograms based on pairwise Nei (D_a) genetic distances (Nei and Chesser 1983) were
206 made with POPTREE2 (Takezaki et al. 2010). Confidence estimates of tree topology were
207 calculated by bootstrap resampling of loci 1,000 times. Thirdly, to detect hierarchical genetic
208 structure among sites, Analyses of Molecular Variance (AMOVA) (Excoffier et al. 1992)
209 were conducted in GenAlEx 6.41. Molecular variance was partitioned into three levels:
210 among clusters, among populations within clusters and within populations. Significance was
211 determined using 1,000 permutations. Fourthly, the pattern of isolation by distance (IBD) was
212 assessed by plotting pairwise $F_{ST}/(1-F_{ST})$ values against the logarithm of the geographic
213 distances (Euclidian distance and marine geographic distance i.e. nearest waterway distance)
214 between all sample sites, as recommended for a two-dimensional model of IBD (Rousset
215 1997). The statistical significance of the correlation coefficient was tested using a Mantel test
216 implemented in GENEPOP, with 10,000 permutations. The analyses were carried out at the
217 global scale and within each cluster. The 3 hatchery samples were excluded from those
218 analyses. Finally, we applied a non-spatial Bayesian model-based clustering algorithm,
219 implemented in STRUCTURE v.2.3.3 (Pritchard et al. 2000), to further assess genetic
220 structure. It infers the number of genetic clusters K and assigns individuals to clusters from
221 the individual's genotypes dataset, while minimizing Hardy Weinberg disequilibrium and
222 gametic phase disequilibrium between loci within groups. Ten independent runs were
223 performed for each K using 1,000,000 iterations and a burn-in period of 100,000, with the
224 model allowing for admixture and correlated allele frequencies between clusters (Falush et al.
225 2003), with and without sample group information (Hubisz et al. 2009). The number of
226 clusters was estimated using the ΔK method (Evanno et al. 2005) as performed in
227 STRUCTURE HARVESTER (Earl and von Holdt 2012). CLUMPP v.1.1.2 (Jakobsson and

228 Rosenberg 2007) was used to average the assignment scores over the 10 runs and results were
229 visualized in DISTRUCT v.1.1 (Rosenberg 2004).
230
231 mtDNA data analysis
232 MNR sequences were checked visually and aligned using CodonCode Aligner software
233 (CodonCode Corporation). Genetic diversity was assessed by calculating the numbers of
234 haplotypes (H_t), singletons (H_u) and polymorphic sites (S); haplotype (H_d) and nucleotide (π)
235 diversities (Nei 1987) using DnaSP v.5 (Rozas et al. 2003). Population differentiation was
236 assessed by calculating pairwise genetic differentiation (F_{ST}) using Arlequin v.3.1 (Excoffier
237 et al. 2005), with the pairwise difference distance method.
238 We performed Spatial Analysis of Molecular Variance (SAMOVA 1.0, Dupanloup et
239 al. 2002, available at http://cmpg.unibe.ch/software/samova), which aims to define groups of
240 populations that are geographically homogeneous, by maximizing F_{CT} value (i.e., the
241 proportion of total genetic variance due to differences between groups of populations).
242 Subdivisions were tested with a range of K values from two to eleven geographic groups
243 using 100 simulated annealing processes.
244 In order to attempt to compare genetic patterns with the history of introductions, a
245 haplotype distribution map was generated, covering the British Isles, Japan (overall source
246 for the introductions in Europe), the European Wadden Sea (location of the genetic break in
247 Europe) and British Columbia (secondary introduction to the UK). A median-joining network
248 (Bandelt et al. 1999) was built using NETWORK v.4.6.1 to reconstruct the phylogenetic
249 relationships among haplotypes.
250
251 **RESULTS**
252
253 Genetic diversity
254 Significant linkage disequilibrium was found in 138 out of 1375 pairwise comparisons among
255 11 loci for all populations. After Bonferroni correction, 47 tests remained significant.
256 However, most of the genotypic linkage disequilibrium detected was due to the hatchery
257 stocks (39 significant tests). Overall, there were significant heterozygote deficiencies for the
258 22 feral samples, and for 1 out of 3 hatchery batches, mainly due to loci Cg49, CGE007,
259 Cgsili39 and Cg108. When those 4 loci were removed from the analyses, significant
260 heterozygote deficiencies remained for only 2 out of the 22 feral samples, FRB (F_{IS} 0.051)
261 and GO (F_{IS} 0.070) (Table 1a). Based on the remaining 7 microsatellites, there were

262 heterozygote excesses in the three hatchery batches (F_{IS} between -0.081 and -0.052; Table
263 1a). As most analyses rely on Hardy Weinberg equilibrium assumptions, all further analyses
264 were done with the 7 microsatellites conforming to Hardy Weinberg equilibrium expectations
265 (CGE009, AMY, Cgsili46, Cgsili44, Cgsili50, Cgsili4 and L10).
266 Genetic diversity and heterozygosity per sample are given in Table S2, for each locus.
267 Overall estimates, averaged over the 7 loci, are given in Table 1a. Mean allelic richness
268 ranged from 13.68 (WGS)-22.34 (DY) for the 22 studied samples, and from 11.10-12.54 for
269 the 3 hatchery batches. The mean number of private alleles ranged from 0 (KB, KR, KHB,
270 SLG, WGS, ILS, ESS, EWM, EBW, EB, DAN)-1.71 (FRB) for the feral samples, and 0-0.14
271 for the hatchery batches. Mean expected heterozygosity (H_e) ranged from 0.880 (KB)-0.931
272 (GO) for the feral populations, and from 0.853-0.874 for the hatchery batches.
273 The MNR aligned dataset, derived from 273 individuals, consisted of 674 nucleotides
274 with 109 (16.2%) variable positions, including 88 transitions, 7 transversions, and 15 indels.
275 Of the 81 haplotypes identified, 19 were identical to those previously reported by Moehler et
276 al. (2011). The 23 randomly-chosen singletons that were re-amplified and re-sequenced led to
277 identical sequences. Two shared singletons (H10, H34) and 5 private singletons (H16, H28,
278 H39, H45, H58) found in this study were identical to haplotypes found in Moehler et al.
279 (2011). In the hatchery batches, 4-8 haplotypes were found, with no singletons. The samples
280 DS, DY, FRB, GO, JM and SLG exhibited a high number of haplotypes (12-18) and
281 singletons (4-15). In contrast, DAN, EWM, KB and ILS exhibited a lower number of
282 haplotypes (6-9) with just 1 or no singletons (Table 1b). Haplotype diversity ranged from
283 0.695 (Hatchery A) to 0.963 (DS), and nucleotide diversity from 0.00219 (GO) to 0.00552
284 (EWM and Hatchery B) (Table 1b).
285
286 Genetic differentiation
287 Based on the microsatellite markers, significant global genetic differentiation was detected
288 among sites. θ and G_{st_est} values were very similar therefore only G_{st_est} values are reported in
289 Table S3. Global θ was 0.014 (95% CI: 0.012-0.018), G_{st_est} was 0.017 (95% CI: 0.016-0.017)
290 and global D_{est} was 0.156 (95% CI: 0.151-0.160). Genetic differentiation between each pair of
291 samples is shown in Figure 2A as heat maps. Pairwise θ and D_{est} values are reported in Table
292 S4, which showed very similar patterns of genetic differentiation. Two main groupings were
293 observed; a Northeast cluster (sites from East England, Northern Ireland, Ireland, and
294 Denmark) and a Southwest cluster (sites from South Wales, South West England, Spain and

295 France). It was clear from the heat maps that the hatchery batches were more closely related
296 to the Northeast cluster than to the Southwest cluster.
297 The neighbor-joining phylogram also revealed two clusters, Southwest and
298 Northeast / UK hatcheries cluster (Figure 3A).
299 Bayesian clustering analysis further confirmed this pattern: two clear genetic clusters
300 were identified (K=2) (Figure 3B-C). In the majority of cases, each sample was composed of
301 individuals with a high probability of belonging to only one of the 2 clusters (Figure 3C). For
302 the Irish (SLN, SLG, IGB, ILS) sites, there seemed to be a certain level of admixture of the
303 two genetic clusters (Figure 3C): each individual was cross-assigned to both clusters. For
304 Garron Pill sample (MGP), the genetic signature observed in the STRUCTURE analysis
305 (Figure 3C) confirmed the sampled oysters were from two different origins (Figure S1).
306 Based on the mtDNA marker, pairwise F_{ST} values highlighted two groups: Southwest /
307 Japan (GO, FRB, DY, DS, JM) and Northeast (KB, EWM, ILS, SLG, DAN) (Figure 2B).
308 Within the Southwest / Japan cluster, F_{ST} values ranged from 0.0005-0.0075. Within the
309 Northeast cluster, F_{ST} values ranged from 0.0130 to 0.0679. Between those two clusters, F_{ST}
310 values were between 0.0187 (SLG-DY) and 0.1683 (ILS-FRB). The two hatchery stocks
311 exhibited a higher genetic differentiation from the Southwest / Japan cluster than to the
312 Northeast cluster. The F_{ST} value between the two hatchery stocks was 0.1072-0.1486 (Table
313 S5). Among the 81 haplotypes identified, 13 were shared among sites. Haplotype frequencies
314 are visualized on a geographic map in Figure 4A. DS, DY, FRB, GO and JM (Southwest /
315 Japan cluster) were characterized by a high proportion of singletons (52 to 64%) and the
316 dominance of haplotype H8 (20 to 39%). KB, EWM, ILS, SLG and DAN (Northeast cluster)
317 shared almost all their haplotypes with the hatchery stocks (e.g., H1, H2, H3, H4, H5), and
318 had a more balanced haplotypes distribution. A median-joining network analysis revealed
319 shallow divergence among the 81 haplotypes. Most haplotypes differed only by a single
320 nucleotide substitution from the central haplotype H8, resulting in a star-like pattern (Figure
321 4B).
322
323 Genetic diversity and differentiation of the inferred clusters
324 The Wilcoxon paired sample test revealed a significantly higher allelic richness (A) and
325 expected heterozygosity (H_e) in the Southwest cluster (A: 42.08, H_e: 0.928) than in the
326 Northeast / Hatcheries cluster (A: 24.94, H_e: 0.891). Also, there were significantly more
327 private alleles in the Southwest cluster (14.429 ± 1.850) than in the Northeast / Hatcheries
328 cluster (1.000 ± 0.378). F_{IS} was 0.032 (p=0.0036) for the Southwest cluster and -0.003

