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Genetic characterization of hybrid mussel (*Mytilus*) populations on Irish coasts

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The blue mussel, Mytilus edulis*, and the Mediterranean mussel,* Mytilus galloprovincialis*, occur widely over much of northern Europe, and wherever they are sympatric they hybridize. The hybrid zone is large, ranging from western France to the north of Scotland, and is spatially complex, containing a mixture of pure, hybrid and introgressed individuals. Results from an Irish study in 1981, using partially diagnostic allozyme markers, indicated that mussels on the Irish Sea coast were solely* M. edulis*, but on Atlantic wave-exposed shores, and to a much lesser extent on wave-protected shores, mussels comprised an interbreeding mixture of* M. edulis *and* M. galloprovincialis*. In this study mussels were analysed from 20 locations on Irish coasts, using the* Me15/16 *nuclear DNA marker. The results showed a high frequency of* M. galloprovincialis *(0.378 ± 0.198) and hybrid (0.429 ± 0.175) genotypes, and correspondingly low frequencies of the* M. edulis *genotype (0.194 ± 0.107) at both exposed and sheltered locations on Atlantic coasts, indicating no apparent advantage for the* M. edulis *genotype at wave-protected sites.* Mytilus galloprovincialis *was virtually absent from the Irish Sea. Mussels in this area may be a self-recruiting population of* M. edulis *due to thermal front development at the northern and southern entrances to the Irish Sea in late spring, thereby preventing an influx of spring-spawned* Mytilus *larvae. The apparent change in the genetic composition of mussels on Atlantic coasts since the early 1980s could be related to climate change, or to aquaculture practice in Ireland whereby mussels from exposed shores are used to seed ropes in wave-protected bays and estuaries.*

Keywords: *Mytilus edulis*, *Mytilus galloprovincialis*, *Me15/16* DNA marker, hybrid zone, hybridization, geographical variation

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INTRODUCTION

The blue mussel, *Mytilus edulis* Linnaeus 1758, and the Mediterranean mussel, *Mytilus galloprovincialis* Lamarck 1819, occur widely over much of northern Europe, and wherever they are sympatric they hybridize (Skibinski *et al.*, 1983; Gosling, 1992a, b). The hybrid zone is large, spanning more than 1400 km of coastline from western France to the north of Scotland, and is spatially complex, containing a mixture of pure, hybrid and introgressed individuals (Skibinski *et al.*, 1983; Gosling, 1992b; Bierne *et al.*, 2003a). Partial ecological segregation of *M. edulis* and *M. galloprovincialis* is said to account for most of the small-scale genetic patchiness within the hybrid zone, with *M. edulis* occupying sheltered estuarine habitats, and *M. galloprovincialis* largely found in wave-exposed habitats (Gosling & Wilkins, 1981; Skibinski *et al.*, 1983, Bierne *et al.*, 2002; Hilbish *et al.*, 2003). Post-settlement habitat-dependent selection (Hilbish *et al.*, 2003) or habitat choice by settling larvae (Bierne *et al.*, 2003b), have been suggested as agents responsible for ecological segregation of the taxa.

There is no single morphological character that can be reliably used to separate mixed populations, particularly at wave-exposed locations where hybridization and introgression between *M. edulis* and *M. galloprovincialis* generate large numbers of intermediate forms (Seed, 1974). About four protein (allozyme) marker loci are well differentiated but not diagnostic between any pair of blue mussel taxa (McDonald *et al.*, 1991; Gosling, 1992b). Two polymerase chain reaction (PCR)-based DNA markers, *Me15/16* and *Glu-5′* have now been developed, which diagnostically identify the three taxa, *M. edulis*, *M. galloprovincialis* and *Mytilus trossulus*, and allow accurate estimation of the frequency of parental and hybrid mussel classes within hybrid zones (Inoue *et al.*, 1995; Rawson *et al.*, 1996).

