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Genetics of Scottish populations of the native oyster, *Ostrea edulis*: gene flow, human intervention and conservation

Andy Beaumont[1,a], Manuela Truebano Garcia[1], Stephan Hönig[1] and Paula Low[2]

[1] School of Ocean Sciences, University of Wales, Bangor, Menai Bridge, Gwynedd, LL59 5AB, UK
[2] University Marine Biological Station Milport, Isle of Cumbrae Scotland, KA28 0EF, UK

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Abstract – The European, native or flat oyster, *Ostrea edulis,* has been the subject of human-mediated translocation and aquaculture in Europe for centuries and may have diluted or masked natural population genetic structure. Samples of *O. edulis* from 10 sites in Scotland and The Netherlands, Brittany and Norway were collected and genotyped at up to six microsatellite loci. Numbers of alleles and heterozygosity per locus were high in all populations and were consistent with previous microsatellite studies. There is no evidence that extensive traditional oyster aquaculture has led to loss of allelelic diversity. Deficiencies of heterozygotes against Hardy-Weinberg predictions were common and were probably mainly due to the presence of null alleles. Overall, population differentiation (F_{st} = 0.05) was estimated to be higher than previous studies and could be resolved into four main genetic groups (1) Norway, (2) The Netherlands and Brittany, (3) Scotland apart from (4) Skye. The genetic distinctness of Norway oysters agrees with previous findings. The distinctiveness of the Skye population could be partly due to an artefact of small sample sizes and partly due to the founder effect of importation of Brittany oysters in the 1950s. Further studies are required to ascertain whether the Skye population may be deserving of special conservation status. The results suggest that human aquaculture activities over recent centuries have probably diluted any original local genetic differentiation within Scotland, but that potentially important genetic differentiation still exists at the wider scale across Europe

Key words: Genetics / Microsatellites / Conservation / Oysters / *Ostrea edulis* / Scotland

Résumé – Génétique des populations d'huîtres plates, *Ostrea edulis* natives d'Ecosse : le flux des gènes, l'intervention humaine et la conservation. L'huître d'origine européenne ou huître plate, *Ostrea edulis,* a été l'objet de transferts par l'intervention humaine, et d'aquaculture en Europe depuis des siècles ; ce qui a pu diluer ou masquer la structure génétique de la population naturelle. Des échantillons de *O. edulis* de 10 sites en Ecosse et aux Pays-Bas, France (Bretagne) et Norvège ont été récoltés, et génotypés jusqu'à six locus microsatellites. Les nombres d'allèles et de gènes hétérozygotes par locus sont élevés dans toutes les populations, et sont en accord avec les études antérieures sur les microsatellites. Il n'y a pas d'évidence que l'aquaculture extensive traditionnelle ait conduit à la perte de diversité en allèles. Des déficiences des hétérozygotes par rapport aux prévisions de Hardy-Weinberg sont communes et probablement dues à la présence d'allèles nuls. Au total, la différentiation des populations (F_{st} = 0.05) est estimée plus élevée que celle les études antérieures et peut être résolue en quatre principaux groupes génétiques (1) Norvège, (2) Pays-Bas et Bretagne, (3) Ecosse à l'exception de celui de (4) l'île de Skye. La distinction génétique des huîtres de Norvège correspond avec les précédentes études. La distinction de la population de Skye pourrait être due, en partie, à un artefact causé par la taille réduite de l'échantillon et en partie, aux premières importations d'huîtres de Bretagne dans les années cinquante. D'autres études sont nécessaires pour confirmer si la population de Skye mériterait un statut de conservation particulier. Les résultats laissent penser que les activités d'aquaculture effectuées depuis des siècles ont probablement dilué toute différentiation génétique initiale et locale en Ecosse mais des différentions génétiques potentiellement importantes existeraient à plus grande échelle en Europe.

1 Introduction

The European flat oyster, *Ostrea edulis* L., is a mollusc that has supported important aquaculture and fisheries activities throughout Europe for hundreds of years. However, what was once a widely abundant species has now become relatively rare in nature, almost certainly due to man's activities. Dramatic declines in population sizes were experienced across Europe towards the end of the 19th century and in the early part of the 20th century. Probable causes of this decline were over-fishing, disease epidemics and anthropogenic degradation of the estuarine habitat.

[a] Corresponding author: a.r.beaumont@bangor.ac.uk

Like other sedentary marine bivalves, oysters have a life history that involves a planktonic larval dispersal phase. Depending on the length of larval life, the prevailing oceanographic conditions and the availability of suitable habitat, marine bivalve species are to a greater or lesser extent subdivided into populations that are relatively genetically distinct. Where there is little gene flow (i.e. few larval exchanges) per generation between populations forces such as random genetic drift (non-directional) and selection (directional) will change allele frequencies at gene loci leading to identifiable and quantifiable genetic differences between populations. Such population genetic differentiation is an important part of natural within-species biodiversity and is of critical value both for recovery in the event of serious population decline and also as a repository of the future evolutionary potential of the species.

Because failing stocks of oysters in one region of Europe were often replenished with oysters from other regions (Millar 1961; 1963; Magennis et al. 1983), the likely consequences will have been a homogenization of any naturally evolved underlying population genetic differentiation. In the particular case of Scotland there have been recorded (and unrecorded) translocations of significant numbers of oysters into the region from Europe, and between sites within the region. A literature search has identified some of these oyster movements, they are illustrated in Figure 1. As part of the Native Oyster Biodiversity Action Plan, and in relation to stock regeneration (Laing et al. 2005), it is important to establish whether human mediated translocations or other aquaculture activities have effectively homogenized any original population genetic differentiation, or whether there remain any sites where unique genetic populations can be identified. The parasite *Bonamia ostreae* has caused very serious declines in European *O.edulis* populations, but *B. ostreae* has not been reported to occur in Scotland.

Genetic differentiation of populations can be identified using a number of genetic markers (Carvalho and Hauser 1998; Beaumont and Hoare 2003). Studies using allozymes (e.g. Johannesson et al. 1989; Saavedra et al. 1993, 1995) did not detect fine-scale genetic differentiation in European stocks of *O. edulis*. Relative to other bivalves, Johannesson et al. (1989) and Saavedra et al. (1993) identified low allozyme genetic variation in *O. edulis*. Little genetic differentiation was detected in Atlantic stocks but Saavedra et al. (1995) reported evidence for two ancient Atlantic and Mediterranean stocks that have subsequently become merged. Allozymes have limitations as markers for identifying genetic differentiation because they generally have low variability and such variability may be determined and maintained by selection rather than random genetic drift (Karl and Avise 1992). This can lead to false conclusions about true gene flow between populations.

