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Genetic differentiation between populations of the dogwhelk, Nucella lapillus, from sheltered and exposed shores as shown by profiling of DNA microsatellite loci

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ABSTRACT

An initial analysis of genetic diversity using Nine primer pairs of DNA microsatellite loci (Nlw2, Nlw3, Nlw8,, Nlw11, Nlw14, Nlw17, Nlw21, Nlw25, Nlw27) was carried out along the North Wales coast, the tow Nucella lapillus populations is from a sheltered shore (Llanfairfechan) and that from Cable Bay is exposed. The 9 primer pairs were optimized none failed to produce amplification products under any of the conditions tested. All nine microsatellite loci tested were polymorphic for both populations and the number of alleles per population per locus ranged from 8 to 14, with a total number of 117 alleles in the global sample. Levels of genetic variability were similar across samples. All individual loci, show higher observed than expected heterozygosity. The observed heterozygosity (H_O) was 0.8614 and 0.8833 and expected heterozygosity (H_E) across all loci per population was 0.8409 and 0.8533, and in the both groups H_O was higher than H_E for all loci.

A global test for concordance with HWE revealed no deviations from HWE in any locus. F_{ST} values per locus ranged from 0.007 to 0.034, and the global F_{ST} was 0.081 ($P<0.001$) revealing significant structuring.

In the present study the distribution of genetic variability among Nucella populations estimated and characterized in tow selected spatial scales sites. The average observed heterozygosity in Nucella (Ho = 0.869) it was differ significantly using data generated by the same set of microsatellite loci, population was genetically affected. Global F_{ST} indicated genetic differentiation among populations (4%), indicating the occurrence of past and/or present gene flow among them.

Analysis of molecular variance revealed that most of the genetic variation is distributed within the populations (96%), indicating that presence of high genetic exchange and ecological adaptations to different environments are important for species survival

Variation at nine microsatellites loci was used to asses whether seasonal and geographical factors as well as Tributyltin (TBT)contamination have an impact on genetic diversity on Nucella lapillus populations. There were obvious genetic variations at these loci in this species (mean expected heterozygosity = 0.84 and 0.85; mean number of alleles = 10.22and 9.67), as significantly different allele frequencies were found, between samples from the two studied populations. Indicating that Nucella lapillus around North Wales comprise two genetically heterozygous populations.

INTRODUCTION

Nucella lapillus (dogwhelk) is a gastropod mollusc that is found on wave exposed to sheltered rocky shores. It is widely distributed on both sides of the North Atlantic where there is suitable habitat[1].

In sheltered shore habitats, snails with elongated shell Figure 1a. relatively small foot, smaller aperture, thickened shell wall impede the attack by crabs, while in wave exposed habitat, snails are of smaller size, rela-

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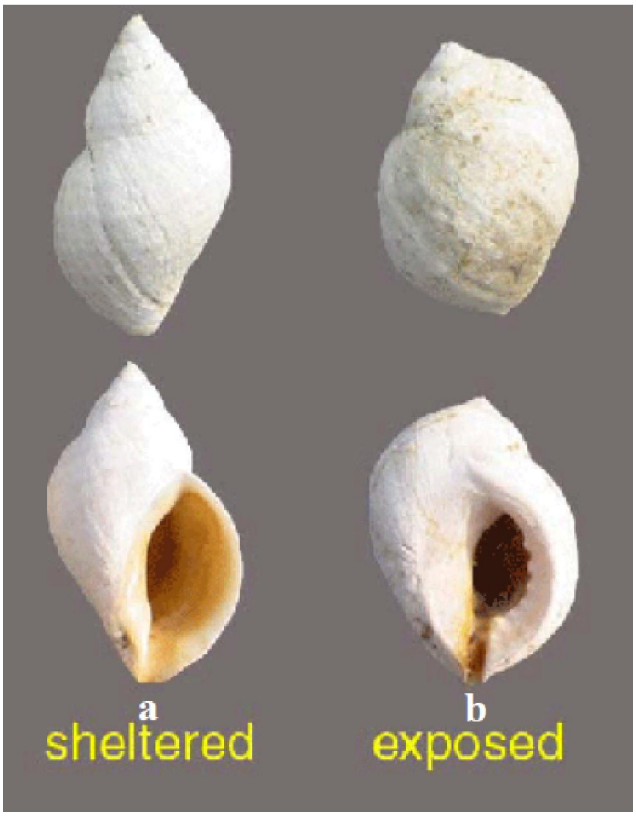
tively large foot,larger and more rounded aperture, stronger attachment and greater resistance to dislodgment by waves Figure 1b. Shells from dogwhelk living in sheltered habitats where predation pressure is likely to be strong, are stronger than shells from more wave-struck places. The animals liable to be attacked by crabs had strong shells and relatively small bodies. Animals on more exposed shores, where crabs were rare, had larger bodies (in particular large feet which helped them hang on) but weaker shells[2].