329 (p=0.8107) for the Northeast / Hatcheries cluster. The F_{ST} between those two clusters was
330 0.017 (95% CI: 0.014-0.021).
331 After exclusion of the GP sample (mixture of individuals belonging to different
332 clusters) from the analysis, hierarchical AMOVA attributed the majority of variation to
333 intrapopulation differences (96-97.2%, p=0.001). However, the AMOVA revealed a
334 significant genetic structuring among the two STRUCTURE identified groups (3.2%,
335 p=0.001).
336 At the global scale, the correlation between $F_{ST}/(1-F_{ST})$ and the logarithm of
337 geographic distance was significant using Euclidian distance (r=0.479, P<0.001) or using the
338 marine geographic distance (r=0.432, P<0.001). Within each cluster, correlations were also
339 significant for the Northeast cluster (Euclidian distance: r=0.665, P<0.001; marine distance:
340 r=0.639, P<0.001), but not for the Southwest cluster (Euclidian distance: r=0.195, P=0.118;
341 marine distance: r=0.206, P=0.099). Genetic (F_{ST}) and marine geographic distances were
342 plotted within and between clusters. For a given geographic distance, genetic differentiation
343 between pairs of samples from different clusters was larger than that between pairs within the
344 cluster (Figure 5). Therefore, genetic differentiation between the Northeast and Southwest
345 clusters was not solely attributable to geographic distance.
346 Based on the mtDNA marker, there was a much higher number of haplotypes and
347 haplotype diversity in the Southwest / Japan cluster (H_t: 69, H_d: 0.879 ± 0.031) than in the
348 Northeast / Hatchery B cluster (H_t: 18, H_d: 0.851 ± 0.017) and Hatchery A (H_t: 4, H_d: 0.695 ±
349 0.079). Pairwise F_{ST} values were 0.118 between Northeast / Hatchery B and Southwest /
350 Japan clusters (P<0.001), 0.066 between Northeast / Hatchery B cluster and Hatchery A
351 (P=0.009), and 0.148 between Southwest / Japan cluster and Hatchery A (P<0.001). The
352 SAMOVA analysis led to an optimal number of four geographic groups: ILS-KB-HB1-HB2
353 (Ireland / East England / Hatchery B), HA (Hatchery A), DAN-EWM (Denmark / East
354 England) and DS-DY-FRB-GO-JM-SLG (Southwest / Japan / North Ireland) (F_{CT}=0.12232,
355 P<0.001). Finally, 12.23% of the total genetic variation was explained by variation among
356 groups of samples, whereas variation within samples accounted for 87.77%.
357
358 **DISCUSSION**
359
360 There is a need for a better integrated understanding of how genetic diversity can be
361 reshuffled by anthropogenic activities during the invasion process (Keller et al. 2012).
362 Elucidating the factors that determine the invasive capacity of species is important not only to

363 help predict the likeliness of an invasion, but also to propose management strategies and
364 mitigation measures to minimize ecological impact. The introduction of *C. gigas* in Europe
365 is relatively well-documented (Figure 1) and therefore constitutes a rare opportunity to
366 confirm (or invalidate) by genetic analysis its introduction history and genetic signature and
367 to document the relationships between propagule pressure, aquaculture practices and genetic
368 diversity.
369
370 Genetic diversity and differentiation of *C. gigas* in the British Isles
371 In our study, we combined two types of markers, nuclear (microsatellites) and mitochondrial
372 (Major Noncoding Region: MNR). In our study, results from both types of markers were
373 highly correlated. Based on the history of introductions (Figure 1), we hypothesized that there
374 should be genetic homogeneity among sites in the British Isles. However, the fact that two
375 clear genetic clusters were observed in the UK (Figures 3 and 4) suggests that the source of
376 *C. gigas* in Southwest England and South Wales was not solely UK hatcheries as initially
377 presumed. Possible explanations include natural dispersal from North Brittany, importation of
378 seed from natural recruitment from France for cultivation purposes, unintentional
379 introduction by hull fouling or release of larvae from ship ballast water. However, we do not
380 have evidence to favor one possibility over the others.
381 The lower genetic diversity in the Northeast / Hatcheries cluster (Table 1) was
382 however expected as this has frequently been reported in hatchery shellfish seed (e.g. Gaffney
383 et al. 1992; Taris et al. 2006; Hara and Sekino 2007; Lind et al. 2009). The presence of two
384 genetic clusters poses the question of the implications of the level of genetic diversity on their
385 adaptive potential (i.e., invasion success). It is generally accepted that introduction events can
386 be accompanied by founder events and that associated loss of genetic diversity may result in
387 inbreeding depression, which could reduce ability of the invasive species to adapt to its new
388 environment. However, there have been several cases of introduced populations with low
389 genetic diversity (genetic bottlenecks) that have been successful (ant *Linepithema humile*:
390 Tsutsui et al. 2000, Yang et al. 2012; mosquitofish *Gambusia affinis*: Purcell et al. 2012). The
391 "genetic paradox" concept in invasion biology questions how newly founded populations can
392 overcome low genetic diversity and associated constraints on evolutionary potential outside
393 their native range (Roman and Darling 2007). However, in the majority of successful aquatic
394 invasions, introduced populations are characterized by no observed reduction in genetic
395 diversity compared with the native range and, on some occasions, exhibit even higher genetic
396 diversity due to multiple introductions from various sources (Roman and Darling 2007;

397 Dlugosch and Parker 2008). The role of multiple introductions in facilitating biological
398 invasions is now well recognized (Geller et al. 2010): multiple introductions from various
399 sources can increase genetic diversity in invaders, hence avoiding the potential negative
400 impacts of genetic bottlenecks (Roman and Darling 2007; Dlugosch and Parker 2008); also
401 the conversion of among-population genetic diversity in the native range to within-population
402 genetic diversity in the introduced range can promote range expansion (European green crab
403 *Carcinus maenas*: Roman 2006; nassariid gastropod *Cyclope neritea*: Simon-Bouhet et al.
404 2006) and create novel allelic combinations which can drive phenotypic diversification
405 (Cuban lizard *Anolis sagrei*: Kolbe et al. 2007). Therefore, the relationship between level of
406 genetic diversity and invasion success is not straightforward and requires further
407 investigation in *C. gigas*.
408
409 <u>Genetic diversity patterns are consistent with history of introductions and aquaculture</u>
410 <u>practices</u>
411 Combining our results with previous studies centered on the Wadden Sea (Moehler et al.
412 2011) or performed at a larger European scale (Meistertzheim et al. 2013; Rohfritsch et al.
413 2013), two distinct European genetic clusters were identified. Our results suggest that genetic
414 diversity patterns are generally consistent with the history of introductions (Figure 1).
415 However, the genetic pattern observed does not reflect some reported introduction events,
416 namely the introduction of seed from France and the Netherlands to Denmark (Nehring
417 2006), suggesting that those importations did not result in considerable spatfall.
418 Part of the genetic diversity patterns observed in the present study can be explained by
419 aquaculture practices. In France, large scale introductions took place resulting in rapid
420 establishment (Robert and Gérard 1999). The fact that populations from Brittany and Galicia
421 clustered with those from Miyagi and British Columbia (Figure 4A-C) shows that the high
422 genetic diversity present in the native range was maintained during the introductions, as
423 previously proposed by Huvet et al. (2000b). In contrast, in the UK, having three small
424 introductions of adult oysters, founder effects and resulting genetic bottlenecks occurred.
425 Subsequently, the breeding practices performed in the quarantine facilities of MAFF (Utting,
426 pers. com.) might have led to further shifts in allele and haplotype frequencies, resulting from
427 the high variance in reproductive success known to occur in this species (Boudry et al. 2002).
428 Overall, our results show that its genetic diversity patterns are mostly consistent with
429 its known history of introductions. This study highlights how the number and size of
430 introduction events, aquaculture practices (natural recruitment versus hatchery-produced

seed), genetic drift and natural dispersal can act concurrently to shape the genetic diversity and differentiation of introduced populations. The elucidation of pathways of introduction and dissemination of *C. gigas* in Europe contributes to the establishment of future management strategies that might also be relevant for other invasive marine species.

Acknowledgements

This research was partially financed by the European Union Atlantic Area Transnational Programme (2007-2013), project SEAFARE, grant agreement n°2009-1/123. The authors thank José Luis Gomez and Lasse Fast Jensen for providing oysters samples from Galicia and Limfjord. The authors are very grateful to Mike Camplin, Roger Covey, Claire Guy, Philip Hollyman, Ron Jessop, Judith Kochmann, Sven Laming, Hywel Lloyd, Willie McKnight, Nigel Mortimer, Hanna Nuuttila, Francis O'Beirn, Jack Sewell, Romy Wild and Nadescha Zwerschke for help during the field work. The Bayesian clustering analysis (STRUCTURE) was conducted on the University of Oslo Bioportal. Finally, the authors are grateful to Mathias Wegner for sharing the haplotype sequences and frequency table of the Wadden Sea samples. We thank associate editor Carol Stepien for correcting the English.

References

Aranishi F, Okimoto T (2005) Sequence polymorphism in a novel noncoding region of Pacific oyster mitochondrial DNA. J Appl Genet 46 (2):201-206

Astanei I, Gosling E, Wilson J, Powell E (2005) Genetic variability and phylogeography of the invasive zebra mussel, *Dreissena polymorpha* (Pallas). Mol Ecol14 (6):1655-1666

Audzijonyte A, Wittmann KJ, Väinölä R (2008) Tracing recent invasions of the Ponto-Caspian mysid shrimp *Hemimysis anomala* across Europe and to North America with mitochondrial DNA. Divers Distrib14 (2):179-186

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16 (1):37-48

Boudry P, Collet B, Cornette F, Hervouet V, Bonhomme F (2002) High variance in reproductive success of the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed by microsatellite-based parentage analysis of multifactorial crosses. Aquaculture 204 (3-4):283-296

464 Carlton JT (1989) Man's role in changing the face of the ocean: biological invasions and
465 implications for conservation of near-shore environments. Conserv Biol 3 (3):265-273
466 Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation,
467 adaptive evolution, and the role of multiple introductions. Mol Ecol 17 (1):431-449
468 Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the
469 genetic structure of populations. Mol Ecol 11 (12):2571-2581
470 Dupont L, Ellien C, Viard F (2007) Limits to gene flow in the slipper limpet *Crepidula*
471 *fornicata* as revealed by microsatellite data and a larval dispersal model. Mar Ecol Prog
472 Ser 349:125-138
473 Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for
474 visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet
475 Resour 4 (2):359-361
476 El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness
477 among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco.
478 Theor Appl Genet 92 (7):832-839
479 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using
480 the software STRUCTURE: a simulation study. Mol Ecol 14 (8):2611-2620
481 Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software
482 package for population genetics data analysis. Evol Bioinform 1:47-50
483 Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from
484 metric distances among DNA haplotypes: application to human mitochondrial DNA
485 restriction data. Genetics 131:479-491
486 Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus
487 genotype data: linked loci and correlated allele frequencies. Genetics 164 (4):1567-1587
488 Gaffney PM, Davis CV, Hawes RO (1992) Assessment of drift and selection in hatchery
489 populations of oysters (*Crassostrea virginica*). Aquaculture 105 (1):1-20
490 Geller JB, Darling JA, Carlton JT (2010) Genetic perspectives on marine biological
491 invasions. Annu Rev Mar Sci 2:367-393
492 Gerlach G, Jueterbock A, Kraemer P, Deppermann J, Harmand P (2010) Calculations of
493 population differentiation based on G_{ST} and D: forget G_{ST} but not all of statistics! Mol
494 Ecol19 (18):3845-3852
495 Gillis NK, Walters LJ, Fernandes FC, Hoffman EA (2009) Higher genetic diversity in
496 introduced than in native populations of the mussel *Mytella charruana*: evidence of
497 population admixture at introduction sites. Divers Distrib15 (5):784-795