Microsatellite DNA markers are expected to be neutral, are highly polymorphic and have high mutation rates making them ideal for identifying fine-scale population genetic structure (Bruford and Wayne 1993; Zane et al. 2002). Several microsatellite loci have now been developed for *O. edulis* (Naciri et al. 1995; Morgan et al. 2000: Sobolewska et al. 2001; Launey et al. 2002) and they have subsequently been used to investigate population genetic differentiation in *O. edulis* (Launey et al. 2002; Sobolewska and Beaumont 2005; Vercaemer et al. 2006). Launey et al. (2002) demonstrated significant genetic variation based on 5 microsatellite loci between two major regions – the Atlantic-western Mediterranean region and the eastern Mediterranean region. Within each region there was much less variation and little to discriminate between Atlantic populations except at the northern limit of the species distribution (Norway) agreeing with an isolation-by-distance model. Data from a mitochondrial 12S-rRNA marker developed by Diaz-Almela et al. (2004) also supported the two major regions identified by Launey et al. (2002) and a pattern of isolation by distance.

Using 4 different microsatellite loci to Launey et al. (2002), Sobolewska and Beaumont (2005) focused more on North Atlantic populations including some that were known to be hatchery-sourced. Overall genetic differentiation was not extensive with Norwegian oysters being the most genetically distinct from all others. Hatchery-sourced populations were less genetically variable and relatively distinct from other wild populations.

Using 5 of Launey et al.'s (2002) loci, Vercaemer et al. (2006) investigated genetic differentiation within North American *O. edulis* stocks and compared these with European stocks. Their results supported the known history of stock translocation and also identified genetic erosion in artificially propagated populations in Canada. Although genetic erosion in the form of loss of alleles at microsatellite loci is an expected consequence of the reduced effective population size involved in aquaculture activity, Hedgecock et al. (2007) have demonstrated that low effective population size can be a feature in the reproduction of natural oyster populations.

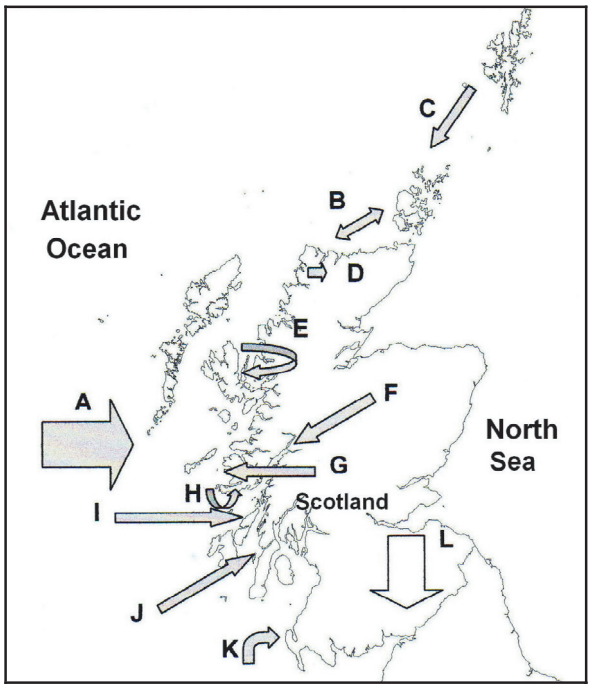
Here, we present population genetic analysis based on data from 6 microsatellite loci in ten *O. edulis* populations from around the Scottish coast and from Norway, Brittany and The Netherlands. We consider the data in relation to the natural processes of gene flow and also in relation to the recorded importations of oysters into Scotland over the last two centuries.

Materials and methods

Sampling and molecular methods

Oysters collected from sites in Scotland (Fig. 2a) were dissected at the University Marine Biological Station, Millport (UMBSM) and samples of gill tissue in 90% alcohol were transported to the School of Ocean Sciences (SOS) during 2004. Norwegian oyster samples (Fig. 2b) were provided in a similar way, as tissue in 90% alcohol. Oysters from The Netherlands and Brittany (Fig. 2b) were sent either fresh or frozen to SOS, dissected there and preserved in 100% alcohol. All samples were held at 4–6 °C until use. Oyster DNA was extracted from gills using a standard phenol-chloroform method (Sambrook et al. 1989) following proteinase K and cationic detergent cetyl-trimethylammonium bromide (CTAB) treatment (Wilding et al. 1997).

Following optimisation of published methods, six microsatellite loci were selected for amplification of oyster samples, three from Launey et al. (2002) (*Oedu*.J12, *Oedu*.T5 and *Oedu*.U2) and three from Morgan et al. (2000) (Oe1/63, Oe1/64 and Oe2/71). Methods followed the published originals except that annealing temperature for amplification of



Area	Date	Details of oyster movements
A	1900s	Unrecorded quantities from Skye and Holland laid along West Coast (Anon. 1885-1977).
	1950s	Thousands of Brittany oysters laid in 19 locations along West Coast (Millar 1961).
B	1990s	Loch Eriboll broodstock used in Orkney hatchery: offspring used to stock a cultivation programme in the Kyle of Tongue (A. MacKay, pers. comm.).
C	1800s	Unknown source, unknown numbers, laid in Orkney (Anon. 1885–1977).
	1912	800 000 oysters from an unknown location laid in Orkney (Millar 1961).
	1920s	Danish oysters laid in Orkney beds (Millar 1961).
D	19th C& 1990s	Loch Eriboll stock relaid in Kyle of Tongue (A. MacKay, pers. comm.).
E	20th C	Oysters from Loch Ainort removed to stock Broadford Bay (Millar 1961).
F	1880s	Unknown quantities from Morbihan, France, to Loch Creran (Anon. 1885-1977).
	1894	Two consignments from Holland to Loch Creran (Anon. 1885-1977).
G	From 1970s	Stock from Seasalter (England) laid in Mull (D. Wathen, pers. comm.).
H	1990s	Oysters moved from south Ulva to other locations around Ulva. Oysters from the Isle of Colonsay also laid around Ulva (J. Howard, pers. comm.).
I	1890s	40 000 oysters from Arcachon, France, via Whitstable, England, laid in Loch Sween (Smith 1895; Millar 1961).
	1947	Few thousand oysters from Brittany laid in Loch Sween (Millar 1961).
J	1886	700 000 oysters from Loch Sween, the Hebrides and France laid in West Loch Tarbert (Anon. 1885-1977). Further restocking from unknown areas in the late 1800s (Millar 1961).
	1950s	200 000 oysters translocated from Brittany to West Loch Tarbert (Millar 1961).
K	1800s	Unknown quantities from France, Holland and Essex laid in Loch Ryan (Millar 1961).
	1958-1960s	Thousands of oysters from Brittany laid in Loch Ryan (Millar 1963).
L	18thC& 19th C	Millions exported from Firth of Forth and laid in England, France and Holland (Fulton 1895; Millar 1961).
	1870s	30 000 oysters unknown source laid within the Firth of Forth (Fulton 1895).