(Photos by R. N. Hughes, Molecular Ecology and Fisheries Genetics Laboratory School of Biological Sciences MEFGL website / Bangor University UK)

Figure 1 : Nucella lapillus

The differences are remarkably clear at those weights where shells may begin to move into a size refuge, the shells from sheltered habitats are more than twice as strong, weight for weight than shells from exposed habitats. The shells of dogwhelk protect the soft internal body of the animal from environmental hazards, such as mobile rocks, or biological hazards, such as predatory crabs and TBT contamination[3].

The dog whelk Nucella lapillus, is an oviparous gastropod which lays sessile egg masses with direct development[4]. N.lapillus has limited dispersal ability which owing to non planktonic larvae and an adult ambit size[5]. Natural selection can produce considerable differences in the physiology or morphology of organisms over quite short distances[6].

Usually it is difficult to determine the selective force acting. However, many intertidal and sublittoral animals are subject to predation by crabs and different environmental factors to greatly differing extents, and this has marked effects.

N. lapillus is able to adapt to suit local environments and is often so sedentary in its reproduction and dispersal that populations within metres of one another can receive little genetic interchange[7-8]. Gene flow between neighbouring populations is not great, they are, therefore, well placed by their breeding system to respond to local differences in habitat such as waves, temperature and contamination[6-9].

In order to complement and extend previous work, characterization of fine-scale population structure analysis based on several unlinked nuclear loci is desirable. The first set of 14 microsatellites DNA markers for Nucella lapillus was published by Kawai[2]. The main objective of the present study were to amplified the known 9 markers for Nucella lapillus, study the genetic differentiation and population structure for Nucella lapillus at selected spatial scales (30 Km range from East Bangor coast to North Bangor coast), within its native range and explore the relationships between genetic differentiation, contamination and hydrodynamic / topographic barriers at regional scales. Therefore, the aim of this work was to study alteration in dogwhelk at the genomic level by environmental change, man made TBT pollution and natural effect wave action and crab predation. This is a preliminary study based on a limited number of comparison (only two populations), but the correlation between additive variation and mean number of alleles suggests that both could be similarly impacted upon during the demographic history of these populations, compared levels of genetic diversity and genetic structuring between two sites representing sheltered and exposed shores. Perhaps the most interesting contribution of this study is to show potential use of quantitative variation for assessing genetics of anthrogenic activities.

MATERIALS AND METHODS

Dog whelks were collected from two localities

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across North-Wales, sampling regions representative of sheltered and exposed shores populations were chosen for the present study. For sheltered shores populations samples were collected from Llanfairfechan and for exposed shored populations samples were collected from Cable Bay. These two sites are separated by approximately 30 km cover water distance Figure 2 and represented close range scale of dispersal TABLE 1.

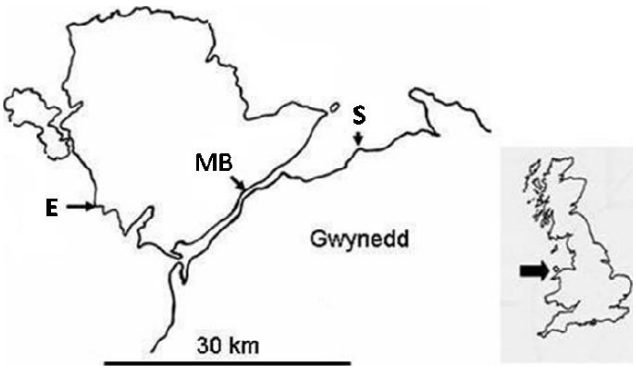

Figure 2 : Location of study sites along the North Wales coastline. S = Llanfairfechan (53° 15.456´ N, 03° 58.085´ W, exposure index = 1); MB = Menai Bridge (53° 13.272´ N, 04° 09.861´ W, exposure index = 0); E = Cable Bay (53° 18.357´ N, 04° 08.293´ W, exposure index = 13). The wave exposure index is based on mean annual wind energy and fetch together with environmental modifiers (10).