498 Goudet J (2002) Fstat 2.9.3.2. URL: http://www2.unil.ch/popgen/softwares/fstat.htm.
499 Grizel H, Héral M (1991) Introduction into France of the Japanese oyster (*Crassostrea*
500 *gigas*). J Cons int Explor Mer 47:399-403
501 Grosholz E (2002) Ecological and evolutionary consequences of coastal invasions.Trends
502 Ecol Evol 17 (1):22-27
503 Hara M, Sekino M (2007) Genetic Differences Between Hatchery Stocks and Natural
504 Populations in Pacific Abalone (*Haliotis discus*) Estimated Using Microsatellite DNA
505 Markers. Mar Biotechnol 9:74-81
506 Hayes KA, Joshi RC, Thiengo SC, Cowie RH (2008) Out of South America: multiple origins
507 of non-native apple snails in Asia. Divers Distrib14 (4):701-712
508 Holland BS (2000) Genetics of marine bioinvasions. Hydrobiologia 420 (1):63-71
509 Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure
510 with the assistance of sample group information. Mol Ecol Resour 9 (5):1322-1332
511 Huvet A, Boudry P, Ohresser M, Delsert C, Bonhomme F (2000a) Variable microsatellites in
512 the Pacific oyster *Crassostrea gigas* and other cupped oyster species. Anim Genet 31
513 (1):71-72
514 Huvet A, Lapègue S, Magoulas A, Boudry P (2000b) Mitochondrial and nuclear DNA
515 phylogeography of *Crassostrea angulata*, the Portuguese oyster endangered in Europe.
516 Conserv Genet 1 (3):251-262
517 Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program
518 for dealing with label switching and multimodality in analysis of population structure.
519 Bioinformatics 23 (14):1801-1806
520 Jost L (2008) G_{ST} and its relatives do not measure differentiation. Mol Ecol 17 (18):4015-
521 4026
522 Kajita Y, O'Neill EM, Zheng Y, Obrycki JJ, Weisrock DW (2012) A population genetic
523 signature of human releases in an invasive ladybeetle. Mol Ecol 21 (22):5473-5483
524 Keller SR, Gilbert KJ, Fields PD, Taylor DR (2012) Bayesian inference of a complex
525 invasion history revealed by nuclear and chloroplast genetic diversity in the colonizing
526 plant, *Silene latifolia*. Mol Ecol 21 (19):4721-4734
527 Kochmann J, Carlsson J, Crowe TP, Mariani S (2012) Genetic evidence for the uncoupling of
528 local aquaculture activities and a population of an invasive species - a case study of
529 Pacific oysters (*Crassostrea gigas*). J Hered 103 (5):661-671
530 Kolbe JJ, Larson A, Losos JB (2007) Differential admixture shapes morphological variation
531 among invasive populations of the lizard *Anolis sagrei*. Mol Ecol 16 (8):1579-1591

Kolde R (2012) pheatmap: Pretty Heatmaps. R package version 0.6.1. http://CRAN.R-project.org/package=pheatmap.

Lacoursière-Roussel A, Bock DG, Cristescu ME, Guichard F, Girard P, Legendre P, McKindsey CW (2012) Disentangling invasion processes in a dynamic shipping–boating network. Mol Ecol 21 (17):4227-4241

Li R, Li Q, Cornette F, Dégremont L, Lapègue S (2010) Development of four EST-SSR multiplex PCRs in the Pacific oyster (*Crassostrea gigas*) and their validation in parentage assignment. Aquaculture 310 (1-2):234-239

Lind CE, Evans BS, Knauer J, Taylor JJU, Jerry DR (2009) Decreased genetic diversity and a reduced effective population size in cultured silver-lipped pearl oysters (*Pinctada maxima*) Aquaculture 286 (1-2):12-19

Magoulas A, Ghjetvaj B, Terzoglou V, Zouros E (1998) Three polymorphic microsatellites in the Japanese oyster, *Crassostrea gigas* (Thunberg). Anim Genet 29:69-70

Meistertzheim A-L, Arnaud-Haond S, Boudry P, Thébault M-T (2013) Genetic structure of wild European populations of the invasive Pacific oyster *Crassostrea gigas* due to aquaculture practices. Mar Biol160 (2):453-463

Minchin D, Duggan CB, Holmes JMC, Neiland S (1993) Introductions of exotic species associated with Pacific oyster transfers from France to Ireland. ICES, Copenhagen (Denmark) 11, CM 1993/F:27

Miossec L, Le Deuff RM, Goulletquer P (2009) Alien species alert: *Crassostrea gigas* (Pacific oyster). ICES Cooperative Research Report 299:42 pp.

Moehler J, Wegner KM, Reise K, Jacobsen S (2011) Invasion genetics of Pacific oyster *Crassostrea gigas* shaped by aquaculture stocking practices. J Sea Res 66:256-262

Nehring S (2006) NOBANIS - Invasive Alien Species Fact Sheet – *Crassostrea gigas*. From: Online Database of the North European and Baltic Network on Invasive Alien Species - NOBANIS www.nobanis.org.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89 (3):583-590

Nei M (1987) Molecular Evolutionary Genetics.Columbia University Press, New York

Nei M, Chesser RK (1983) Estimation of fixation indices and gene diversities. Ann Hum Genet 47 (3):253-259

Neilson ME, Stepien CA (2011) Historic speciation and recent colonization of Eurasian monkey gobies (*Neogobius fluviatilis* and *N. pallasi*) revealed by DNA sequences, microsatellites, and morphology. Divers Distrib17 (4):688-702

Peakall ROD, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6 (1):288-295

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155 (2):945-959

Purcell K, Ling N, Stockwell C (2012) Evaluation of the introduction history and genetic diversity of a serially introduced fish population in New Zealand. Biol Invasions 14 (10):2057-2065

Quayle DB (1988) Pacific oyster culture in British Columbia. Can B Fish Aquat Sci 218:241 pp.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248-249

Reise K (1998) Pacific oysters invade mussel beds in the European Wadden Sea. Senck Marit 28:167-175

Reise K, Dankers N, Essink K (2005) Introduced species. In: Essink K, Dettmann, C., Farke, H., Laursen, K., Lüerßen, G., Marencic, H. and Wiersinga, W. (ed) Wadden Sea Quality Status Report 2004. Wadden Sea Ecosystem No. 19.pp 155-161

Rius M, Turon X, Ordóñez V, Pascual M (2012) Tracking invasion histories in the sea: facing complex scenarios using multilocus data. PLoS One 7 (4):e35815

Robert R, Gérard A (1999) Bivalve hatchery technology: The current situation for the Pacific oyster *Crassostrea gigas* and the scallop *Pecten maximus*in France. Aquat Living Resour 12 (2):121-130

Rohfritsch A, Bierne N, Boudry P, Heurtebise S, Cornette F, Lapègue S (2013) Population genomics shed light on the demographic and adaptive histories of European invasion in the Pacific oyster, *Crassostrea gigas*. Evol Appl 6 (7):1064-1078

Roman J (2006) Diluting the founder effect: cryptic invasions expand a marine invader's range. Proc Roy Soc B 273 (1600):2453-2459

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22 (9):454-464

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Mol Ecol Notes 4 (1):137-138

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145 (4):1219-1228

Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8 (1):103-106

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19 (18):2496-2497.

Shatkin G, Shumway SE, Hawes R (1997) Considerations regarding the possible introduction of the Pacific oyster (*Crassostrea gigas*) to the Gulf of Maine: A review of global experience. J Shellfish Res 16 (2):463-477

Simon-Bouhet B, Garcia-Meunier P, Viard F (2006) Multiple introductions promote range expansion of the mollusk *Cyclope neritea* (Nassariidae) in France: evidence from mitochondrial sequence data. Mol Ecol 15 (6):1699-1711

Spencer BE, Edwards DB, Kaiser MJ, Richardson CA (1994) Spatfalls of the non-native Pacific oyster, *Crassostrea gigas*, in British waters. Aquat Conserv Mar Freshwat Ecosyst 4:203-217

Takezaki N, Nei M, Tamura K (2010) POPTREE2: software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. MolBiolEvol27 (4):747-752

Taris N, Ernande B, McCombie H, Boudry P (2006) Phenotypic and genetic consequences of size selection at the larval stage in the Pacific oyster (*Crassostreagigas*). J Exp Mar BiolEcol333 (1):147-158

Troost K (2010) Causes and effects of a highly successful marine invasion: Case-study of the introduced Pacific oyster *Crassostrea gigas* in continental NW European estuaries. J Sea Res 64 (3):145-165

Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. P Natl Acad Sci USA 97 (11):5948-5953

Utting SD, Spencer BE (1992) Introductions of marine bivalve molluscs into the United Kingdom for commercial culture - case histories. ICES Mar Sc194:84-91

Voisin M, Engel CR, Viard F (2005) Differential shuffling of native genetic diversity across introduced regions in a brown alga: aquaculture vs. maritime traffic effects. P Natl Acad Sci USA 102 (15):5432-5437

Walne PR, Helm MM (1979) Introduction of *Crassostrea gigas* into the United Kingdom. In: Mann R (ed) Exotic species in mariculture. MIT Press, Cambridge, Massachusetts.363 pp., pp 83-105

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370

Wilson JRU, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM (2009) Something in the way you move: dispersal pathways affect invasion success. Trends Ecol Evol 24 (3):136-144

Yang C-C, Ascunce MS, Luo L-Z, Shao J-G, Shih C-J, Shoemaker D (2012) Propagule pressure and colony social organization are associated with the successful invasion and rapid range expansion of fire ants in China. Mol Ecol 21 (4):817-833

Zhan A, Perepelizin PV, Ghabooli S, Paolucci E, Sylvester F, Sardiña P, Cristescu ME, MacIsaac HJ (2012) Scale-dependent post-establishment spread and genetic diversity in an invading mollusc in South America. Divers Distrib18 (10):1042-1055