Fig. 1. Recorded translocations and anecdotal records of translocations of *Ostrea edulis* into and within Scotland.

*Oedu.*J12 was increased from 50 °C to 55 °C, the number of secondary polymerase chain reaction (PCR) cycles for Oe1/63 was reduced from 25 to 22 and minor changes were made to concentrations of template DNA and deoxynucleotide triphosphates (dNTPs). PCR products were separated and quantified using a LiCor DNA sequencer and gel images were carefully checked by eye to ensure that the automatic analysis was detecting and selecting fragment sizes correctly.

Genetic analyses

Microsatellite genotype data were analysed using TFPGA (Miller 1997), F-STAT (Goudet 2001) and BOTTLENECK (Cornuet and Luikart 1996). Agreement with the Hardy Weinberg model was tested using exact tests and Wright's (1965) F statistics were calculated according to Weir and Cockerham (1984). Probabilities of multiple tests of the same hypothesis

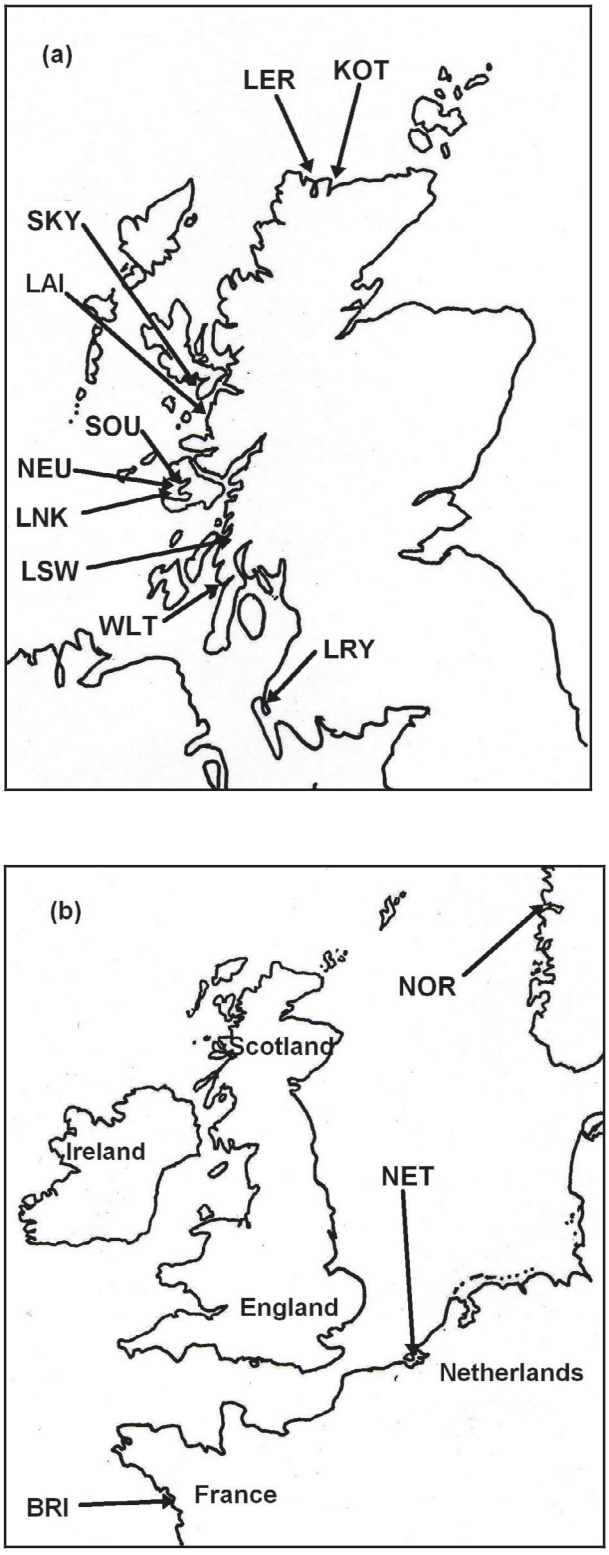


Fig. 2. Sample sites in Scotland and Europe of populations of *O. edulis* for genetic analysis at 6 microsatellite loci. Population abbreviations (from North to South): Norway (**NOR**), Loch Eriboll (**LER**), Kyle of Tongue (**KOT**), Skye (**SKY**), Loch Ailort (**LAI**), Sound of Ulva (**SOU**), North East Ulva (**NEU**), Loch na Keal (**LNK**), Loch Sween (**LSW**), West Loch Tarbert (**WLT**), Loch Ryan (**LRY**), The Netherlands (**NET**) and Brittany (**BRI**). (a) Sample sites in Scotland. (b) Sample sites in northern Europe.

were Bonferroni adjusted using the Hochberg (1988) method. Nei's (1978) unbiased genetic distances were calculated for all pairwise comparisons between populations and used within an Unweight Pair Group Method with Arithmetic (UPGMA) routine to produce a dendrogram illustrating the genetic relatedness between oyster populations. For each population Cornuet and Luikart's (1996) method was used to estimate the probability that a population had recently undergone a genetic bottleneck. Mantel tests were used to check for isolation by distance across populations by looking at correlations between pair-wise geographical distances (nearest coastline distance) and pair-wise genetic distance measures, i.e. Nei's (1978) D and Rousset's (1997) $F_{st} / (1–F_{st})$.

Results

Microsatellite variation

Tables 1a-f show allele frequencies at up to six loci across 13 populations. Sample sizes were small for some populations and because some samples failed to amplify at certain loci only two loci were successfully genotyped for Loch na Keal and data for some other loci are missing for other populations. Analyses were carried out separately on a "6 locus" data set from 8 populations and a "3 locus" data set from 12 populations.

Mean numbers of alleles across all populations range from 3.3 for locus Oe2/71 up to 18.8 for locus *Oedu*.U2 (Table 2). Because there is variation in sample sizes between populations, the numbers of alleles per locus are best described by the measure of allelic richness (Oe2/71 = 3.06, *Oedu*.U2 = 14.65). Mean heterozygosity is highest at the *Oedu*.J12 locus (0.919) and lowest at Oe2/71 (0.472) (Table 2).

Agreement with the Hardy-Weinberg model

In every case where there is a significant deviation from the Hardy-Weinberg model (12 out of 61 tests: $p < 0.001$ following Bonferroni adjustment), the deviation is always in the direction of deficiency of heterozygotes (Ho < He) (Table 2). Across all 61 tests, there are 51 instances of Ho < He (-ve) and only 10 instances of Ho>He (+ve) and this is a significant ($p < 0.05$) difference from an equal number of positives and negatives that would be expected if loci were generally in Hardy Weinberg equilibrium in all populations. Overall values of mean F_{is} are positive for both the 3 locus (F_{is} = 0.193) and the 6 locus (F_{is} = 0.148) data set confirming an overall deficiency of heterozygotes relative to the expectations of the Hardy-Weinberg model.