At each locality we collected individuals from sites the demographic history of Nucella lapillus is well documented[4], including sites where shores are sheltered or exposed. TABLE 1

TABLE 1 : Sampling sites details

Site	Sample size	Latitude	Longitude	Wave exposure index
Llanfairfechan	42	53° 15.456´ N	03° 58.085´ W	1
Cable Bay	42	53° 18.357´ N	04° 08.293´ W	13

DNA extraction, screening and microsatellite analysis

Total genomic DNA was extracted from foot muscle tissue in periopods of all Nucella lapillus using the DNeasy Tissue Kit (Qiagen)[11]. Following initial trials, nine microsatellite loci described and developed by Kawai[2], Each population sample was genotyped at nine microsatellite loci were tested after they were amplified for Polymerase chain reaction (PCR) conditions.

DNA extraction / CTAB

Molecular variability was estimated by genotyping nine microsatellite loci, Total genomic DNA was extracted from foot muscle tissue using the CTAB - DNA extraction method (Pascoal'Sónia, 11) and amplified for the following loci (Nlw2, Nlw3, Nlw8, Nlw11, Nlw14, Nlw17, Nlw21, Nlw25, Nlw27), described in Kawai et al[2]. A total of 96 samples of Nucella adult individuals were collected (48 from each population) for genetic analysis from two sites, Cable Bay (Exposed shore) and Llanfairfechan (Sheltered shore) during May2009 and stored in ethanol until DNA extraction.

Levels of genetic diversity within and between lapillus from the two above sites were typed for the nine microsatellite loci were assessed using variation at nine microsatellite loci: Nlw2, Nlw3, Nlw8, Nlw11, Nlw14, Nlw17, Nlw21, Nlw25 Nlw27, developed by Kawai [(2)]. Molecular variability was estimated by genotyping nine microsatellite loci.

Whole DNA (total nucleic acid was extracted from ethanol preserved tissue using a CTAB-chloroform / IAA method (2% Hexadecyltrimethyl ammonium Bromide)[12]. PCR reaction were performed under the following conditions: 120 s at 95CË% then 30 cycles of 30 s at 95CË%, 30 s at the specific annealing temperature (see Shaw and Adcock[13] and Shaw et al[14] and 1s at 72 CË%, using a Hybaid Omnigene thermal cycler. Reaction mixes contained 0.5µl template DNA, 0.75 µl MgCl2, 0.32 µl each nucleotide,0.2 lM of each primer (forward primer 5' end-labelled with a Cy5 fluorescent dye group), 0.05 µl Taq polymerase (Bioline UK) with the manufacturer's supplied 2.5 µl buffer (160 mM (NH4)2 SO4, 670 mM Tris-HCl), in a final reaction volume of 12 µl. Amplified products were resolved on 1% agarose gels run on an ALF express (Pharmacia Biotech) automated sequencer, product sizes being determined against internal standard size markers using Fragment Manager v.1.2 (Pharmacia Biotech).

Measuring DNA

DNA size measured by a Nanodrop spectrophotometer (ND-1000 Technologies Inc.19810 USA. The Nanodrop ND-1000 is a full-spectrum (220-750nm) with high accuracy and reproducibility. The ND-1000 has the capability to measure highly concentrated samples without dilution (50X higher concentration than the samples measured by a standard cuvette spectrophotometer.

PCR – RELP (Restriction fragment length polymorphism)

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Analysis on PCR –amplified DNA fragments, PCR product was digested with restriction enzymes. The resulting DNA fragment was analysed by agarose gel (1%) electrophoresis.. DTT (dithiothretiol) stabilise the enzyme[15].