Table 1. a) Genetic diversity indices based on7microsatellite markers. Sites locations and their abbreviations (Abbr.), sample size (*N*), habitat in which the oysters were collected, as well as key summary genetic parameters: allelic richness (*A*), number of private alleles (N_p), expected heterozygosity (H_e) with standard deviations (SD) and F_{IS} values. Significant F_{IS} values are in bold (5,500 randomizations). b) Genetic diversity indices of *C. gigas* MNR mitochondrial DNA sequences. *N*: number of samples successfully sequenced. H_t: number of all haplotypes. H_u: number of singletons (haplotypes found only once at a single site). *S*: number of polymorphic sites. H_d: haplotype diversity. π: nucleotide diversity. SD: standard deviation. Northeast cluster includes KB, EWM, SLG, ILS and DAN. Southwest cluster includes DS, DY, FRB and GO.

a)

	Abbr.	**Geographic region**	**Location**	***N***	**Habitat/substrate**	***A* ± SD**	**N_p ± SD**	**H_e ± SD**	**F_{IS}**
Feral	KB	Kent (E England)	Birchington	100	Chalk reef	13.82 ± 3.99	0.00 ± 0.00	0.880 ± 0.03	-0.010
	KR	Kent (E England)	Ramsgate	100	Groyne	13.89 ± 4.14	0.00 ± 0.00	0.881 ± 0.03	-0.002
	KHB	Kent (E England)	Herne Bay	100	Flint boulders	13.93 ± 3.91	0.00 ± 0.00	0.888 ± 0.03	0.007
	SLN	Co Down (N Ireland)	Strangford Lough	100	Gravel and shingle	15.67 ± 4.02	0.14 ± 0.14	0.897 ± 0.04	-0.008
	SLG	Co Down (N Ireland)	Strangford Lough	100	Gravel and shingle	16.35 ± 4.96	0.00 ± 0.00	0.897 ± 0.04	-0.007
	WGS	The Wash (E England)	Gat Sand	100	Mussel bed	13.68 ± 4.54	0.00 ± 0.00	0.882 ± 0.05	-0.002
	ILS	Co Donegal (Ireland)	LoughSwilly	200	Mussel bed	15.37 ± 3.67	0.00 ± 0.00	0.894 ± 0.03	-0.016
	IGB	Co Galway (Ireland)	Galway Bay	95	Harbour wall	15.55 ± 4.45	0.14 ± 0.14	0.895 ± 0.04	-0.005
	MGP	Milford Haven (Wales)	GarronPill	100	Mud and shingle	20.28 ± 7.03	0.14 ± 0.14	0.915 ± 0.04	0.021
	MPP	Milford Haven (Wales)	Pennar Point	37	Rock	21.19 ± 7.42	0.29 ± 0.18	0.928 ± 0.04	0.014
	DS	Devon (SW England)	Salcombe estuary	95	Rock	21.62 ± 6.78	1.00 ± 0.44	0.930 ± 0.04	0.024
	DPS	Devon (SW England)	Plymouth	100	Rock	21.39 ± 7.60	1.00 ±	0.925 ±	0.015

			Sound				0.31	0.04	
	DY	Devon (SW England)	Yealmestuary	100	Rock	22.34 ± 7.56	0.86 ± 0.34	0.930 ± 0.04	0.034
	CF	Cornwall (SW England)	Falestuary	75	Rock	21.30 ± 6.13	0.71 ± 0.18	0.925 ± 0.03	0.013
	ESS	Essex (E England)	Southend on Sea	100	Gravel and shingle	14.06 ± 4.21	0.00 ± 0.00	0.888 ± 0.04	0.001
	EWM	Essex (E England)	West Mersea	98	Mud and shingle	14.31 ± 4.24	0.00 ± 0.00	0.891 ± 0.03	-0.016
	EBW	Essex (E England)	Blackwater	100	Mud and shingle	13.86 ± 4.38	0.00 ± 0.00	0.885 ± 0.04	0.004
	EB	Essex (E England)	Brightlingsea	100	Mud and shingle	14.42 ± 4.26	0.00 ± 0.00	0.888 ± 0.04	0.013
	FRB	Brittany (France)	Rade de Brest	99	Rock	22.10 ± 8.48	1.71 ± 0.52	0.928 ± 0.04	**0.051**
	DAN	Nordjylland (Denmark)	Limfjord	100	Mussel bed	15.01 ± 4.63	0.00 ± 0.00	0.887 ± 0.05	0.027
	GO	Galicia (Spain)	Ria de Ortigueira	40	Rock	22.26 ± 6.76	0.57 ± 0.20	0.931 ± 0.04	**0.070**
	GC	Galicia (Spain)	Ria de Celeiro	38	Rock	21.31 ± 7.01	0.57 ± 0.20	0.920 ± 0.04	0.045
Hatchery	HA	United Kingdom	A – year 2008	50	n/a	11.57 ± 3.47	0.14 ± 0.14	0.853 ± 0.04	-0.058
	HB1	United Kingdom	B – year 2005	46	n/a	11.10 ± 2.21	0.00 ± 0.00	0.854 ± 0.04	**-0.081**
	HB2	United Kingdom	B – year 2006	49	n/a	12.54 ± 3.10	0.00 ± 0.00	0.874 ± 0.04	-0.052

b)

Population	***N***	H_t	H_u	***S***	H_d **(SD)**	π **(SD)**

DS	20	16	11	20	0.963 (0.033)	0.00330 (0.00043)
DY	22	17	14	29	0.935 (0.047)	0.00424 (0.00065)
FRB	21	14	11	14	0.786 (0.096)	0.00227 (0.00050)
GO	18	12	10	13	0.817 (0.095)	0.00219 (0.00047)
JM	25	18	15	24	0.880 (0.064)	0.00335 (0.00056)
DAN	21	6	0	11	0.862 (0.032)	0.00484 (0.00050)
EWM	19	7	1	13	0.830 (0.054)	0.00552 (0.00044)
KB	21	9	1	14	0.843 (0.057)	0.00509 (0.00038)
SLG	21	13	4	19	0.943 (0.031)	0.00436 (0.00054)
ILS	24	6	1	11	0.790 (0.047)	0.00475 (0.00034)
HA	20	4	0	8	0.695 (0.079)	0.00414 (0.00066)
HB1	20	8	0	11	0.821 (0.072)	0.00500 (0.00033)
HB2	21	7	0	12	0.748 (0.084)	0.00552 (0.00044)
Northeast / Hatchery B	147	18	7	24	0.851 (0.017)	0.00515 (0.00012)
Southwest / Japan	106	69	61	80	0.879 (0.031)	0.00311 (0.00027)
Overall	273	81	68	94	0.923 (0.008)	0.00462 (0.00016)

652 **Figure 1**.History of introductions of the Pacific oyster *Crassostrea gigas* in Europe.
653
654 **Figure 2**. Pairwise genetic differentiation heat maps. (A) Based on 7 microsatellite loci.
655 Above diagonal: θ, estimator of F_{ST} (Weir and Cockerham 1984). Below diagonal: D_{est}, bias-
656 corrected differentiation index (Jost 2008). (B) Based on mtDNA MNR marker. Estimator of
657 F_{ST}.
658
659 **Figure 3**.Between samples genetic structure based on 7 microsatellite markers. (A) Neighbor-
660 joining tree based on Nei (D_a) genetic distances among the 25 samples of *Crassostrea gigas*.
661 Nodes supported by bootstrap values > 50% in 1,000 pseudoreplicates are indicated with
662 filled circles. Branches are color-coded according to clusters identified in STRUCTURE
663 analysis. (B) Map showing the distribution of sampling sites of *Crassostrea gigas*, with
664 colored pie charts showing the contribution of the two main genetic clusters identified by
665 STRUCTURE analysis. (C) Bayesian individual clustering of *Crassostrea gigas* performed in
666 STRUCTURE, using the admixture model with correlated allele frequencies, and using
667 sample group information (Hubisz et al. 2009). Each individual is indicated by a thin vertical
668 line, with coloured bars representing proportions of membership to each cluster. Bold vertical
669 lines separate sampling sites, with site abbreviations shown below the plot (see Table 1 for
670 full names). Results shown are for K=2.
671
672 **Figure 4**.Between sample genetic structure based on the Major Noncoding Region (MNR) of
673 the mitochondrial genome. The ten most common shared haplotypes are color-coded. (A, C)
674 Maps of MNR haplotype frequencies. At each site, singletons have been pooled for graphical
675 representation (pSingl: private singletons; sSingl: singletons shared between samples). The
676 numbers of private singletons are written on the pie charts. (A) Samples analyzed in this
677 study (see Table 1 for nomenclature). (C) Samples analyzed by Moehler et al. (2011). (B)
678 Median-joining network for the 81 MNR haplotypes of *Crassostrea gigas*. Connecting lines
679 represent single mutation change and black dots represent inferred missing haplotypes. The
680 size of the circles is proportional to the number of individuals observed for each haplotype.
681
682 **Figure 5**. Pairwise F_{ST} versus marine geographic distance within and between clusters
683 identified with STRUCTURE, for 22 feral samples of *Crassostrea gigas*, based on 7
684 microsatellites.
685