Genetic differentiation

Genetic differentiation measured as Wright's (1965) F_{st} is 0.048 for the 6 locus (8 population) data set and 0.051 for the 3 locus (12 population) data set indicating that the reduction of loci to 3 and the inclusion of a further 4 populations does not

Table 1 (a). Allele frequencies at the *Oedu*.J12 locus in 10 Scottish and 3 European populations of *O. edulis*. Alleles are given as size in base pairs. For population abbreviations see Figure 2. Allele frequencies above 0.1 are given in bold.

Population Allele	NOR	LER	KOT	SKY	LAI	SOU	NEU	LNK	LSW	WLT	LRY	NET	BRI
202							0.011						
204						0.025							
206					0.029				0.012				
208					0.014								
210										**0.141**			
212			0.019		0.014		0.011		0.036				
214							0.023		0.060		0.088		
216		**0.125**		0.056	0.043	**0.100**		0.021	0.024	0.031	**0.132**		**0.147**
218			0.039		0.071		0.023	0.063		**0.203**		**0.100**	
220			**0.115**		0.014		**0.148**	0.021	**0.202**	0.047		0.050	
222	**0.141**		0.019	**0.278**	**0.143**		0.057	0.063	**0.107**	0.094	0.044	0.050	**0.147**
224	0.016	0.063	**0.173**	**0.111**	0.014	**0.150**	0.068	**0.188**	0.036		**0.118**	**0.100**	**0.177**
226	0.016	**0.125**		0.056	0.043	**0.200**	0.011	0.063	0.036	0.063		0.050	0.059
228		**0.188**	0.019		0.057	**0.175**	0.046	0.063		0.016	0.088		
230	0.031	**0.250**	0.058		0.043			0.083	0.083	0.031	0.059		
232			0.039		**0.100**	0.025	0.057	0.042	0.095		0.029		0.029
234	0.094		0.077	0.056		0.050	0.034	0.021		**0.109**	0.059	**0.100**	0.059
236		0.063			0.057		0.034		0.012	0.016	0.044		0.029
238	**0.203**	0.063	0.039		0.029		0.034	0.042	0.060	0.063	0.059		**0.118**
240	0.047	0.063	**0.154**	0.056	0.014	0.050	0.057	0.021	0.024				
242			0.019		0.014		0.034	0.083	0.024	0.016	0.074	**0.150**	
244	0.016		0.019		0.086	**0.150**	0.091	0.063	0.083	0.016	0.059		0.059
248	**0.281**		0.077	**0.111**	0.057		0.057	0.021	0.012		0.015	0.050	0.059
250	0.063		0.039	**0.111**	0.014	0.025	0.011	0.042		0.031	0.044	0.050	0.059
252	0.016		0.039		0.043	0.025	0.034	0.083	0.036		0.044	**0.150**	0.029
254	0.047				0.043	0.025	0.011			0.031			
256	0.016	0.063	0.058		0.014		0.057		0.012		0.029		0.029
258	0.016			**0.111**			0.011		0.012			**0.150**	
260					0.014		0.023	0.021		0.031	0.015		
262					0.014		0.011		0.012	0.031			
264					0.014				0.024	0.016			
266										0.016			
268							0.046						
276				0.056									

Table 1 (b). Allele frequencies at the *Oedu*.T5 locus in 10 Scottish and 3 European populations of *O. edulis*. Alleles are given as size in base pairs. For population abbreviations see Figure 2. Allele frequencies above 0.1 are given in bold.

Population Allele	NOR	LER	KOT	SKY	LAI	SOU	NEU	LNK	LSW	WLT	LRY	NET	BRI
104							0.022						
106	0.088	**0.206**	**0.107**	**0.181**	**0.368**	0.068	**0.111**	0.043	**0.318**	**0.119**	**0.293**	0.053	0.033
108												0.053	**0.100**
110			0.018				0.011	**0.114**		0.036			
112						0.011			0.080		0.012		
114							0.011	0.086				0.026	0.033
116					0.053			0.043					
118	0.029		**0.250**	**0.318**	**0.132**	**0.409**	**0.189**	**0.229**	**0.136**	**0.333**	0.098	0.053	
120		**0.118**			0.092	0.011	**0.111**	0.071	0.034	0.012	0.061	0.079	0.067
122		**0.177**					0.022	0.029	0.011				
124			**0.143**	0.091	**0.145**	**0.102**	**0.111**	0.014	0.046	0.060	0.073	0.026	0.033
126		**0.324**	0.018		0.013	0.023			**0.102**	0.036	0.061	0.053	0.067
128		0.029	0.018		0.040				0.046		0.037	0.053	
130		0.029	0.018			0.023	0.022	0.029	0.023		0.012	**0.132**	0.033
132	0.074	0.029	0.089	0.046		0.034	**0.156**	0.043	0.034	0.071	0.012	0.026	
134			**0.161**	**0.136**	0.026	0.046	0.011	0.014	0.011		0.061		
136					0.013	**0.102**	0.067		0.034	**0.107**	0.012	**0.132**	0.033
138	**0.250**		0.054		0.053	0.068	0.044	0.029	0.011	0.071	0.061	0.079	**0.233**
140	**0.132**				0.013	0.023	0.022	0.029	0.034	0.012		0.053	**0.133**
142	0.029			0.046		0.011	0.033	0.029	0.011	0.060			0.033
144	0.029			0.091	0.013	0.011	0.033	**0.100**	0.023	0.036	0.049		
146	**0.265**	0.088	0.036			0.011		0.029	0.011		0.024		
148			0.054	0.046				0.014	0.023	0.012	0.037	**0.105**	0.033
150	0.059		0.018	0.046	0.013	0.023	0.022			0.024	0.024		
152			0.018		0.013	0.011		0.029	0.011	0.012	0.024	0.026	0.067
154	0.015							0.014				0.053	0.033
156	0.029				0.013						0.012		0.033
158						0.011					0.024		
160													0.033
178								0.014			0.012		

Table 1 (c). Allele frequencies at the *Oedu*.U2 locus in 10 Scottish and 3 European populations of *O. edulis*. Alleles are given as size in base pairs. For population abbreviations see Figure 2. Allele frequencies above 0.1 are given in bold.