Primers amplification

Various permutations of PCR conditions were tested on all 9 primer pairs to optimize locus-specific amplification conditions and test their utility as genetic markers. Each pair was subjected to a round of PCR optimization performing cycling with annealing temperatures ranging from 45°C to 62°C PCR amplifications were carried out in 12.5 µl reactions containing 50–100 ng of genomic DNA (0.5 µl), 0.6 µl of each primer (Nlw2, Nlw3, Nlw8,, Nlw11, Nlw14, Nlw17, Nlw21, Nlw25, Nlw27), 2.5µl PCR buffer, 0.75µl mM MgCl2, 0.3 µl mM of each dNTP (Promega),7.2 µl dH_2O_2 and 0.05 U Taq DNA polymerase (Promega) on an MJ Mini Thermal-Cycler (Biorad). Thermal cycling parameters were: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, primer-specific annealing temperature for 1 min (TABLE 2), extension at 72°C for 1 min and final extension at 72°C for 10 min. Microsatellite fragments were then resolved on 1 % agarose gels.

Primer labelling and multiplex amplify loci using qiagen multiplex kits

Multiplex PCR is a powerful technique that enables amplification of two or more products in parallel in a single reaction tube. It is widely used in genotyping applications and different areas of DNA testing in research laboratories. Multiplex can also be used for qualitative and semi-quantitative gene expression analysis using cDNA as a starting template (analysis of satellite DNA, short tandem repeat[STR].

Following PCR multiplex amplification, the extension products were resolved on 2% agarose gels.

Visualizing and quantifying DNA by electrophoresis

Whole genomic DNA (total nucleic acid) extracted was visualized by electrophoresis on 1% agarose gel, rough estimate was made of the quantity extracted by comparison with known quantitation standard or mass ladder (marker). The location of the DNA within the gel can be determined directly by including a low concentration of the fluorescent dye, eithdium bromide (Et Br) in the gel. A Transilluminator was used to shine ultraviolet (UV) light of wave length 300nm on the gel and the fluorescent emission (590nm) is photographed[15].

Genotyping performed on an ABI 3010 xl genetic analyzer (Applied Biosystems) and alleles were sized to an internal size standard (ROX GS 400HD; Applied Biosystems) Using GENESCAN 3.7(Applied Biosystems). Automated sequencers product sizes being determined against internal standard size markers using 3130 xl Genetic analyzer (AB applied Biosystems-Hitachi) Genetic Analyzer (applied Biosystems/ Hitachi). In order to determine levels of polymorphism of these markers, we screened 96 individual samples collected from Cable Bay and Llanfairfechan. Extension products were resolved on an ABI PRISM 3130 Genetic Analyser (Applied Biosystems) and alleles were sized relative to an internal size standard (ROX GS 400HD; Applied Biosystems) using the Gene Mapper® Software (Applied Biosystems)[11].

Statistical analysis

Genetic data analysis

Genotypes at all pairs of loci were tested for genotypic linkage disequilibrium, and within loci within samples for deviation from Hardy-Weinberg out-crossing expectations, using exact tests with significance determined by a Markov chain method (genepop v.3.2[16]. Genetic differentiation among samples was analysed using F_{ST}[17], which is a measure of genetic variation distributed between samples compared to that within samples, and varies from 0 (identical gene frequencies) to 1 (samples fixed for different allelic forms).. Levels of FST, estimated by 0[18] both globally and pairwise between samples, were calculated and tested for significant departure from zero (no differentiation) using permutation procedures within FSTAT v.2.9.3.2[19]. Where multiple tests were conducted significance levels were adjusted according to a Bonferroni correction[20].

The raw data was analysed with Microcheker[21] to check microsatellites for null alleles and scoring errors. Excel microsatellite Toolkit (30 Park SDE 2001) was used to calculate allelic frequencies, mean number of alleles per locus and observed (Ho) and expected heterozygosity (Hg) under Hardy-Weinberg assump-

tion. Tests for deviations from Hardy-Weinberg proportions, heterozygote deficiencies, genotypic linkage equilibrium and genic heterogeneity among populations were estimated using the exact test of GENEPOP version 3.4[22]. Estimates of FST, FIS, and their significance per population over all loci were calculated using FSTAT version 2.9.3.2[23]. Finally, hierarchical analysis of molecular variance (AMOVA) was performed using GenAlEx version 6.2[24] in order to test for possible regional structure.