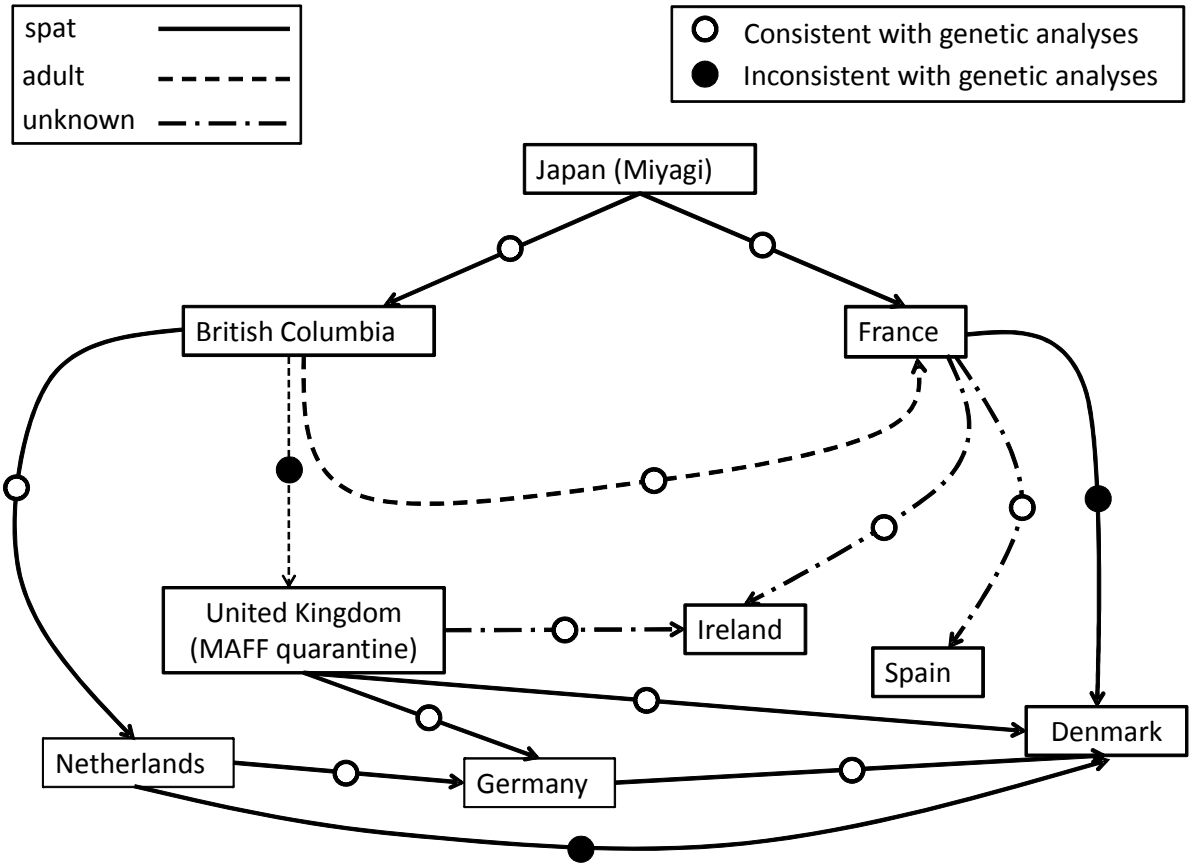
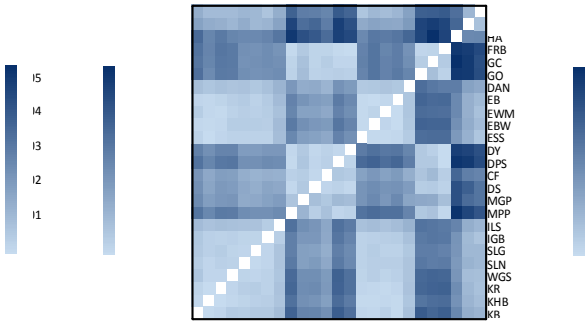

spat
adult
unknown
Consistent with genetic analyses
Inconsistent with genetic analyses
Japan (Miyagi)
British Columbia
France
United Kingdom
(MAFF quarantine)
Ireland
Spain
Denmark
Netherlands
Germany

Figure 1

(a)


FRB
GC
GO
DAN
EB
EWM
EBW
ESS
DY
DPS
CF
DS
MGP
MPP
ILS
IGB
SLG
SLN
WGS
KR
KHB

(b)

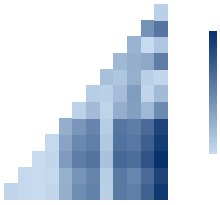


Figure 2

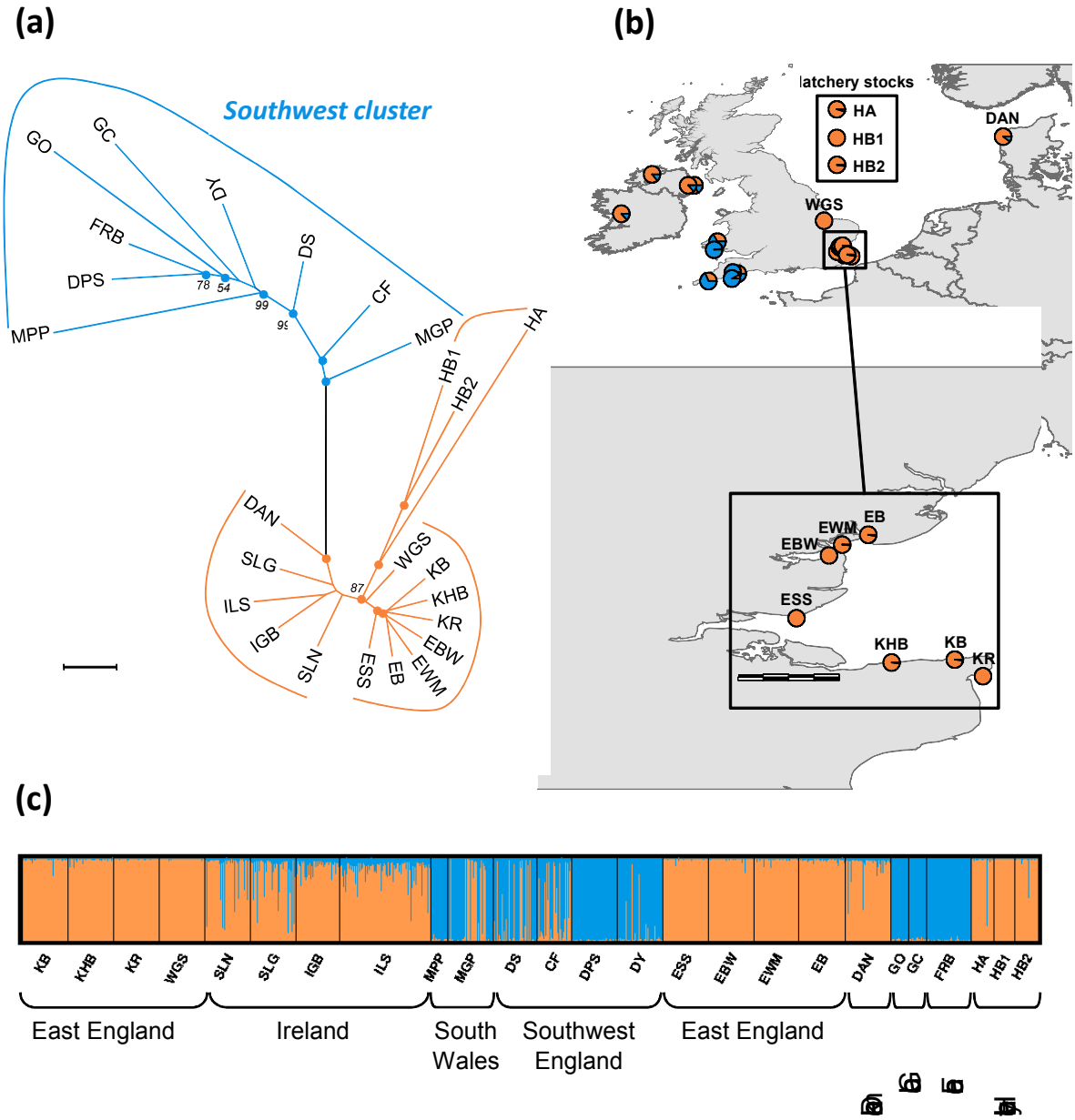

(a)
Southwest cluster
GO
GC
DY
FRB
DPS
DS
CF
MPP
MGP
HA
HB1
HB2
78
54
99
87
DAN
SLG
ILS
IGB
SLN
WGS
KB
KHB
KR
EBW
EWM
EB
ESS
(b)
Hatchery stocks
HA
HB1
HB2
DAN
WGS
EWM
EB
EBW
ESS
KHB
KB
KR
(c)
KB
KHB
KR
WGS
SLN
SLG
IGB
ILS
MPP
MGP
DS
CF
DPS
DY
ESS
EBW
EWM
EB
DAN
GO
GC
FRB
HA
HB1
HB2
East England
Ireland
South Wales
Southwest England
East England

Figure 3

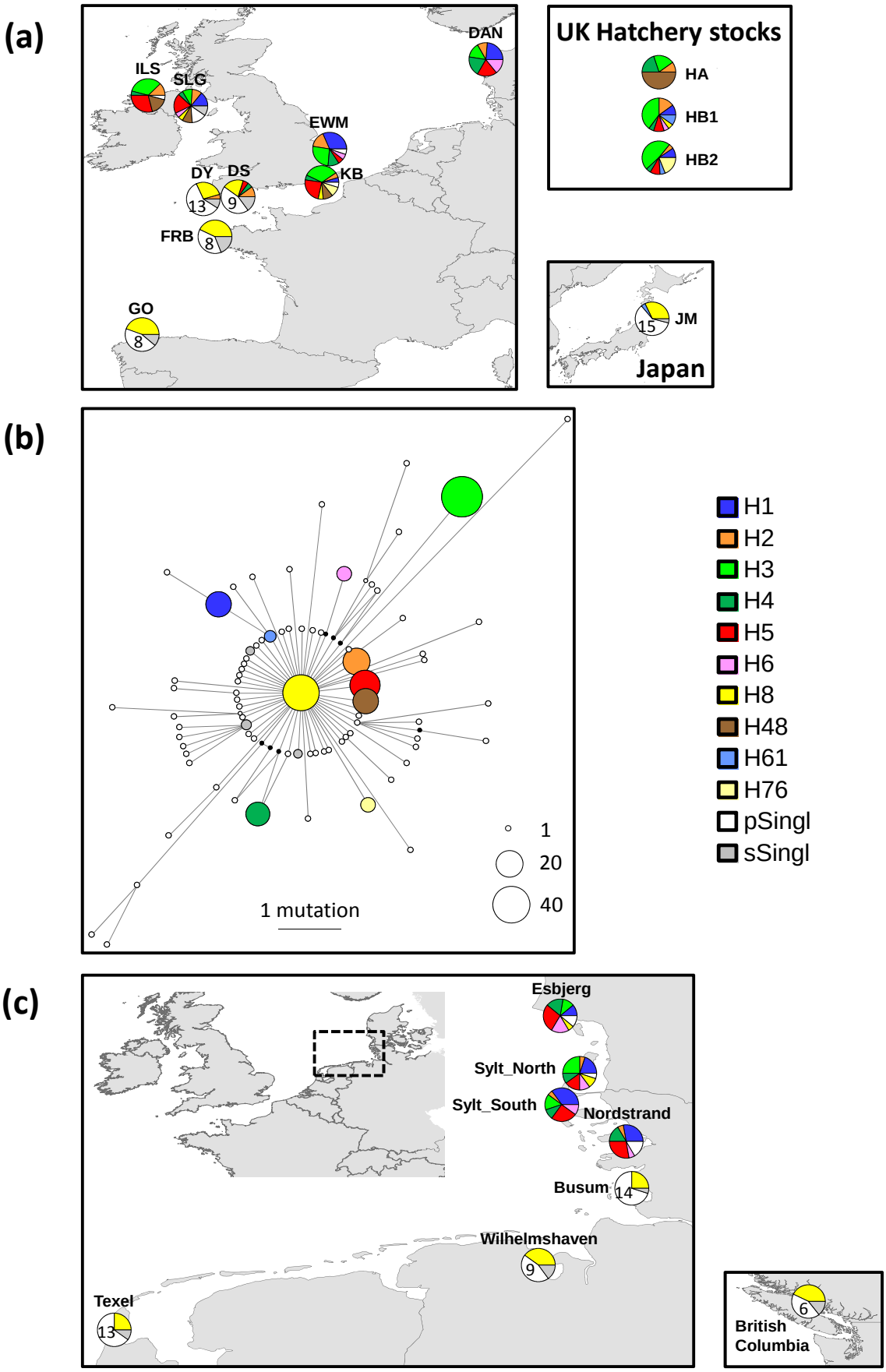

(a)
DAN
ILS
SLG
EWM
DY
DS
KB
13
9
FRB
8
GO
8
UK Hatchery stocks
HA
HB1
HB2
JM
15
Japan
(b)
H1
H2
H3
H4
H5
H6
H8
H48
H61
H76
pSingl
sSingl
1
20
40
1 mutation
(c)
Esbjerg
Sylt_North
Sylt_South
Nordstrand
Busum
14
Wilhelmshaven
9
Texel
13
6
British
Columbia