Recently, Coghlan & Gosling (2007) used the *Me15/16* marker to investigate the genetic composition of mussels from two wave-exposed sites and one sheltered, estuarine site, in Galway Bay, western Ireland. In spat and adults the frequency of the *M. galloprovincialis* allele was high (0.56–0.80), due to high frequencies of *M. galloprovincialis* (>37%) and hybrid (>33%) genotypes, and there were correspondingly low frequencies of the *M. edulis* genotype (<11%). They concluded that the three sites, all within 6 km of each other, share a common larval source, that recruitment is genetically homogeneous, and that the *M. edulis* genotype has no apparent advantage in sheltered, estuarine conditions. In contrast, results from an earlier study by Gosling & Wilkins (1981) on *Mytilus* from 26 sites on Irish coasts, using partially diagnostic allozyme markers (*Gpi, Pgm* and *Lap-2*), showed that mussels in the Irish Sea constituted a single panmictic population of *Mytilus edulis* alone, whereas on Atlantic wave-exposed shores, and to a much lesser extent on wave-

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protected shores, mussels comprised an interbreeding mixture of *M. edulis* and *Mytilus galloprovincialis*. A later study by Skibinski *et al.* (1983), using a larger number of loci, confirmed our observations for Irish Sea and Atlantic Ocean sites.

Since Coghlan & Gosling (2007) only sampled a small part of the distribution of *Mytilus* on Irish coasts, the aim of this study was to carry out a broader survey of 20 sites to see if the genetic structure established for Galway Bay *Mytilus* is typical of other populations on Irish Atlantic coasts. Also, because a number of sites in Gosling & Wilkins (1981) and Gosling & McGrath (1990) were also sampled in the current study this provided us with the opportunity to qualitatively compare allozyme and molecular data sets for the sites in common.

MATERIALS AND METHODS

Mussels were collected from 20 sites on the Irish Sea and Atlantic coasts of Ireland. At each site the sample was collected from one spot on the shore, usually the mid-shore (Table 1), and an attempt was made to collect a representative size-range of mussels. Among the 20 sites, three paired geographically close sites were sampled: Achill Island (exposed (E) Dooega, and sheltered (S) Saula; 5.5 km apart), Southern Connamara (Ballynahown E and S; 900 m apart) and Northern Clare (Blackhead (E) and Coolsiva, semi-exposed; 2 km apart). Because the sample size in this study was generally small (N = ~40), we tested the reliability of our results by comparing allele and genotype frequencies at a single site (Ballynahown sheltered; N = 36; July 2005) with four larger samples from the same site (N = 133–150) collected in January, February, March and April 2006.

Once collected, mussels were either preserved in 100% alcohol or frozen at −20°C. DNA extraction, PCR amplification and electrophoresis followed methods outlined in Coghlan & Gosling (2007).

Conformance to Hardy–Weinberg equilibrium (HWE) and comparisons of allele and genotype distributions between pairs of populations were assessed using Fisher's exact tests as implemented in GENEPOP 3.4 (Raymond & Rousset, 1995), with specified Markov chain parameters of 10 000 dememorization steps, followed by 100 batches of 5000 iterations per batch for all populations. To correct for multiple pairwise comparisons the false discovery rate procedure of Benjamini & Yekutieli (2001) was applied. This method accommodates large numbers of potentially dependant tests while balancing risks of Type I and Type II errors and is a good alternative to the very conservative Bonferroni correction (Rice, 1989), which is effective in reducing Type I, but not Type II errors (Narum, 2006).

RESULTS

Table 2 lists the genotype and allele frequencies for 1306 individuals from the 20 locations. All samples were in HWE with the exception of four Galway Bay sites: Coolsiva ($P < 0.001$), Ballynahown wave-exposed ($P < 0.05$) and wave-protected ($P < 0.05$), and Carraroe ($P < 0.05$), plus Belfast Lough ($P < 0.001$) and Carnsore Point ($P < 0.001$), which are situated at entry points to the Irish Sea. Departures from HWE were due to deficits of heterozygotes, with the exception of Carraroe where an excess of heterozygotes was observed.

The samples collected from Irish Sea sites were exclusively *Mytilus edulis*, with the exception of Belfast Lough (Table 2;

Table 1. Details of sites and dates of *Mytilus* sample collection.