RESULTS

The 9 primer pairs (Nlw2, Nlw3, Nlw8, Nlw11, Nlw14, Nlw17, Nlw21, Nlw25, Nlw27) were optimized, non was failed to produce amplification products under any of the conditions tested and amplified reliably (TABLE 2). To confirm that the primers were useful, various permutations of PCR conditions were tested on all 9 primer pairs to optimize locus-specific amplification conditions and test their utility as genetic markers.

The annealing temperature of locus Nlw14 was low ($45C^{O}$). Preliminary genotyping of individuals showed that locus polymorphism ranged from 8 to 16 alleles (N = 13) and observed heterozygosity (H_O) per locus ranged from 0.833to 0.95 with the expected heterozygosity (H_E) from 0.78 to 0.88. No evidence of linkage disequilibrium was observed, and a test for concordance with Hardy-Weinberg equilibrium (HWE) revealed no deviation from HWE except in locus Nlw11 and Nlw14. The primers described by Kawai et al[2] yielded generally uniform amplifications of putative loci in the Nucella population.

Microsatellite analysis

A total of 96 Nucella lapillus retrieved from the two sites were typed for the nine microsatellite loci. The Micro-checker analysis did not detect scoring errors due to stuttering, no evidence of large allele drop out. No evidence of null alleles and no corresponding significant positive values of F_{IS} (TABLE 2)

All nine microsatellite loci were polymorphic for the studied populations and the number of alleles per population per locus ranged from 8 to 16, with a total number of 117 alleles in the global sample. Levels of genetic variability were similar across samples. All individual loci, show higher observed than observed heterozygosity. The observed heterozygosity (HO) across all loci per population was ranged from 0.833 to 0.95, and the expected heterozygosity (HE)) was 0.78 to 0.88 in the Llanfairfechan and Cable Bay Group respectively (TABLE 2). A global test for concordance with HWE revealed no deviations from HWE in all locus (TABLE 2). Testing HWE for individual populations and loci revealed that this disequilibrium remained not significant within populations (all populations locus)(2008). Global F_{IS} value was -0.046, suggesting excess of heterozygotes in the sampling area. F_{ST} values per locus ranged from -0.0063 and 0.066, and the global FST was 0.026 ($P<0.001$) revealing significant structuring. Nine microsatellite loci tested were polymorphic (TABLE 2), with 9-21 alleles per locus. The observed heterozygosity per locus (H_O) varied from 0.35 to 0.45 (mean 0.4) and expected heterozygosity (H_E) from 0.81 to 0.92 9 mean 0.86). Ho was lower than HE for all loci.

A global test for concordance with HWE revealed no deviations from HWE in all locus except for Nlw11 and Nlw14, nine microsatellite loci tested were polymorphic with 8-14 alleles per locus (TABLE 2). HWE for individual populations and loci revealed that this disequilibrium remained significant within populations (all populations at locus Nlw11 and Nlw14). Evidence of linkage disequilibrium was observed in pair wise loci Nlw11 and Nlw14 in the global population test. Global F_{IS} value in all loci were range from -0.002 to 0.105 (mean -0.046). suggesting an excess of heterozygotes in the sampling area. F_{ST} values per locus ranged from 0.007 and 0.034, and the global F_{ST} was 0.018 ($P<0.001$) (TABLE 2), revealing significant structuring.

Additionally, hierarchical AMOVA (TABLE 3) revealed that most of the genetic variance was found within populations (96%, P,0.001).

however, a significant fraction (4%) Figure 3 was also found among populations within sites (partition among biogeographic sites,Llanfairfechan and Cable Bay coast Group)

DISCUSSION

The populations of most, if not all, species show some levels of genetic structuring, which may be due to a variety of non mutually exclusive agents. Environmental barriers, historical processes and life histories (e.g. mating system) may all, to some extent, shape the ge-

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TABLE 2 : Main genetic variability measure by locus of Nucella lapillus from North-Wales coast.