Figure 4

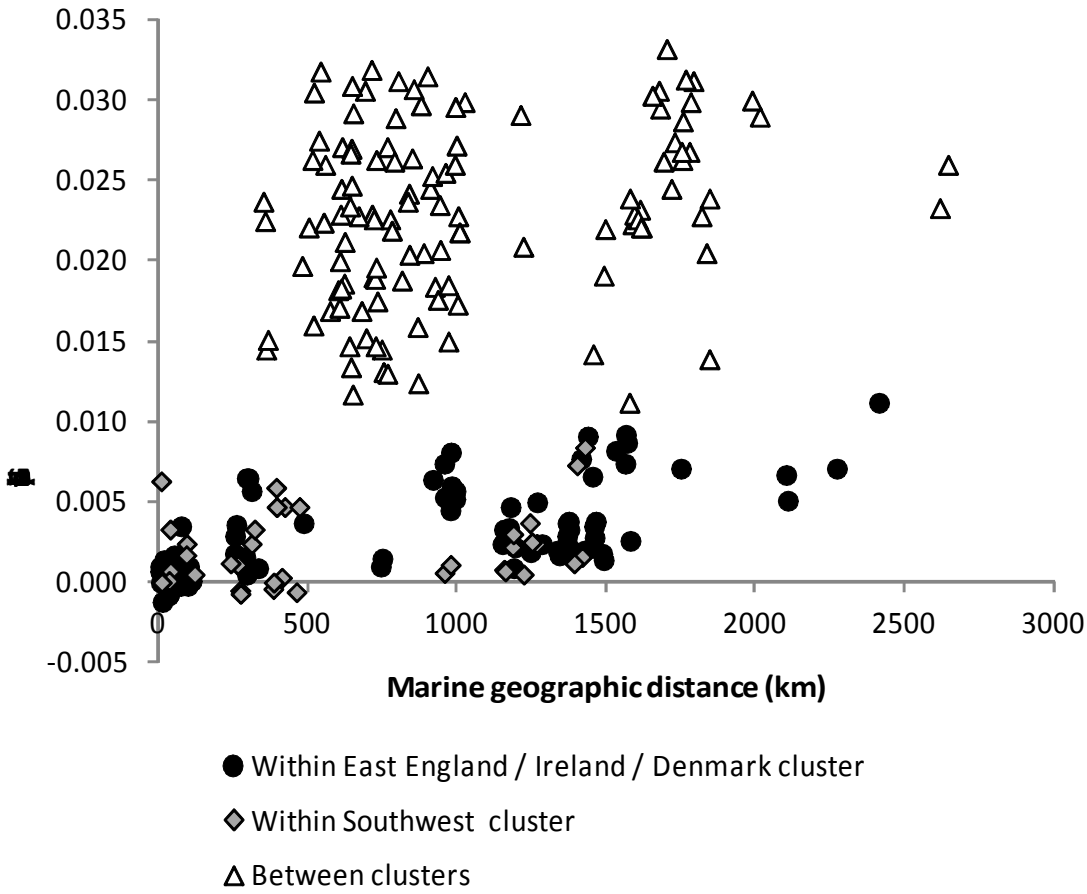

0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
-0.005
0
500
1000
1500
2000
2500
3000
Marine geographic distance (km)
Within East England / Ireland / Denmark cluster
Within Southwest cluster
Between clusters

Figure 5

Supplementary Table S1. Geographic information, abbreviation (Abbr.) and sampling date of each feral population collected in this study.

Abbr.	Geographic region	Location	Site	Latitude	Longitude	Date
KB	Kent (E England)	Birchington	Epple Bay	N 51° 22’56.20”	E 001° 18’44.97”	10/09/2010
KR	Kent (E England)	Ramsgate	Western Undercliff	N 51° 19’33.82”	E 001° 24’27.67”	10/09/2010
KHB	Kent (E England)	Herne Bay	Hampton Pier	N 51° 22’19.98”	E 001° 05’54.54”	11/09/2010
SLN	Co Down (N Ireland)	Strangford Lough	Nendrum	N 54° 31’24.96”	W 005° 39’27.24”	23/09/2010
SLG	Co Down (N Ireland)	Strangford Lough	Greyabbey	N 54° 31’23.64”	W 005° 34’25.44”	24/09/2010
WGS	The Wash (E England)	Sutton Bridge	Gat Sand	N 52° 55’24.60”	E 000° 10’41.22”	13/10/2010
ILS	Co Donegal (Ireland)	Lough Swilly	Shellfield	N 55° 01’15.50”	W 007° 34’41.40”	23/11/2010
IGB	Co Galway (Ireland)	Galway Bay	Marine Institute Pier	N 53° 14’38.47”	W 008° 58’01.88”	25/11/2010
MGP	Milford Haven (Wales)	Lawrenny	Garron Pill	N 51° 43’56.40”	W 004° 52’59.10”	19/02/2011
MPP	Milford Haven (Wales)	Pembroke Dock	Pennar Point	N 51° 41’14.43”	W 004° 58’35.63”	20/02/2011
DS	Devon (SW England)	Salcombe estuary	Snapes Point	N 50° 14’21.48”	W 003° 45’38.82”	19/03/2011
DPS	Devon (SW England)	Plymouth Sound	Jennycliff	N 50° 21’00.90”	W 004° 07’20.10”	21/03/2011
DY	Devon (SW England)	Yealm estuary	Noss Mayo	N 50° 18’40.02”	W 004° 03’05.64”	22/03/2011
CF	Cornwall (SW England)	Fal estuary	Turnaware Point	N 50° 12’07.32”	W 005° 02’00.96”	20/03/2011

ESS	Essex (E England)	Southend on Sea	Shoebury Common	N 51° 31’20.22”	E 000° 46’32.70”	17/05/2011
EWM	Essex (E England)	West Mersea	Beach huts	N 51° 46’18.00”	E 000° 55’52.44”	19/05/2011
EBW	Essex (E England)	Blackwater	Bradwell Waterside	N 51° 44’05.81”	E 000° 53’09.51”	18/05/2011
EB	Essex (E England)	Brightlingsea	Sailing boat club	N 51° 48’17.88”	E 001° 01’08.16”	19/05/2011
FRB	Brittany (France)	Rade de Brest	L’Auberlac’h	N 48° 19’31.60”	W 004°26’53.00”	02/08/2011
DAN	Nordjylland (Denmark)	Limfjord	Agger Tange	N 56° 43’16.99”	E 008°15’26.34”	19/10/2009
GO	Galicia (Spain)	Ria de Ortigueira		N 43°40′59.00″	W 007°51′00.00″	12/2008
GC	Galicia (Spain)	Ria de Celeiro		N 43°40'06.00"	W 007°35'36.00"	12/2008

Supplementary Table S2. Genetic diversity and heterozygosity within 22 feral populations and 3 hatchery batches of the Pacific oyster *Crassostrea gigas* at 7 microsatellite markers. N_a: number of alleles. A: allelic richness. H_o: observed heterozygosity. H_e: expected heterozygosity (Nei 1978 unbiased). F_{IS}: estimator of fixation index per locus and population using FSTAT. P-value for F_{IS} within samples was based on 5500 randomisations (FSTAT). Indicative adjusted nominal level (5%) for one table is 0.0002. In bold are significant F_{IS} values (P<0.0002). In italics are F_{IS} values with P<0.05.