Population Allele	NOR	LER	KOT	SKY	LAI	SOU	NEU	LNK	LSW	WLT	LRY	NET	BRI
146			0.036			**0.229**							
148									0.019				
152	**0.103**						0.048		0.019		0.018		
154	0.059										0.036		
156	0.015		**0.179**		0.018	0.063			0.093	0.021	0.054		
158			0.036		0.054	0.021	0.032			0.042	0.071		
160	0.029		0.071		0.054	0.021	0.032		0.093	0.063	**0.143**		
162	**0.235**		0.071		0.036	0.021	0.048		0.093	**0.104**	0.018		
164	0.015		0.071		0.071	0.042	0.097				0.071		
166	0.015		0.089				0.032		0.019	0.042	0.071		
168	0.074		0.036			0.021	0.016			0.042	0.054		
170			0.089		0.071	**0.125**	0.065		**0.130**	**0.146**	0.071		
172			0.036		0.036	0.021	0.081		0.074	0.021	0.054		
174			0.018		0.018	0.042	0.032		0.074	0.063	0.036		
176	0.029				0.071	0.042	0.081		0.037	**0.104**	0.018		
178			0.036		**0.161**	0.083	0.065			0.021	0.089		
180			0.018		0.036				0.074	0.063			
182					0.071		0.048		0.056	0.063	0.036		
184	0.088		0.036		0.036		0.032		0.037	0.021			
186	0.074		0.054		0.036	**0.146**	**0.113**		0.056	0.021	0.054		
188			0.054		0.054		0.016			0.042	0.036		
190	0.059		0.054		0.054		0.048		0.019	0.021			
192	**0.147**				0.054		0.032		0.074	0.083	0.018		
194	0.029				0.018	0.042	0.016						
196						0.021	0.016		0.019		0.036		
198	0.029				0.036	0.021	0.032						
200									0.019		0.018		
202			0.018		0.018	0.021	0.016						
208						0.021							
226										0.021			

Table 1 (d). Allele frequencies at the Oe1/63 locus in 10 Scottish and 3 European populations of *O. edulis*. Alleles are given as size in base pairs. For population abbreviations see Figure 2. Allele frequencies above 0.1 are given in bold.

Population Allele	NOR	LER	KOT	SKY	LAI	SOU	NEU	LNK	LSW	WLT	LRY	NET	BRI
90						0.081							
92									0.011				
94						0.032	**0.141**		0.054		0.043		
96				**0.750**	0.052	0.032	0.094			0.013	0.043	**0.375**	**0.333**
98		0.063	**0.125**		**0.155**		**0.266**			0.040	0.029		
100	**0.265**	**0.125**	**0.146**	**0.125**	0.086	**0.129**	**0.109**		0.065	**0.145**	0.043	**0.188**	0.083
102	**0.235**	0.063	**0.125**	0.063	**0.121**	0.065	**0.188**		0.033	**0.250**	0.071		0.083
104	**0.162**	**0.219**	0.063		**0.172**	**0.242**	0.016		**0.359**	**0.184**	**0.100**	**0.250**	0.083
106	**0.132**	0.094	0.083	0.063	**0.172**	**0.145**			**0.109**	**0.184**	**0.300**	0.063	0.083
108	**0.147**	**0.219**	0.063		**0.172**	**0.210**	**0.125**		**0.217**	0.092	**0.229**		**0.333**
110	0.059	**0.219**	**0.375**		0.035	0.048	0.031		**0.120**	0.040	**0.129**	0.063	
112			0.021		0.035				0.022	0.053	0.014	0.063	
114						0.016	0.031		0.011				

Table 1 (e). Allele frequencies at the Oe1/64 locus in 10 Scottish and 3 European populations of *O. edulis*. Alleles are given as size in base pairs. For population abbreviations see Figure 2. Allele frequencies above 0.1 are given in bold.

Population Allele	NOR	LER	KOT	SKY	LAI	SOU	NEU	LNK	LSW	WLT	LRY	NET	BRI
130											0.040		
132											0.020		
134											0.040		
136					0.042	0.022							
138											0.080		
140						0.022					0.080		
142											0.040		
144	0.059				0.021	0.022			0.036				
146	**0.221**		**0.136**		**0.125**	**0.130**	**0.107**		**0.196**		0.040		
148	**0.118**		**0.159**		**0.229**	**0.174**	**0.179**		**0.143**	**0.104**	0.060		
150	0.044					**0.109**				**0.271**	0.040		
152	0.088				**0.188**	0.022			0.071	0.021	0.020		
154	0.059				**0.375**	**0.326**	**0.393**		**0.554**		**0.220**		
156	**0.103**		**0.300**			**0.109**	**0.321**			**0.313**	**0.300**		
158	0.015		**0.364**		0.021	0.022				**0.104**	0.020		
160	**0.265**		0.023			0.022				**0.188**			
162	0.015					0.022							
164	0.015												
174			0.023										

Table 1 (f). Allele frequencies at the Oe2/71 locus in 10 Scottish and 3 European populations of *O. edulis*. Alleles are given as size in base pairs. For population abbreviations see Figure 2. Allele frequencies above 0.1 are given in bold.

Population	NOR	LER	KOT	SKY	LAI	SOU	NEU	LNK	LSW	WLT	LRY	NET	BRI
Allele													
300							0.036			0.022	0.080		
302											0.020		
304					**0.105**		0.036		0.056		0.080		
306	**0.833**		**0.833**		**0.579**		**0.643**		**0.630**	**0.630**	**0.500**		
308	**0.152**		**0.167**		**0.316**		**0.286**		**0.315**	**0.348**	**0.320**		
310	0.015												

dramatically alter the estimate of the level of genetic differentiation. Mean F_{st} for all Scottish populations is 0.038 and this value falls slightly to 0.030 if the Skye population is excluded.

Pairwise comparisons between 12 populations (excluding Loch na Keal) using Nei's (1978) unbiased genetic distance (D) and Wright's (1965) F_{st} are given in Table 3. The highest values of D and of F_{st} are found when comparing the Skye population with all others (mean D = 0.8107, mean F_{st} = 0.068): this compares with the next highest mean D of 0.5877 and mean F_{st} of 0.051 for the Norway population. These high values of D indicate that these two populations are relatively distinct, genetically, from all others. Figure 3 shows a dendrogram of 12 populations based on data at three loci which illustrates the genetically distinct nature of the Skye and Norway samples. The Netherlands and Brittany samples cluster together suggesting that they are quite similar to one another, but they differ from Scottish and Norwegian samples. There appear to be two main groupings among the Scottish sites (apart from Skye): a cluster that includes Loch Ailort, Loch Ryan, Loch Sween and Loch Eriboll and a second group consisting of the Ulva region, West Loch Tarbert and the Kyle of Tongue. Because of the small samples sizes and high variability of the microsatellite loci used, it is unlikely that any significance can be attached to these two groupings. The Loch na Keal sample is excluded from Figure 3 because there are only data for two loci, but when included (dendrogram not shown) Loch na Keal oysters cluster within the geographically proximate Ulva grouping.