Locus	Primer Sequence5?-3?	Repeat	T (C)	Size range (bp)	Na	H_E	H_O	F_{IS}	F_{ST}	H-W P-val
Nlw2	F:GGGCAGGTATCCATAGTTAC R:CGGTCGGTGGTAGCATGTCC	(GT)8(4 bp)(GT)4TT	55	110	12	0.828	0.842	-0.016	0.012	0.0046
Nlw3	F:GGGCAGGTATCCATAGTTAC R:TTTCTCTGGCCACTTCCTCC	(GT)19(GC)2(GT)13G	55	180	11	0.851	0.951	-0.117	0.016	0.04
Nlw8	F:GATCTGAATTTGTCGCATGTA R:TTTCTCTGGCCACTTCCTCC	(CA)3CC (CA)9AA	57	150	12	0.848	0.902	-0.064	0.007	0.0115
Nlw11	F:ATATCATATGCGGTGGGAGG R:GATCCTGTGGAATTTTTGCGTT	(GA)4(CA)2(GA)4GT	52	160	14	0.851	0.833	0.021	0.011	0.008*
Nlw14	F:GATCCAGCATGCACGCGCA R:GATCAACTTTGAAAAGGTTAAGG	(CA)22(6 bp)(CA)5	45	180	11	0.833	0.807	0.031	0.018	0.552*
Nlw17	F:GATCTACAGTATACTCTATAT R:CCCGTGACCAGAATGAATCC	(GT)25	49	140	14	0.832	0.833	-0.002	0.017	0.0291
Nlw21	F:TTTCTGAGTCTGTCCCTGTC R:GAGAAACGCAAGACGTACAC	(CT)4(GTCT)3CTT	53	190	8	0.799	0.883	-0.105	0.029	0.7439
Nlw25	F:ATCAATGTTAGAGCTTAACATC R:GATCTTTTTTTAGCATAATT	(CA)13(4 bp)(CA)8	50	110	9	0.787	0.867	-0.102	0.034	0.0003
Nlw27	F:TAGTGCCTTAGTGCAAAGAGT R:GATAACCGACACATG	(GT)11(8 bp)(GTCT)2	55	150	16	0.883	0.933	-0.058	0.021	0.2267
All					117	0.831	0.869	-0.046	0.018	

T (°C) : annealing temperature; bp: base pairs; Na: number of alleles found per locus; HE: expected heterozygosity; HO: observed heterozygosity; FIS: standardised genetic variance within populations at each locus; FST: standardized genetic variance among populations at each locus; H-W: Hardy-Weinberg P values. Microsatellites developed in the present study. (* should not be with equilibrium)

TABLE 3 : Hierarchical AMOVA for N.lapillus means populations in the North-Wales coast.

Source of variation	d.f	SS	MS	Est. Var	(%)	P=value
Among populations	1	21.823	21.823	0.344	4	P< 0.001
Within populations	82	605.940	7.390	7.390	96	0.044
Total	83	627.762	29.212	7.733		P< 0.001

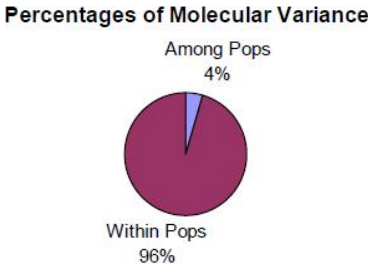

Figure 3 : Analysis of molecular variance

TABLE 4 : Main genetic variability measure for North-Wales coast populations.

roup of pulation	N	Nexp (±SD)	H_{obs} (±SD)	N-all (±SD)	F_{IS}									
	42	0.8409 ± 0.0135	0.8614 ± 0.0194	10.22 ± 2.44	Nlw2	Nl3	Nl8	Nl11	Nl14	Nl17	Nl21	Nl25	Nl27	Me
					-0.016	-.117	-0.064	0.021	0.031	-.002	-0.105	-0.102	0.545	-0.(
	42	0.8533 ± 0.0087	0.8833 ± 0.0182	9.67 ± 1.66										

N: sample size; Hexp: unbiased heterozygosity according to Hardy-Weinberg; Hobs: observed heterozygosity; N-all: mean number of alleles per locus and standardised genetic variance within populations (FIS) at each locus for each population; SD: Standard deviation.

netic structure of populations. In addition, as species' geographical distributions are typically more extended than an individual's dispersal capacity, populations are often genetically differentiated through isolation by distance[25]. Accurate knowledge of local population structure can provide important insights into species biology, including dispersal behaviour of individuals[8]. DNA microsatellite markers are routinely used to investigate the genetic structuring of natural populations. The knowledge of how genetic variation is partitioned among populations may have important implications not only in evolutionary biology and ecology, but also in conservation biology[25]. Many researchers have experienced difficulties in isolating micro-satellite loci from marine invertebrate species, mainly because microsatellite repeats in invertebrates are typically less abundant and shorter than in vertebrates[26].