Site	Coordinates	Date
Irish Sea coast		
Belfast Lough, Antrim (S)	54° 41′ 01″N 5°46′ 41″W	February 2005
Carlingford Lough, Down (S)	54° 03′ 09″N 6° 10′ 36″W	February 2005
Clogherhead, Louth (SE)	53° 51′ 35″N 6° 14′ 15″W	March 2005
Blackrock, Co. Dublin (S)	53° 17′ 43″N 6° 09′ 06″W	March 2005
Atlantic coasts		
Carnsore Point, Wexford (E)	52° 09′ 22″N 6° 21′ 59″W	March 2005
Garrettstown, Cork (E)	51° 38′ 26″N 8° 34′ 19″W	February 2005
Lough Hyne, Cork (S)	51° 30′27″N 9° 18′59″W	February 2005
Blackhead, Clare* (E)	53° 09′ 12″N 9° 15′ 55″W	July 2005
Coolsiva Quay, Clare* (S)	53° 09′04″N 9° 15′ 27″W	July 2005
Ballyloughan, Galway (S)	53° 16′ 06″N 9° 01′ 02 ″W	November 2005
Nimmo's Pier, Galway (S)	53° 16′ 00″N 9° 02′ 49″W	February 2006
Ballynahown, Galway* (E)	53° 13′ 16″N 9° 31′ 26″W	May 2003
Ballynahown, Galway* (S)	53° 13′ 18″N 9° 31′ 06″W	May 2005
Carraroe (E)	53° 14′ 04″N 9° 35′ 09″W	October 2003
Dooega, Achill* (E)	53° 55′07″N 10° 00′ 55″W	July 2005
Saula, Achill* (S)	53° 57′ 25″N 09° 56′38″W	July 2005
Louisburg (SE)	53° 46′ 16″N 09° 49′20″W	February 2006
Inishcrone (SE)	54° 12′ 41″N 09 05′50″W	November 2005
Kincaslough, Donegal (SE)	55° 02′03″N 08° 24′ 28″W	July 2005
Creeslough Donegal (S)	55° 08′14″N 07° 52′ 24″W	February 2006

S, sheltered; E, exposed; SE, semi-exposed; *paired sampling sites.

Table 2. Genotype and allele frequencies in 20 samples of *Mytilus* on Irish coasts. N, sample size. Figure in parentheses is the percentage of the hybrid genotype. Asterisks indicate samples that show significant deviations from Hardy–Weinberg equilibrium; $^{*}P < 0.05$; $^{***}P < 0.001$.

Site	N	Genotype frequencies			Allele frequencies	
		E/E	E/G	G/G	E	G
Belfast Lough	38	29	3 (8)	6	0.80	0.20***
Carlingford	38	38	0 (0)	0	1.00	0.00
Clogherhead	38	38	0 (0)	0	1.00	0.00
Blackrock	38	38	0 (0)	0	1.00	0.00
Carnsore Point	38	3	1 (3)	34	0.09	0.91***
Garrettstown	38	1	15 (39)	22	0.22	0.78
Lough Hyne	38	3	13 (34)	22	0.25	0.75
Blackhead	36	9	12 (33)	15	0.42	0.58
Coolsiva Quay	37	13	7 (19)	17	0.45	0.55***
Ballyloughan	38	7	25 (66)	6	0.51	0.49
Nimmo's Pier	69	10	35 (51)	24	0.40	0.60
Ballynahown E	34	8	10 (29)	16	0.38	0.62*
Ballynahown S	36	9	9 (25)	18	0.38	0.62*
Carraroe	33	0	20 (61)	13	0.30	0.70*
Dooega	71	16	38 (54)	17	0.49	0.51
Saula	35	13	19 (54)	3	0.64	0.36
Louisburgh	36	12	15 (42)	9	0.54	0.46
Inishcrone	38	6	25 (66)	7	0.49	0.51
Kincaslough	35	6	19 (54)	10	0.44	0.56
Creeslough	16	4	9 (56)	3	0.53	0.47