A second pairwise comparison (Fig. 4) is made between 8 populations for which there are data at all 6 loci (or 5 loci – Sound of Ulva). As in Figure 2, the Norway sample is clearly different from all others. Of the Scottish samples, Loch Ryan, Loch Sween and Loch Ailort group together as they did in the 3 locus analysis and Kyle of tongue and North East Ulva oysters cluster together. West Loch Tarbert oysters appear relatively different from other Scottish populations.

No populations of oysters showed any evidence of a recent bottleneck based on Cornuet and Luikart's (1996) method. Mantel tests for correlation between two measures of genetic distance and actual linear geographic distances between populations were not significant, i.e. Nei's (1978) D: r = 0.1579, $p > 0.05$; Rousset's (1997) F_{st} / (1–F_{st}) : r = −0.1267, $p > 0.05$).

Discussion

Using just 20 oysters trawled from Southampton Water (UK), Morgan et al. (2000) recorded 7 alleles at the Oe1/63 locus, 4 at the Oe1/64 locus and 3 at the Oe2/71 locus. We have detected a further 6, 15 and 3 alleles respectively for these loci. Launey et al. (2002) do not provide total numbers of alleles detected at their three loci, *Oedu*.J12, *Oedu*.T5 and *Oedu*.U2, that we have used in our study.

We did not achieve a full data set of genotypes at 6 loci for all the 13 populations sampled. Reasons could include variable quality of extracted DNA, contaminant-inhibition of PCR, difficulty in identification of bands due to "stutter" and the existence of homozygotes for null alleles. It is probable that all

Table 2. Number of individuals scored (N), number of alleles (Na), allelic richness (AR), heterozygosity expected (He), heterozygosity observed (Ho) and probability (*p*) of Hardy Weinberg Equilibrium (significant in bold) at six microsatellite loci in 13 populations of *O. edulis*. Population abbreviations, Figure 2.

Population Locus	NOR	LER	KOT	SKY	LAI	SOU	NEU	LNK	LSW	WLT	LRY	NET	BRI	Mean
Oedu.J12 N	32	8	26	9	35	20	44	24	42	32	34	10	17	25.6
Na	14	9	17	10	24	12	25	18	21	19	17	11	13	16.2
AR	9.85	7.64	13.38	7.94	16.4	7.01	16.03	13.30	13.67	13.35	13.75	8.68	8.41	11.47
He	0.854	0.908	0.923	0.909	0.948	0.889	0.948	0.936	0.920	0.915	0.938	0.936	0.918	0.919
Ho	0.594	0.875	0.808	0.889	0.771	0.600	0.705	0.792	0.762	0.656	0.765	0.700	0.765	0.745
p	**<0.001**	0.768	0.035	0.805	**<0.001**	**<0.001**	**<0.001**	0.011	0.001	0.002	**<0.001**	0.006	0.618	
Oedu.T5 N	34	17	28	11	38	44	45	35	44	42	41	19	15	31.8
Na	11	8	14	9	15	18	17	20	19	15	20	16	16	15.2
AR	8.83	5.55	10.48	6.69	9.59	6.48	11055	130.2	11.76	10.58	13.02	7.78	8.95	9.56
He	0.842	0.822	0.879	0.861	0.819	0.805	0.902	0.913	0.862	0.851	0.887	0.945	0.924	0.870
Ho	0.618	0.706	0.821	1.000	0.553	0.705	0.733	0.800	0.750	0.810	0.781	0.947	0.867	0.776
p	0.003	0.158	0.252	0.642	**<0.001**	0.055	**<0.001**	0.008	0.231	0.604	0.416	0.009	0.618	
Oedu.U2 N	34	0	28	0	28	24	31	0	27	24	28	0	0	31.5
Na	15	-	18	-	20	18	22	-	18	19	20	-	-	18.8
AR	11.46		14.45	-	15.71	14.45	16.26	-	14.19	15.10	15.59	-	-	14.65
He	0.896	-	0.936	-	0.947	0.908	0.954	-	0.942	0.944	0.949	-	-	0.935
Ho	0.912	-	0.750	-	0.893	0.958	0.871	-	0.889	0.875	0.786	-	-	0.867
p	0.438	-	0.001	-	0.082	0.020	0.349	-	0.133	0.111	0.005	-	-	
Oe1/63 N	34	16	24	8	29	31	32	0	46	38	35	8	6	25.6
Na	6	7	8	4	9	10	9	-	10	9	10	6	6	7.8
AR	5.88	5.80	7.42	3.45	8.30	6.29	7.76	-	7.5	7.69	8.32	5.96	5.24	6.63
He	0.818	0.851	0.809	0.442	0.875	0.858	0.849	-	0.798	0.845	0.832	0.800	0.818	0.806
Ho	0.706	0.750	0.250	0.250	0.655	0.613	0.625	-	0.783	0.711	0.571	0.750	0.833	0.625
p	0.116	0.151	**<0.001**	0.145	0.004	**<0.001**	**<0.001**	-	0.044	0.017	0.008	0.725	1.000	
Oe1/64 N	34	0	22	0	24	23	14	0	28	24	25	0	0	24.3
Na	11	-	6	-	7	12	4	-	5	6	13	-	-	8.0
AR	8.75	-	5.27	-	5.99	9.92	4.00	-	4.70	5.57	10.60	-	-	6.85
He	0.821	-	0.753	-	0.769	0.838	0.725	-	0.640	0.788	0.853	-	-	0.773
Ho	0.882	-	0.636	-	0.583	0.652	0.286	-	0.714	0.667	0.680	-	-	0.638
p	**<0.001**	-	0.194	-	0.097	0.015	**<0.001**	-	0.016	0.355	0.004	-	-	
2/71 N	33	0	24	0	19	0	14	0	27	23	25	0	0	23.6
Na	3	-	2	-	3	-	4	-	3	3	5	-	-	3.3
AR	2.42	-	2.00	-	2.99	-	4.00	-	2.90	2.61	4.50	-	-	3.06
He	0.287	-	0.284	-	0.569	-	0.521	-	0.511	0.492	0.647	-	-	0.472
Ho	0.333	-	0.333	-	0.632	-	0.429	-	0.407	0.478	0.800	-	-	0.487
p	1.000	-	1.000	-	0.751	-	0.222	-	0.477	1.000	0.727	-	-	
Mean N	33.5	13.6	25.3	9.3	28.8	28.4	30	29.5	35.7	30.5	31.3	12.3	12.7	
Mean Na	10.0	8.0	10.8	7.7	13.0	14.0	13.5	19.0	12.7	11.8	14.2	11.0	11.7	
Mean AR	7.87	6.33	8.83	6.03	9.79	8.83	9.93	13.16	9.12	9.15	10.96	7.47	7.53	
Mean He	0.758	0.860	0.764	0.737	0.821	0.860	0.816	0.925	0.779	0.806	0.851	0.894	0.887	
Mean Ho	0.674	0.777	0.600	0.713	0.681	0.706	0.608	0.796	0.718	0.699	0.730	0.799	0.822	

Table 3. Pairwise comparisons of genetic differences between populations of *O. edulis* based on up to 6 loci over 12 populations. Above diagonal, Wright's (1965) F_{st}; below diagonal, Nei's (1978) unbiased genetic distance (*D*). For population abbreviations see Figure 2.