The low annealing temperature (45C^O) of locus Nlw14 in this study was considerably low, such low or extreme annealing temperature (41C^O) was also recorded for N. lapillus at locus Nlw25 by Kawai et al[2] and they refer to the low melting temperature of the reverse primer.

The microsatellites in this study generally exhibited high levels of heterozygosity; observed heterozygosity was significantly higher than expected heterozygosity. No significant deviations from Hardy-Weinberg equilibrium between and within populations except for loci Nlw11 and Nlw14, (exact probability test, P,0.05) were recorded, do not record the presence of null alleles or excess of homozygotes at these loci. Results of this study showed a global F_{ST} of 0.018 (P<0.001 between the Llanfairfechan and Cable Bay Groups, suggesting significant structuring, among the sampled populations collected in 2009. Accordingly, significant genetic structure between these two sites was also revealed by Hierarchical AMOVA TABLE (3). global test for concordance with HWE revealed no deviations from HWE in most loci except Nlw11 and Nlw14 (TABLE 2). Testing HWE for individual populations and loci revealed that this disequilibrium remained non significant within populations. Global F_{IS} value was -0.046, suggesting excess of heterozygotes in the sampling area. F_{ST} values per locus ranged from 0.007 to 0.034 and the global F_{ST} was 0.018 (P<0.001) revealing significant structuring Wright[27].

Genotype-based statistics (e.g. F_{IS}) and parentage analysis are severely biased by null alleles Null alleles are generally referred to as alleles that fail to amplify during polymerase chain reaction (PCR) and may cause a locus to deviate from Hardy-Weinberg equilibrium and show a homozygous excess, particularly when the frequency is high (say 10% or more). When some loci are in HWE while other loci show clear disequilibrium, this is interpreted as evidence for random mating and paramixia In such cases, deviation from HWE proportions are assumed to possibly a scoring error or null allele[28].

The results of this study showed that there were no deviations from Hardy-Weinberg proportions for most loci except those that were recorded for loci Nlw11 and Nlw14. This may suggest that the samples represent non panmicitic populations. Results in TABLE (2) showed that H_O was lower than H_E for all loci, which is in accordance with Kawai et al[2] results on Nucella lapillus, where they refer that this perhaps reflects population subdivision or inbreeding.

Analyses based on multi-locus microsatellite genotyping indicate genetic homogeneity of Nucella lapillus populations. Observed F_{ST} values between Llanfairfechan and Cable Bay populations were low and none differed significantly from Zero. Low level of genetic structuring have been previously detected, using DNA and microsatellite data[29].

Current study showed a global F_{ST} of 0.081 (P<0.001 between the Llanfairfechan and Cable Bay Groups, suggesting significant structuring, among the sampled populations collected in 2009. Accordingly, significant regional genetic structure between these two sites was also revealed by AMOVA. For the interpretation of F_{ST}, it has been suggested that a value lying in the range 0.05 indicates little genetic differentiation[27,30].

The interpretation of the two theoretical extremes for F_{ST} (0 and 1) is however, straightforward. A value of zero means that we sampled within a panmictic unit. At the other extreme, a value of one means that there is no diversity within subpopulations and that at least two of the sampled subpopulations are fixed for different alleles. Values between these two extremes will then be interpreted as depicting various levels of structuring. However, it can be difficult and misleading to give a biological meaning for these values. For the interpretation of F_{ST}, it has been suggested that a value lying in the range 0-0.05 indicates little genetic differentiation; a value between 0.05 and 0.15, moderate differentiation; a value between 0.15 and 0.25, great differentiation; and values above 0.25, very great genetic differentiation (27,30). Indeed, the value of F_{ST} (0.018) TABLE (2) in this study is generally considered as reasonably low and investigators[25] interpreted that the structuring between sub-populations is weak. A seemingly low F_{ST} value (0.018) might in fact indicate very important genetic differentiation, this proof was already stressed by Wright[27], he wrote that differentiation is by no means negligible if F_{ST} is as small as 0.05 or less. F_{ST} was used rather than RST[31], because R_{ST} tends to suffer from higher variance with limited numbers of loci[25] and to follow both estimates tend to converge under conditions of high migration rate, departures from a strict Stepwise Mutation Model and low levels of sup-population differentiation (all likely conditions in the present study)

Nevertheless, the present study suggests that, some local constraints to gene flow among populations of N. lapillus may exist, at least during the time period in which sampling was undertaken. Such constraints might result from hydrodynamic or topographic barriers along the

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studied area, with a particular impact of wave and TBT contamination.