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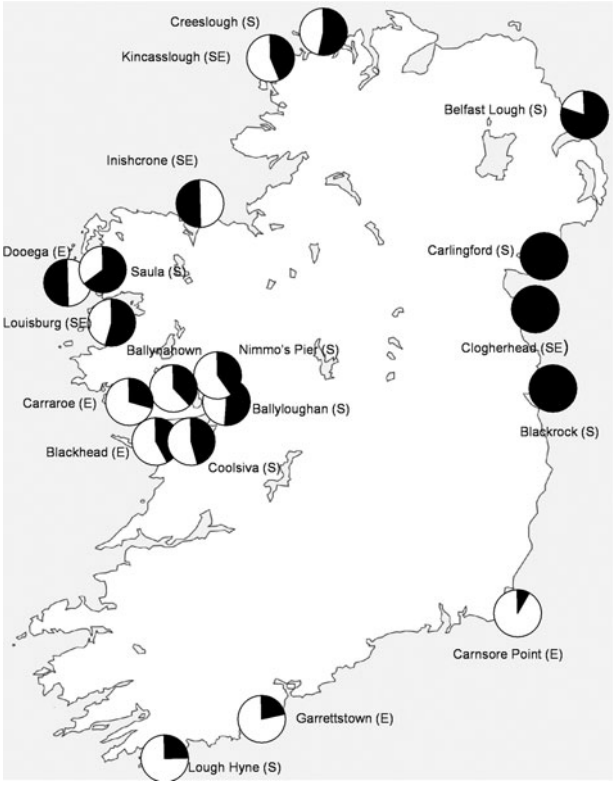

Fig. 1. Frequency of the *Mytilus edulis* allele (black) and the *Mytilus galloprovincialis* allele (white) at Irish sampling sites. Because allele frequencies were identical for the Ballynahown wave-exposed and sheltered sites, only one frequency is presented.

Figure 1), where the frequency of the G allele was moderately high (0.20), with hybrid and M*ytilus galloprovincialis* genotypes at a frequency of 8% and 16%, respectively. As a consequence, pairwise comparisons between Belfast Lough and the other three Irish Sea sites differed significantly in both genotypic ($P < 0.05$) and allelic ($P < 0.001$) proportions.

On Atlantic coasts the frequency of the G allele ranged from 0.36 at Saula, to 0.91 at Carnsore Point (Table 2; Figure 1). All samples differed significantly in both genotypic ($P < 0.001$ to 0.05) and allelic ($P < 0.001$ to 0.01) proportions from Irish Sea sites (Table 3), with the exception of Belfast Lough, which, while significantly different from the majority of Atlantic coastal samples in both genotypic ($P < 0.01$ to 0.05) and allelic ($P < 0.01$ to 0.001) proportions, did not differ significantly from Saula and Creeslough on the west and north-west coasts.

There was a remarkable degree of genetic homogeneity between the 12 samples from west and north-west Atlantic coast sites (Table 3). This was not related to geographical distance between sites, as mussels from sites that were far apart from each other were as genetically similar as those that were collected closer together, irrespective of the exposure level of the shore. However, Saula, which had the highest frequency of the E allele on Atlantic coast sites, differed significantly from mussels at four of the sites in Galway Bay (Tables 2 & 3).

Mussels from the three south coast sites, Carnsore, Garrettstown and Lough Hyne, were genetically similar to each other, but were significantly differentiated from samples ($N = 12$) analysed from the west and north-west Atlantic coast sites (Table 3). The greatest differentiation was observed between Carnsore and the other 12 Atlantic sites ($P < 0.001$ to 0.05) due the high frequency of G (0.91) and the corresponding low frequency (3%) of the hybrid genotype in the Carnsore sample. Garrettstown and Lough Hyne samples were significantly differentiated in both genotypic and allelic ($P < 0.05$ to 0.001) proportions from 6–8 of the west and north-west Atlantic coast samples, most of these from sites situated north of Galway Bay (Table 3).

Because sample sizes were small ($N = 39 \pm 11.4$) we tested the reliability of our results by comparing genotypic and allelic frequencies for the Ballynahown sheltered site ($N = 36$) with four larger samples collected from the same site (Table 4). There were no significant differences in either genotypic or allelic proportions for any pairwise comparison, which demonstrates that taking small samples gives as good an estimate of genetic structure as larger ones. Also, it is clear that the genetic structure of mussels does not vary significantly either within or between the two years, at least at this particular site.