Population	NOR	LER	KOT	SKY	LAI	SOU	NEU	LSW	WLT	LRY	NET	BRI
NOR		0.051	0.040	0.099	0.378	0.059	0.039	0.040	0.044	0.040	0.051	0.058
LER	0.758		0.039	0.097	0.020	0.047	0.046	0.035	0.027	0.047	0.010	0.051
KOT	0.276	0.615		0.059	0.023	0.020	0.010	0.013	0.030	0.029	0.026	0.032
SKY	1.230	1.871	0.586		0.058	0.081	0.070	0.017	0.099	0.078	0.076	0.019
LAI	0.339	0.401	0.200	0.754		0.036	0.011	0.032	0.010	0.027	0.013	0.047
SOU	0.807	0.545	0.240	0.837	0.302		0.033	0.034	0.044	0.033	0.037	0.059
NEU	0.335	0.861	0.060	0.717	0.124	0.330		0.009	0.030	0.025	0.021	0.041
LSW	0.388	0.276	0.210	1.219	0.063	0.305	0.187		0.046	0.027	0.033	0.006
WLT	0.267	0.756	0.240	0.836	0.295	0.406	0.234	0.384		0.043	0.025	0.071
LRY	0.391	0.255	0.142	0.907	0.119	0.330	0.164	0.181	0.216		0.033	0.047
NET	0.793	0.867	0.640	0.215	0.678	0.646	0.737	0.609	0.602	0.712		0.056
BRI	0.400	0.637	0.283	0.332	0.499	0.533	0.614	0.653	0.748	0.352	0.199	

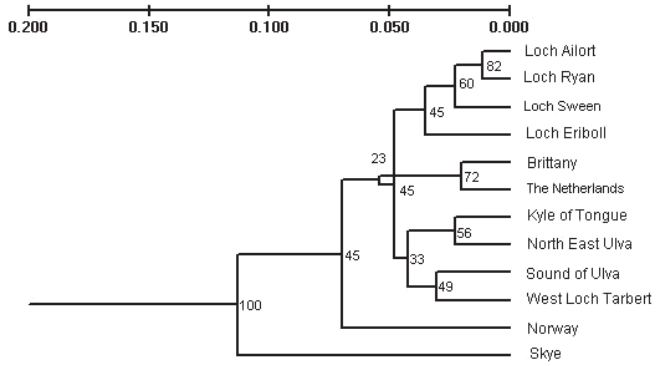


Fig. 3. Dendrogram of genetic relatedness – Nei's (1978) Genetic Distance, *D* – between populations of *O. edulis* based on data at 3 microsatellite loci. Numbers at nodes are proportions of permuted data sets that support the node.

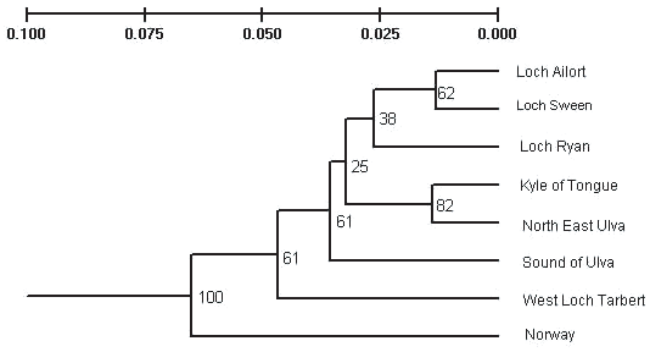


Fig. 4. Dendrogram of genetic relatedness – Nei's (1978) Genetic Distance, *D* – between populations of *O. edulis* based on data at 6 microsatellite loci (5 for SOU). Numbers at nodes are proportions of permuted data sets that support the node.

four reasons have contributed to the reduced data set in this instance. In addition, for some localised populations only a few individuals could be obtained.

For populations at which all 6 loci were scored, mean numbers of alleles per locus ranged from 10.0 to 13.5 which is less than the mean value reported by Launey et al. (2002) but within the range previously reported by Sobolewska and Beaumont (2005) for 4 (entirely different) microsatellite loci in northern European populations. At highly polymorphic loci, the number of alleles identified is very dependent upon the sample size and in some cases the sample sizes in this study are really quite small. A significant relationship between sample size and number of alleles detected is evident at three of the loci (J12, $p < 0.001$; T5, $p < 0.02$; 1/63, $p < 0.01$), but not at the other three loci (U2, 1/64 and 2/71, $p > 0.05$). The measure of allelic richness takes sample sizes into account (Table 2).

Using larger sample sizes, Hedgecock et al. (2007) detected a mean of 23 alleles per microsatellite locus in an adult *O. edulis* population, and 13.75 in a sample of juveniles from a (12 day) natural settlement in the same area. Because of the differences in sample sizes, and the fact that only two of the microsatellite loci used were common to Hedgecock et al.'s (2007) study and ours, it would be unwise to draw conclusions from any direct comparison of genetic variability based on mean numbers of alleles per locus.

As in other studies of highly polymorphic microsatellites, mean expected heterozygosity is high at most loci (ranging from 0.773 to 0.919). The exception is locus Oe2/71 where there are only 3 common alleles and mean expected heterozygosity is 0.472.

The general deficiency of heterozygotes relative to the Hardy-Weinberg model was a very common finding in allozyme studies of marine bivalves (Zouros and Foltz 1984; Gaffney 1994). The same pattern seems to be emerging at microsatellite loci in flat oysters (Launey et al. 2002; Sobolewska and Beaumont 2005; Hedgecock et al. 2007; Vercaemer et al. 2006) and cupped oysters (McGoldrick et al. 2000; Rose et al. 2006).

It is very unlikely that the significant heterozygote deficiencies in our data are caused by some systematic factor such as inbreeding because there is no consistent pattern at particular loci or in particular populations (Table 1). Significant deficiencies occur at all loci except Oe2/71, and are not restricted to particular populations. Also the Wahlund effect, the accidental sampling of cryptic population substructure within populations, is ruled out because of the general lack of large allele frequency differences between populations.