		Feral populations	Hatchery batches																							
Locus		KB	KHB	KR	WGS	SLN	SLG	IGB	ILS	MPP	MGP	DS	CF	DPS	DY	ESS	EBW	EWM	EB	DAN	GO	GC	FRB	HA	HB1	HB2
L10	N_a	30	24	27	28	29	32	30	30	33	40	44	35	46	46	25	28	26	26	30	32	32	50	18	15	18
	A	20.39	19.36	20.77	21.41	20.81	23.69	21.28	20.59	32.22	28.74	31.26	27.92	31.59	33.03	20.61	21.31	20.50	20.32	22.76	30.13	30.93	34.71	16.05	13.26	16.28
	H_o	0.940	0.930	0.930	0.930	0.960	0.970	0.925	0.975	1.000	0.950	0.926	0.960	0.980	0.970	0.970	0.950	0.980	0.910	0.970	0.900	0.947	0.939	0.940	0.956	0.959
	H_e	0.926	0.925	0.919	0.935	0.934	0.938	0.933	0.928	0.970	0.962	0.967	0.959	0.969	0.972	0.937	0.937	0.933	0.924	0.950	0.963	0.967	0.973	0.908	0.873	0.899
	F_{IS}	-0.015	-0.005	-0.012	0.006	-0.028	-0.034	0.008	*-0.050*	-0.031	0.013	*0.042*	-0.001	-0.011	0.002	-0.035	-0.013	*-0.050*	0.016	-0.021	0.066	0.02	0.035	-0.036	-0.096	-0.067
Cgsili46	N_a	14	15	14	14	20	20	22	24	21	27	27	28	26	28	16	14	14	14	19	20	19	24	10	12	14
	A	11.18	12.01	11.24	10.94	15.06	14.23	15.98	15.26	20.61	18.79	21.21	22.05	19.52	21.43	11.80	11.15	11.40	10.57	13.42	19.30	18.43	18.28	9.31	11.46	12.93
	H_o	0.870	0.850	0.740	0.810	0.820	0.790	0.883	0.860	0.865	0.820	0.853	0.840	0.810	0.850	0.810	0.760	0.867	0.800	0.760	0.750	0.579	0.848	0.800	0.889	0.898
	H_e	0.849	0.861	0.840	0.822	0.871	0.872	0.884	0.876	0.936	0.868	0.936	0.916	0.920	0.928	0.857	0.848	0.860	0.842	0.854	0.927	0.894	0.904	0.857	0.862	0.860
	F_{IS}	-0.024	0.013	*0.119*	0.014	0.059	*0.094*	0.001	0.019	0.077	0.055	*0.089*	*0.084*	*0.120*	*0.084*	0.056	*0.105*	-0.009	0.051	*0.111*	*0.193*	**0.356**	*0.062*	0.067	-0.031	-0.045
Cgsili44	N_a	16	16	15	13	15	16	15	17	15	18	22	20	24	23	16	15	16	17	16	19	18	22	12	11	12
	A	13.97	14.04	13.50	12.20	13.81	13.65	13.71	14.36	14.88	15.65	18.17	17.54	17.08	17.79	13.96	13.26	14.44	14.43	14.50	17.98	17.72	17.29	11.03	10.73	11.81
	H_o	0.940	0.920	0.900	0.960	0.930	0.900	0.905	0.930	0.919	0.950	0.895	0.907	0.940	0.920	0.940	0.900	0.918	0.890	0.930	0.900	0.892	0.889	0.918	1.000	0.959
	H_e	0.901	0.889	0.888	0.899	0.913	0.890	0.909	0.902	0.920	0.926	0.924	0.924	0.916	0.927	0.888	0.882	0.899	0.901	0.905	0.903	0.916	0.920	0.842	0.870	0.876
	F_{IS}	-0.044	-0.035	-0.014	*-0.068*	-0.019	-0.011	0.005	-0.031	-0.008	-0.026	0.032	0.019	-0.026	0.008	*-0.059*	-0.021	-0.021	0.012	-0.028	0.004	0.027	0.034	-0.091	*-0.152*	-0.096
AMY	N_a	20	21	20	21	23	28	23	26	28	35	34	33	35	37	21	20	23	23	23	29	24	40	16	13	13
	A	16.21	16.81	16.47	16.82	18.34	20.31	18.36	17.50	27.34	27.50	26.40	25.08	26.57	27.46	17.16	16.34	18.10	18.74	17.90	27.55	23.34	28.76	13.70	12.39	12.11
	H_o	0.960	0.860	0.930	0.910	0.920	0.950	0.958	0.905	0.865	0.970	0.936	0.947	0.940	0.970	0.850	0.920	0.929	0.900	0.880	0.925	0.974	0.918	0.940	0.913	0.878
	H_e	0.899	0.907	0.910	0.908	0.914	0.923	0.919	0.893	0.956	0.960	0.960	0.947	0.959	0.961	0.919	0.921	0.918	0.922	0.925	0.959	0.945	0.963	0.852	0.873	0.895
	F_{IS}	*-0.068*	0.052	-0.022	-0.002	-0.007	-0.029	-0.042	-0.013	*0.096*	-0.010	0.025	0.000	0.020	-0.009	*0.076*	0.002	-0.012	0.024	0.049	0.036	-0.031	*0.047*	*-0.104*	-0.047	0.019
CGE009	N_a	11	12	11	11	16	14	16	16	15	18	22	21	21	23	11	11	13	13	11	20	18	20	8	9	11
	A	9.98	9.93	10.06	10.43	12.36	12.31	12.22	11.81	14.83	14.40	16.68	16.02	14.57	16.57	9.84	10.03	10.82	11.21	10.47	19.19	17.51	15.85	7.95	8.69	10.59
	H_o	0.840	0.870	0.890	0.840	0.880	0.900	0.894	0.910	0.919	0.880	0.958	0.893	0.940	0.890	0.840	0.890	0.908	0.900	0.820	0.900	0.947	0.869	0.860	0.913	0.878

	H_e	0.839	0.860	0.854	0.872	0.871	0.893	0.872	0.881	0.895	0.896	0.911	0.903	0.895	0.909	0.850	0.844	0.855	0.869	0.847	0.931	0.906	0.910	0.823	0.833	0.850
	F_{IS}	-0.001	-0.012	-0.042	0.037	-0.011	-0.008	-0.025	-0.033	-0.027	0.018	*-0.052*	0.010	-0.050	0.021	0.011	-0.054	-0.062	-0.036	0.032	0.034	-0.047	0.046	-0.046	-0.097	-0.033
Cgsili50	N_a	10	9	11	9	12	13	10	12	13	14	14	15	16	17	10	9	9	11	10	13	12	15	8	8	8
	A	9.24	8.74	9.16	8.26	9.83	10.25	8.34	10.03	12.78	11.08	11.49	12.40	11.70	11.76	8.88	8.78	8.71	9.29	9.20	12.37	11.60	11.50	7.64	7.74	7.63
	H_o	0.830	0.830	0.850	0.850	0.880	0.900	0.830	0.845	0.892	0.820	0.851	0.920	0.838	0.800	0.880	0.790	0.806	0.850	0.810	0.800	0.895	0.775	0.875	0.848	0.939
	H_e	0.847	0.844	0.843	0.821	0.834	0.827	0.824	0.845	0.869	0.851	0.853	0.862	0.849	0.851	0.846	0.834	0.855	0.832	0.811	0.868	0.854	0.859	0.796	0.764	0.813
	F_{IS}	0.02	0.017	-0.008	-0.036	-0.048	*-0.088*	-0.008	0.000	-0.026	0.036	0.002	-0.067	0.013	0.061	-0.040	0.053	0.057	-0.022	0.001	0.079	-0.048	*0.098*	-0.100	-0.110	*-0.156*
Cgsili4	N_a	20	20	19	18	24	26	26	24	26	35	32	38	37	40	19	21	20	20	21	30	30	35	17	14	18
	A	15.74	16.60	16.05	15.68	19.48	19.96	18.93	18.02	25.66	25.76	26.15	28.07	28.68	28.31	16.14	16.14	16.15	16.36	16.80	29.31	29.61	28.33	15.28	13.45	16.41
	H_o	0.840	0.910	0.940	0.890	0.940	0.910	0.905	0.930	0.946	0.880	0.936	0.920	0.930	0.890	0.920	0.960	0.929	0.880	0.870	0.895	0.917	0.929	0.979	0.935	0.918
	H_e	0.899	0.926	0.915	0.918	0.937	0.934	0.926	0.928	0.960	0.945	0.961	0.960	0.965	0.964	0.919	0.926	0.917	0.923	0.914	0.969	0.955	0.965	0.892	0.903	0.921
	F_{IS}	*0.066*	0.018	-0.028	0.031	-0.003	0.026	0.023	-0.002	0.015	*0.069*	0.026	0.042	0.037	*0.077*	-0.001	-0.037	-0.012	0.047	0.049	*0.077*	0.040	0.038	*-0.099*	-0.036	0.003
Overall	N_a	17.29	16.71	16.71	16.29	19.86	21.29	20.29	21.29	21.57	26.71	27.86	27.14	29.29	30.57	16.86	16.86	17.29	17.71	18.57	23.29	21.86	29.43	12.71	11.71	13.43
	A	13.82	13.93	13.89	13.68	15.67	16.35	15.55	15.37	21.19	20.28	21.62	21.30	21.39	22.34	14.06	13.86	14.31	14.42	15.01	22.26	21.31	22.10	11.57	11.10	12.54
	H_o	0.889	0.881	0.883	0.884	0.904	0.903	0.900	0.908	0.915	0.896	0.908	0.912	0.911	0.899	0.887	0.881	0.905	0.876	0.863	0.867	0.879	0.881	0.902	0.922	0.918
	H_e	0.880	0.888	0.881	0.882	0.897	0.897	0.895	0.894	0.928	0.915	0.930	0.925	0.925	0.930	0.888	0.885	0.891	0.888	0.887	0.931	0.920	0.928	0.853	0.854	0.874
	F_{IS}	-0.010	0.007	-0.002	-0.002	-0.008	-0.007	-0.005	*-0.016*	0.014	*0.021*	*0.024*	0.013	0.015	*0.034*	0.001	0.004	-0.016	0.013	*0.027*	**0.070**	0.045	**0.051**	*-0.058*	***-0.081***	*-0.052*

Supplementary Table S3. Global genetic differentiation based on 7 microsatellites. H_{T_est}: heterozygosity of the pooled subpopulations; H_{S_est}: mean heterozygosity of the individual subpopulations (Nei & Chesser 1983). CI: 95% confidence interval (1,000 bootstrap resamplings).

Locus	G_{st_est} [CI]	H_{T_est}	H_{S_est}	D_{est} [CI]
L10	0.012 [0.011-0.013]	0.952	0.940	0.201 [0.184-0.220]
Cgsili46	0.015 [0.014-0.016]	0.891	0.878	0.114 [0.103-0.126]
Cgsili44	0.013 [0.012-0.014]	0.913	0.901	0.124 [0.112-0.136]
AMY	0.013 [0.012-0.014]	0.936	0.924	0.165 [0.151-0.182]
CGE009	0.017 [0.016-0.019]	0.890	0.875	0.127 [0.117-0.139]
Cgsili50	0.012 [0.010-0.014]	0.848	0.838	0.065 [0.056-0.075]
Cgsili4	0.010 [0.009-0.011]	0.944	0.934	0.156 [0.139-0.172]
Multilocus	0.017 [0.016-0.017]	0.907	0.893	0.156 [0.151-0.160]

Supplementary Table S4. Pairwise genetic differentiation table based on 7 microsatellites. Upper matrix: F_{ST} values (θ Weir & Cockerham 1984); in bold are significant values after 6000 permutations (FSTAT). Lower matrix: D_{est} (Jost 2008); in bold are significant values (95% CI does not include the zero, after 1000 bootstraps – DEMEtics). See Table 1 for populations' abbreviations.