DISCUSSION

Results for the 16 Atlantic coast sites supported those reported by Coghlan & Gosling (2007) for Galway Bay *Mytilus*: (1) a high frequency of the *M. galloprovincialis* (G) allele and genotype (mean: 0.592 ± 0.134; 0.378 ± 0.198, respectively); (2) a high frequency of the hybrid genotype (0.429 ± 0.175), although our frequencies ranged from a low of 0.03 at Carnsore Point, to 0.66 at two sites on the west coast (Table 2); (3) correspondingly low frequencies of the *M. edulis* genotype (0.194 ± 0.107); and (4) no relationship between allele or genotype frequency and exposure level of the shore, i.e. no support for the partial ecological segregation of the two taxa reported by Gosling & Wilkins (1981) for Irish Atlantic coast sites.

In contrast to Atlantic coasts, Irish Sea samples were almost exclusively *M. edulis*. What factors are responsible for the virtual absence of *M. galloprovincialis* in this region are unknown. From satellite imagery a distinct thermal front develops during late spring at the boundary between the stratified Celtic Sea and the mixed waters of the southern Irish Sea (Brown *et al.*, 2003). This breaks down with the onset of winter cooling and increased wind-induced mixing. Stratification also occurs within the Irish Sea, especially to the west of the Isle of Man where the water is deeper and the tides weaker than to the east of the island. This front also breaks down in winter and re-forms in spring. Mussels in the Irish Sea may thus be a self-recruiting population, with the thermal fronts at the northern and southern entrances preventing the influx of spring-spawned *Mytilus* larvae. The importance of fronts as biogeographical boundaries for phytoplankton, zooplankton and planktonic stages of fish and invertebrate species in European waters has been well documented (Holligan, 1981; Quesada *et al.*, 1995; Munk *et al.*, 1999; Duran *et al.*, 2004). However, because there is inter-annual variation in the timing of formation and break-up of the fronts (Xing & Davies, 2001), we cannot rule out the possibility that larvae enter but due to a scarcity of suitable substrates may not recruit to sites along the south-eastern or north-eastern coasts, or, alternatively, larvae may only settle in the sublittoral region. But if the

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Table 3. Pairwise comparisons of genotypic proportions at the *Me15/16* locus in samples of *Mytilus* on Irish coasts. Asterisks indicate significant pairwise comparisons from Fisher's exact tests, after the Benjamini & Yekutieli (2001) procedure: $^{*}P < 0.0086$; $^{**}P < 0.0017$; $^{***}P < 0.0002$. Where significance levels differed between genotypic and allelic comparisons, the level is indicated in parentheses.

	Belf	Carl	Clogh	Brock	Carns	Gtown	LHyne	Bhead	Coolsiva	Blough	Nimmo	Bhown^{e}	Bhown^{s}	Carr	Dooega	Saula	Lburg	Incrone	Kincas
Carl	*(***)																		
Clogh	*(***)	np																	
Brock	*(***)	np	np																
Carns	***	***	***	***															
Gtown	***	***	***	***	ns														
LHyne	***	***	***	***	ns	ns													
Bhead	**(***)	***	***	***	**(***)	ns	ns												
Coolsiva	**(***)	***	***	***	**(***)	ns$^{(*)}$	ns	ns											
Blough	**(***)	***	***	***	***	***(**)	**(*)	ns	ns										
Nimmo	***	***	***	***	***	ns	ns	ns	ns	ns									
Bhown^{e}	***	***	***	***	**(***)	ns	ns	ns	ns	ns	ns								
Bhown^{s}	***	***	***	***	**(***)	ns	ns	ns	ns	ns	ns	ns							
Carr	***	***	***	***	*	ns	ns	ns	ns	*(ns)	ns	ns	ns						
Dooega	***	***	***	***	***	***(**)	**	ns	ns	ns	ns	ns	ns	*(ns)					
Saula	ns	***	***	***	***	***(**)	***	ns	ns	ns	**	*	*	***	ns				
Lburg	*(**)	***	***	***	***	**(***)	**	ns	ns	ns	ns	ns	ns	*	ns	ns			
Incrone	**(***)	***	***	***	***	***	**	ns	ns	ns	ns	ns	ns	*(ns)	ns	ns	ns		
Kincas	***	***	***	***	***	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
Creesl	ns	***	***	***	***	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