Although microsatellite loci are generally expected to be neutral, some evidence that indirect selection, where the microsatellite locus is linked to a coding locus, has been detected

in oysters by Boudry et al. (2002). This could be the cause of some of the heterozygote deficiencies, but by far the most likely cause is the presence of null alleles. Null homozygotes do not score on gels and null heterozygotes are scored incorrectly as homozygotes leading to a deficiency of heterozygotes. In hatchery crosses, null alleles have sometimes been detected at allozyme loci (Gaffney 1994, Hoare and Beaumont 1995) but null alleles are far more common at microsatellite loci (McGoldrick et al. 2000; Boudry et al. 2002). Non-scoring individuals may be caused by other factors besides null alleles so counting non-scoring individuals is not a very safe method for estimating the frequency of null alleles at microsatellite loci.

Our estimates of mean F_{st} between all 12 populations (0.051) or 8 populations (0.048) is higher than that determined by Launey et al. (2002) for their North Altantic samples (F_{st} = 0.014) and for the northern European populations studied by Sobolewska and Beaumont (2005) (F_{st} = 0.02 excluding hatchery-based populations). The implication is that the suite of microsatellite loci used here is identifying more genetic differentiation than other studies but this may be partly a consequence of our relatively small sample sizes. Our mean F_{st} for all Scottish populations is 0.038 which is similar to that estimated by Vercaemer et al. (2006) for North American populations (F_{st} = 0.033).

The analysis of population genetic differentiation identifies the Skye sample as being rather different to all others and the Norway sample as being different to all except the Skye population. This result in relation to the Norway sample agrees with the findings of Launey et al. (2002) and Sobolewska and Beaumont (2005) who also found Norwegian populations of *O. edulis* to be distinct from other European populations. This current study employs some of Launey et al.'s (2002) loci, but none of those used by Sobolewska and Beaumont (2005). Confirmation of a pattern identified by variation at a number of different microsatellite loci by different authors strengthens the conclusion that Norwegian *O. edulis* may represent a genetic resource that has not been significantly affected by human activity. Conservation of Norwegian oysters as a separate population is therefore of importance and importations of oysters from elsewhere should be controlled.

The small sample size of the Skye population gives rise to concern over the significance of its genetic distinctiveness. Only 11 individuals were available for genotyping, and some failed to amplify at all loci. How important is the sample size?

Of the three loci scored, only one (Oe1/63) shows a large difference in allele frequency from other samples. The Oe1/63 allele [96] is at high frequency (0.750) in Skye, but absent from, or rare in all other Scottish populations (highest frequency 0.094 in North East Ulva). In order for Skye to group among the other populations in its region, it should have a similar frequency of allele Oe1/63 [96]. If the *true* frequency of this allele in the Skye population is around 0.09 we can calculate the probability of sampling, by chance from 8 oysters, individuals that give a frequency of 0.75 for this allele. The probability of this event is $p = \ll 0.001$ and sample size needs to be down as low as 4–5 individuals before this becomes a likely event. It is therefore not safe to conclude that the distinctness of Skye sample is simply an effect due to small sample size.

Although the Oe1/63 [96] allele is rare or absent in Scottish populations (other than Skye) it is more common in the southern samples from the Netherlands (0.375) and Brittany (0.333). In the 1950s unknown quantities of 2–3 year old Brittany oysters were imported to various sites in Scotland including Skye (Millar 1961). It is possible that the high frequency of the Oe1/63 [96] allele in the Skye population is the result of a founder effect from this stocking event.

If this is a real founder effect, it is important to consider why there is no evidence for founder effects at other Scottish sites that were stocked in similar way. This could indicate that gene flow between Skye and other Scottish populations may be less frequent than gene flow within these other Scottish populations.

Considering the Skye data at other loci, Norway has allele *Oedu*.J12[248] at high frequency (0.281) but this is rare (<0.08) or absent at all other sites except Skye where its frequency is 0.111. At the *Oedu*.T5 locus, Skye shares its three most common alleles *Oedu*.T5[106], *Oedu*.T5[118] and *Oedu*.T5[134] with many other Scottish populations, but these alleles are rare or absent in the Brittany sample. This is not what would be expected if there had been a founder effect due to importation of oysters from Brittany.

Because of the small sample size of the Skye population, it is not possible to decide with certainty what may have been the cause of the apparently distinct genetic nature of the sample analysed. However, there are some intriguing possibilities and further work is required to elucidate the true genetic relatedness of the Skye population to others in the region.

There is suggestion of a further subdivision of populations within the Scottish samples (excluding Skye) where Loch Ryan, Loch Ailort, Loch Sween and Loch Eriboll cluster together distinct from the Ulva populations, Kyle of tongue and West Loch Tarbert (Fig. 3). This grouping is partially, but less clearly supported by the 6 loci data set (Fig. 4). Because of the relatively weak bootstrap support for this node in both dendrograms it is probably not safe to treat these as significant groupings. Nevertheless, it is important to note that these suggested groupings have no obvious link to their geographical position – Loch Eriboll, the most northerly Scottish population groups with Loch Ryan the most southerly one and oysters from the Kyle of Tongue, close to Loch Eriboll and probably derived from Eriboll oysters via a hatchery in Orkney, cluster in the other group.

Cornuet and Luikart (1996) developed a test of the expectation that, following a severe reduction in population size, the equilibrium between the number of alleles and heterozygosity at a locus is disturbed such that a slight excess of heterozygosity over that predicted by the Hardy-Weinberg model is expected for several generations. Deficiencies of heterozygotes are a common feature of our data so the test is likely to be unable to effectively detect excess heterozygosity. It is therefore not unexpected that no significant bottlenecks were detected, but this does not mean that there could not have been bottlenecks. Whether or not there have been significant bottlenecks at any time in the populations that were sampled in this study, it is instructive to note that the general level of genetic variation is high. As with microsatellite loci in other organisms (Sunnucks 2000), many variant alleles are present and

heterozygosity is high at the loci tested. This would not support the idea of significant population size reductions having taken place in the recent past.

If there has been little influence of human intervention on population structure in *O. edulis*, we would expect to find that pair-wise genetic distances correlate to some extent with geographic distances between pairs of populations. Mantel tests to check for isolation by distance using Nei's (1978) *D* and Rousset's (1997) index (F_{st} / [1–F_{st}]) were not significant ($p > 0.05$) and this supports the contention that human activities have been sufficiently strong over recent centuries to override any random genetic drift that could establish and maintain natural population differentiation. However, the simplistic measure of geographic distance used in the test does not take into account the potential oceanographic factors that could restrict or enhance larval travel between any two points or populations. Thus the Mantel test result should be taken with caution.

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