	KB	KHB	KR	WGS	SLN	SLG	IGB	ILS	MPP	MGP	DS	CF	DPS	DY	ESS	EBW	EWM	EB	DAN	GO	GC	FRB	HA	HB1	HB2
KB	-	0.000	0.001	**0.006**	**0.003**	0.005	0.005	**0.009**	**0.032**	**0.025**	**0.023**	**0.021**	**0.032**	**0.028**	0.002	0.000	0.004	0.001	**0.008**	**0.033**	**0.031**	**0.032**	**0.035**	**0.017**	**0.014**
KHB	0.002	-	-0.001	0.001	0.001	0.002	0.002	**0.007**	**0.025**	**0.018**	**0.016**	**0.015**	**0.026**	**0.022**	-0.001	0.000	0.000	0.000	**0.006**	**0.025**	**0.026**	**0.026**	**0.026**	**0.014**	**0.009**
KR	0.011	0.001	-	0.001	**0.002**	0.003	0.002	**0.008**	**0.030**	**0.021**	**0.020**	**0.018**	**0.031**	**0.026**	0.001	0.000	0.000	0.001	**0.007**	**0.030**	**0.030**	**0.031**	**0.030**	**0.013**	**0.009**
WGS	**0.038**	0.009	**0.013**	-	0.002	**0.001**	**0.003**	**0.007**	**0.029**	**0.021**	**0.019**	**0.018**	**0.031**	**0.026**	0.002	**0.004**	0.002	0.003	**0.006**	**0.029**	**0.030**	**0.030**	**0.026**	**0.011**	**0.009**
SLN	**0.033**	**0.017**	**0.029**	**0.016**	-	**0.001**	**0.001**	**0.007**	**0.024**	**0.015**	**0.015**	**0.013**	**0.023**	**0.019**	0.002	**0.004**	**0.003**	**0.004**	**0.007**	**0.023**	**0.022**	**0.023**	**0.025**	**0.014**	**0.011**
SLG	**0.042**	**0.027**	**0.033**	**0.013**	0.005	-	**0.002**	**0.007**	**0.023**	**0.015**	**0.013**	**0.012**	**0.023**	**0.019**	**0.002**	**0.003**	0.002	0.002	**0.005**	**0.022**	**0.023**	**0.022**	**0.025**	**0.010**	**0.010**
IGB	**0.044**	**0.029**	**0.021**	**0.021**	**0.011**	**0.014**	-	**0.004**	**0.023**	**0.017**	**0.016**	**0.013**	**0.024**	**0.020**	**0.002**	**0.003**	**0.004**	**0.004**	**0.007**	**0.023**	**0.024**	**0.024**	**0.027**	**0.011**	**0.006**
ILS	**0.075**	**0.059**	**0.064**	**0.055**	**0.057**	**0.055**	**0.032**	-	**0.025**	**0.019**	**0.015**	**0.012**	**0.024**	**0.021**	**0.008**	**0.009**	**0.007**	**0.009**	**0.011**	**0.024**	**0.023**	**0.022**	**0.031**	**0.017**	**0.013**
MPP	**0.308**	**0.274**	**0.301**	**0.291**	**0.252**	**0.236**	**0.238**	**0.273**	-	0.006	0.000	0.002	0.000	0.000	**0.026**	**0.030**	**0.026**	**0.027**	**0.021**	0.002	0.001	-0.001	**0.051**	**0.047**	**0.038**
MGP	**0.223**	**0.191**	**0.202**	**0.197**	**0.152**	**0.152**	**0.174**	**0.197**	**0.096**	-	0.005	0.003	**0.006**	**0.005**	**0.019**	**0.023**	**0.017**	**0.022**	**0.014**	0.008	**0.007**	**0.005**	**0.043**	**0.034**	**0.029**
DS	**0.221**	**0.183**	**0.204**	**0.199**	**0.165**	**0.146**	**0.173**	**0.178**	**0.028**	**0.070**	-	0.000	0.001	0.000	**0.017**	**0.020**	**0.017**	**0.019**	**0.015**	0.002	0.001	0.001	**0.040**	**0.036**	**0.028**
CF	**0.194**	**0.150**	**0.171**	**0.166**	**0.128**	**0.115**	**0.127**	**0.133**	**0.064**	**0.049**	0.011	-	0.002	0.002	**0.015**	**0.020**	**0.015**	**0.018**	**0.011**	0.002	0.004	0.001	**0.034**	**0.029**	**0.026**
DPS	**0.321**	**0.285**	**0.312**	**0.313**	**0.250**	**0.238**	**0.262**	**0.272**	0.013	**0.086**	**0.019**	**0.049**	-	0.000	**0.027**	**0.031**	**0.027**	**0.029**	**0.022**	0.003	0.001	-0.001	**0.048**	**0.048**	**0.040**
DY	**0.280**	**0.249**	**0.275**	**0.273**	**0.209**	**0.205**	**0.226**	**0.246**	**0.021**	**0.075**	0.005	**0.039**	**0.016**	-	**0.023**	**0.027**	**0.023**	**0.025**	**0.019**	0.003	0.001	-0.001	**0.045**	**0.043**	**0.037**
ESS	**0.014**	0.004	**0.014**	**0.017**	**0.021**	**0.021**	**0.022**	**0.067**	**0.269**	**0.182**	**0.181**	**0.147**	**0.292**	**0.249**	-	0.000	0.000	0.000	**0.005**	**0.026**	**0.027**	**0.027**	**0.025**	**0.014**	**0.006**
EBW	0.006	0.003	0.007	**0.029**	**0.031**	**0.033**	**0.026**	**0.074**	**0.287**	**0.212**	**0.199**	**0.174**	**0.308**	**0.272**	0.003	-	0.001	-0.001	**0.005**	**0.031**	**0.031**	**0.031**	**0.030**	**0.017**	**0.010**
EWM	**0.023**	0.008	0.004	**0.017**	**0.028**	**0.022**	**0.030**	**0.063**	**0.276**	**0.173**	**0.185**	**0.146**	**0.283**	**0.255**	0.004	0.010	-	0.000	**0.006**	**0.027**	**0.027**	**0.026**	**0.029**	**0.016**	**0.009**
EB	0.005	0.004	**0.010**	**0.023**	**0.030**	**0.023**	**0.032**	**0.074**	**0.273**	**0.211**	**0.189**	**0.166**	**0.302**	**0.259**	0.006	-0.004	0.000	-	**0.005**	**0.030**	**0.029**	**0.029**	**0.031**	**0.015**	**0.011**
DAN	**0.066**	**0.052**	**0.063**	**0.056**	**0.056**	**0.046**	**0.065**	**0.104**	**0.224**	**0.136**	**0.160**	**0.115**	**0.240**	**0.219**	**0.039**	**0.047**	**0.052**	**0.053**	-	**0.026**	**0.023**	**0.022**	**0.036**	**0.020**	**0.017**
GO	**0.327**	**0.265**	**0.302**	**0.295**	**0.257**	**0.241**	**0.251**	**0.267**	**0.049**	**0.127**	**0.033**	**0.055**	**0.043**	**0.046**	**0.283**	**0.309**	**0.289**	**0.306**	**0.284**	-	0.003	0.001	**0.046**	**0.047**	**0.038**
GC	**0.301**	**0.285**	**0.310**	**0.305**	**0.244**	**0.244**	**0.259**	**0.260**	**0.062**	**0.127**	**0.038**	**0.084**	**0.046**	**0.028**	**0.287**	**0.305**	**0.285**	**0.291**	**0.257**	**0.069**	-	0.001	**0.054**	**0.049**	**0.039**
FRB	**0.317**	**0.287**	**0.315**	**0.303**	**0.245**	**0.229**	**0.256**	**0.253**	0.009	**0.071**	**0.021**	**0.032**	0.001	-0.001	**0.285**	**0.307**	**0.278**	**0.297**	**0.242**	0.023	**0.041**	-	**0.047**	**0.046**	**0.040**
HA	**0.213**	**0.188**	**0.201**	**0.178**	**0.168**	**0.180**	**0.182**	**0.225**	**0.435**	**0.360**	**0.358**	**0.299**	**0.432**	**0.403**	**0.172**	**0.192**	**0.211**	**0.212**	**0.241**	**0.421**	**0.447**	**0.423**	-	**0.036**	**0.033**
HB1	**0.118**	**0.098**	**0.089**	**0.076**	**0.105**	**0.087**	**0.083**	**0.117**	**0.387**	**0.287**	**0.320**	**0.240**	**0.418**	**0.390**	**0.106**	**0.119**	**0.113**	**0.111**	**0.147**	**0.417**	**0.420**	**0.410**	**0.212**	-	**0.011**
HB2	**0.099**	**0.074**	**0.067**	**0.069**	**0.087**	**0.089**	**0.057**	**0.093**	**0.344**	**0.265**	**0.266**	**0.223**	**0.372**	**0.358**	**0.050**	**0.078**	**0.066**	**0.083**	**0.132**	**0.368**	**0.364**	**0.377**	**0.214**	**0.067**	-

Supplementary Table S5. Pairwise F_{ST} values between sampling sites, based on the mtDNA marker. In bold are shown significant values after B-Y method correction (Benjamini & Yekutieli 2001; Narum 2006) (for k=13, P<0.01572); and in italics, F_{ST} values with P<0.05. See Table 1 for populations' abbreviations.

Population	DS	DY	FRB	GO	JM	DAN	EWM	KB	SLG	ILS	HA	HB1	HB2
DS	0.0000												
DY	0.0093	0.0000											
FRB	0.0052	0.0044	0.0000										
GO	0.0024	0.0005	0.0025	0.0000									
JM	0.0067	0.0046	0.0075	0.0051	0.0000								
DAN	**0.0672**	**0.0647**	**0.0952**	**0.0832**	**0.0683**	0.0000							
EWM	**0.1156**	**0.1042**	**0.1386**	**0.1236**	**0.1037**	0.0155	0.0000						
KB	**0.1332**	**0.1286**	**0.1525**	**0.1367**	**0.1289**	0.0478	0.0225	0.0000					
SLG	0.0160	*0.0187*	**0.0365**	*0.0294*	*0.0212*	0.0130	0.0173	0.0416	0.0000				
ILS	**0.1427**	**0.1428**	**0.1683**	**0.1540**	**0.1480**	*0.0679*	0.0459	0.0326	0.0512	0.0000			
HA	**0.1345**	**0.1247**	**0.1601**	**0.1446**	**0.1377**	*0.0886*	**0.0934**	0.0661	*0.0680*	0.0592	0.0000		
HB1	**0.1788**	**0.1664**	**0.1990**	**0.1818**	**0.1655**	0.0512	0.0095	0.0172	*0.0738*	0.0024	*0.1072*	0.0000	
HB2	**0.2385**	**0.2229**	**0.2532**	**0.2346**	**0.2217**	*0.1169*	0.0464	0.0069	*0.1382*	0.0313	**0.1486**	0.0166	0.0000

Supplementary Figure S1. Habitat and morphology of the Garron Pill samples. Two types could be distinguished: oysters that were loose in the sediment had a very thick and cupped shell, while the oysters that were attached to the rock were much flatter and moulded to their substrate.

References

Benjamini Y, Yekutieli D (2001) The Control of the False Discovery Rate in Multiple Testing under Dependency. *The Annals of Statistics*, **29**, 1165-1188.

Narum S (2006) Beyond Bonferroni: Less conservative analyses for conservation genetics. *Conservation Genetics*, **7**, 783-787.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89 (3):583-590

Nei M, Chesser RK (1983) Estimation of fixation indices and gene diversities. *Annals of Human Genetics*, **47**, 253-259.