np, not possible; ns, not significant; Belf, Belfast; Carl, Carlingford; Clogh, Clogerhead; Brock, Blackrock; Carns, Carnsore Point; Gtown, Garrettstown; LHyne, Lough Hyne; Bhead, Black Head; Coolsiva, Coolsiva Quay; Blough, Ballyloughan; Nimmo, Nimmo's Pier; Bhown^{e}, Ballynahown E; Bhown^{s}, Ballynahown S; Carr, Carraroe; Dooega, Dooega; Saula, Saula; Lburg, Louisburg; Incrone, Inishcrone; Kincas, Kincaslough; Creesl, Creeslough.

Table 4. Genotype and allele frequencies in small and large samples of *Mytilus* from the wave-protected shore at Ballynahown, Galway Bay.

Collection date	N	Genotype frequencies			Allele frequencies	
		E/E	E/G	G/G	E	G
May 2005	36	9	9	18	0.38	0.62
January 2006	133	39	50	44	0.48	0.52
February 2006	151	34	70	47	0.46	0.54
March 2006	140	31	56	53	0.42	0.58
April 2006	150	31	79	40	0.47	0.53

latter were true then we would expect to see recruitment of *M. galloprovincialis* at intertidal sites along the south-eastern and north-eastern coasts. This may be what is happening in Belfast Lough, or alternatively, because Belfast is one of the busiest of Irish ports, *M. galloprovincialis* may have been brought into the Lough either as larvae in ballast water or as a fouling organism on boat hulls.

With the exception of Carnsore Point, Belfast Lough and some samples in Galway Bay, the majority of samples were in HWE, suggesting the strong possibility that populations on Atlantic coasts are highly introgressed. Recent analysis of the reproductive cycle has shown that hybrids are fertile (Doherty, unpublished data) and, therefore, both the two-banded and single-banded genotypic classes probably contain some F_2 backcross individuals. This in turn means that for Atlantic coast samples the *Me15/16* locus is less diagnostic than we thought; it is useful for estimating the frequency of E and G alleles, but only provides an approximate estimate of the proportions of *M. edulis*, *M. galloprovincialis* and F_1 individuals in our samples. For this reason comparing hybrid frequencies based on the partially diagnostic allozyme loci of Gosling & Wilkins (1981) with *Me15/16* hybrid genotype frequencies could be problematic. Instead, comparing frequencies of *Me15/16* E and G alleles with frequencies of compound alleles at loci with high discriminatory power, e.g. *Est-D*, could be very worthwhile.

We believe that allele frequencies indicate that *M. galloprovincialis* is increasing, not just at exposed Atlantic sites, but more strikingly, at wave protected locations. A supposed increase might be explained by the increase in sea surface temperature (0.6°C) in the North Atlantic over the last century (Jones *et al.*, 2001). In the north-eastern Atlantic cold-water species of phytoplankton and zooplankton have retreated northwards and warm-water species have followed them; the changes have been so dramatic in the last 20 years that marine scientists are referring to this as 'a regime shift' and one clearly forced by global warming. Other plausible explanations for an increase could be movement of mussel seed for aquaculture. Exposed shore mussels are the primary source for seeding ropes on many sheltered sites on Atlantic coasts. It is interesting that Gardner & Skibinski (1988), in an allozyme study of hybrid mussel populations in south-west England, found little evidence of a change in genetic structure in the period between 1980–1981 and 1986–1987, although a subsequent survey has not been carried out.

To test if *M. galloprovincialis* has increased in frequency over the past 25–30 y we plan to analyse *Mytilus* from the same 15 Irish locations sampled in the late 1970s by Skibinski *et al.* (1983), using *Est-D* and *Odh* in addition to the *Me15/16* DNA marker. These allozyme loci have good discriminatory value (Gosling, 1992b), but were not in use when Gosling & Wilkins (1981) carried out their analyses. The use of allozyme and DNA markers on the same individuals will allow us to evaluate the discriminatory power of the two different marker types.

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