This study raises interesting questions regarding the effect of a temporally and spatially dynamic hydrogeographic regime along the Welsh coastline. However, future studies employing additional hierarchical sampling at larger geographic scales would be desirable to complement our findings and inferences on the potential role of hydrodynamic/topographic barriers. Based on our data, and experience with a range of N. lapillus microsatellite markers derived from geographically disparate populations, In anticipate that the current loci of Kawai et al[2] using the application of an appropriately robust panel of informative polymorphic markers for population genetic studies. Together, they will be useful in examining population differentiation at a range of spatial and temporal scales in this important globally invasive species.

There is a persistent wave gradient as well as TBT contamination gradient and possibly temperature along these sites[6]. However, given that N. lapillus is continuously distributed over a considerably larger range of temperatures and of other environmental conditions with no signature of a genetic break such as observed along the coast of continental Europe and in view of the level of structuring, we suggest that neutral population differentiation is a more likely explanation of the observed differences. N.lapillus has no long planktonic larval phase and is an oviparous gastropod laying sessile egg masses with direct development[4], N. lapillus, therefore has limited dispersal ability. However, the genetic diversity observed here indicates that dispersal ability of Nucella lapillus might be higher than generally assumed[4].

Sampling areas are separated by approximately 30 km (shortest over water distance) According to the oceanographic model the distance between the Llanfairfechan and Cable Bay groups is greater than the predicted megalopa average dispersion radius, potentially contributing to the genetic differentiation observed here[32].

Gene flow between neighbouring populations is not great, they are, therefore, well placed by their breeding system to respond to local differences in habitat[5,6].

Previous detailed comparative analysis of the genetic structure in marine organisms revealed that dogwhelk populations show less genetic structure than most directly developing species for which genetic data are available[33].

Adaptation through successive generations is apparent in N. lapillus and it is able to adapt to suit local environments and is often so sedentary in its reproduction and dispersal that populations within metres of one another can receive little genetic interchange[8]. It is this ability to genetically adapt to environmental conditions that has produced the globose form in the exposed areas, the elongated form in the lesser-exposed areas and the range of intermediate forms. The genetic diversity and genetic structure were observed in the two populations, this may be due to an artifact, however, the superposition of other local factors (could be TBT contamination and temperature effects), influencing the genetic structuring of dogwhelk populations. This results also stress the importance of local factors (environmental or ecological) in determining genetic structure of dogwhelk populations.

In north Wales the wave exposure index is based on mean annual wind energy and fetch together with environmental[10]. However, given that Nucella lapillus is continuously distributed over a considerably larger range of exposed and sheltered areas and of other environmental conditions with no signature of a genetic break such as observed along the coast of continental Europe[34] and in view of the strong level of structuring, we suggest that neutral population differentiation is a more likely explanation of the observed differences.

According to population genetics theory, marine taxa with direct development are estimated to posses higher population genetic structuring, with lower connectivity than those with planktonic larva[9], and therefore gene flow between neighbouring populations is not great. They are, therefore, well placed by their breeding system to respond to local differences in habitat like wind waves effect[6]. This consistent with those based on empirical observations on the strength of coastal currents. Therefore, because most Nucella populations along the Welsh coast are mainly separated by distances of 20 to 60 km, such a dispersal radius is predicted to result in no considerable exchange of individuals among local populations. However, sampling areas are separated by approximately 30 km (shortest over water distance) and, the short distance between the sampling group is contributing to the weak genetic differentiation observed here. The lack of evidence for strong genetic structure reported here can be interpreted as, either the popula-

tions are of recent origin and have not had sufficient time to differentiate genetically or levels of gene flow have been maintained high between breeding populations[29].

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