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Genetic population structure across a range of geographic scales in the commercially exploited marine gastropod *Buccinum undatum*

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ABSTRACT: Marine invertebrates are exploited increasingly as food resources worldwide, but knowledge of genetic population structure is limited for most commercially valuable taxa. We investigated genetic structure in the widely distributed, subtidal gastropod *Buccinum undatum* by screening samples from 28 locations at 5 microsatellite loci. Multidimensional scaling and cluster analysis were applied to study macrogeographic structure, and revealed 4 well-supported groupings of populations: Canada, Iceland, Swedish Skaggerak, and a cluster containing most samples from the European continental shelf (hereafter 'Shelf'). A fifth cluster comprising all 5 samples from the Solent (UK) relied on exceptional differentiation of just 2 alleles, which is consistent with significant evidence for recent bottlenecks in this area. Within the Shelf cluster most pairwise tests of differentiation were significant, although the global F_{ST} of 0.014 was very low for a direct-developing species. Our data suggest that use of highly polymorphic markers caused a relatively minor downward bias to F_{ST}, although historical connectivity of populations that are not in migration-drift equilibrium might be more important. However, significant isolation by distance among British North Sea coast samples (F_{ST} = 0.010) is consistent with approach to equilibrium and suggests recent gene flow, probably between semi-continuous populations. At a microgeographic scale, we found migration to be consistently higher from inshore to offshore within 3 separate areas; a factor that may underpin the lower diversity and greater differentiation observed for bay and inlet populations. Such populations might serve as important sources of genetic diversity, but are likely to be particularly vulnerable to exploitation.

KEY WORDS: Fishery genetics · Whelk · Microsatellites · Isolation by distance · Asymmetric migration · Bottleneck

INTRODUCTION

Marine invertebrates constitute a major global food resource of growing importance (Defra 2004: available at http://statistics.Defra.gov.uk/esg/publications/fishstat/default.asp). Consequent increases in invertebrate fisheries necessitate an improved understanding of the genetic population structure of exploited species, which is considered vital for effective management (Thorpe et al. 2000, Kenchington et al. 2003). Yet, whilst commercially important marine invertebrates often have broad geographic ranges, few species have been investigated in sufficient detail to provide general, rather than potentially area-specific, information about population structure for fishery managers. This presents a problem, as exploited marine invertebrates rep-

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resent a phylogenetically diverse group, rendering extrapolation of data among studies potentially difficult, because the species compared are unlikely to be closely related (Thorpe et al. 2000). In the absence of meaningful genetic data, general predictions of the scale and potential determinants of population structure might be made from consideration of the life histories of exploited species. Indeed, in an extensive meta-analysis, Bohonak (1999) found a strong correlation between levels of genetic differentiation and dispersal potential, assessed from species' life histories. This leads to the prediction that species with limited adult and juvenile dispersal capability are more likely to show a fragmented population structure, especially if this is coupled with low fecundity (Hellberg et al. 2002).

Marine invertebrates have been found to follow a broad trend, whereby species lacking planktonic larvae tend to show genetic substructure at a local-regional scale, whereas those with planktonic larvae tend to be more genetically homogeneous over large distances (see Colson & Hughes 2004 for a recent partial review). However, many exceptions to this rule have been found, for example among marine molluscs (e.g. Grant & da Silva-Tatley 1997, Todd et al. 1998, Shaw et al. 1999, Kyle & Parsons 2000), and in general, potential for larval dispersal appears to be a poor quantitative predictor of genetic differentiation in marine invertebrates (Lambert et al. 2003, Colson & Hughes 2004). Nevertheless, relatively few studies have considered deviations from migration-drift equilibrium, and thus to what extent the genetic distance metrics applied (most commonly F_{ST}) might reflect historical biogeography, which may bear little relationship to contemporary dispersal potential and gene flow. Moreover, much genetic population data on exploited marine invertebrates comes from allozyme studies (see Colson & Hughes 2004), but microsatellites are now preferred for their higher power to detect population differentiation (see Raymond & Rousset 1995a), which is considered vital because migration may be quite high among fishery stocks (Carvalho & Hauser 1994, Waples 1998). Unfortunately, many genetic distance measures, and F_{ST} in particular, are sensitive to marker heterozygosity and mutation rate (reviewed by Balloux & Lougin-Moulin 2002), yet the potential for genetic distance estimates to vary among studies because of differing levels of molecular marker polymorphism has been largely overlooked.

Clearly there is a requirement for detailed, large-scale genetic-population studies of exploited marine invertebrates that consider how results may be determined not only by a species' biology and ecology, but also by historical factors and the properties of molecular markers and distance metrics. Here, we investigate genetic population-structure, and identify potentially important determinants of differentiation, in the commercially-exploited whelk *Buccinum undatum* Linnaeus, 1758, concentrating on the European continental shelf, but with samples spanning the species' geographic range. Our study is the first genetic-population investigation of a buccinoid (Mollusca: Gastropoda: Neogastropoda) to encompass a range of spatial scales, and the first at any scale to employ microsatellites as molecular markers.

Buccinum undatum is a large, carnivorous, subtidal snail with an E Atlantic distribution from northern Norway to the Bay of Biscay and W Atlantic limits between Newfoundland and New Jersey (Taylor & Taylor 1977; Fretter & Graham 1984). Although occasionally found in deep water (>30 m) (Fretter & Graham 1984), *B. undatum* shows a preference for water around 20 to 30 m deep (Valentinsson et al. 1999, Ellis et al. 2000). *B. undatum* exhibits very little potential for dispersal of gametes and larvae since fertilisation is internal, egg capsules are adhered firmly to stones on the sea bed and development is direct (Hancock 1967, Valentinsson 2002a). Moreover, reproduction is slow, and fecundity limited, with 3 to 8 mo required for development of the 1% of eggs from annual reproductive events that hatch; all others are ingested by the developing larvae (Hancock 1967, Valentinsson 2002a). Adults may be long-lived (>10 yr), but direct observations (Himmelmann 1988, Himmelmann & Hamel 1993) suggest very limited movement. Furthermore, in the only major mark-recapture study, off Whitstable (UK), Hancock (1963) recaptured only 1 snail (of 3099) outside its original 8 km^2 release plot over a 3 yr period.

Based on life history and dispersal behaviour, a strong genetic structure would be expected for *Buccinum undatum*, raising concerns for the viability of its exploitation, which occurs throughout the geographical range. Indeed, local depletions of fishery stocks have been reported (Valentinsson et al. 1999, Morel & Bossy 2004), and if the prediction of strong genetic structure proves correct, recolonisation is likely to be very slow and important local adaptations may be lost. Intriguingly, however, such apparently limited dispersal seems at odds with its broad geographical range (Marko 2004), and suggests some current or historical continuity of populations across thousands of kilometres or occasional migrations of much greater distance than found by Hancock (1963). The present paper represents the first major investigation of *B. undatum* population genetics. Since ecological data are quite limited, our primary aim was to characterise genetic structure and diversity at both broad and local scales, and to determine the extent to which such data follow predictions from the species' potential for dispersal. On this basis, our secondary aim was to generate *a posteriori* hypotheses of potential determinants of genetic

structure and diversity, which are examined in this study and will be the subject of future investigations.

MATERIALS AND METHODS

Sample collection and screening. Adult *Buccinum undatum* were collected using baited pots or trawl from 28 locations (Fig. 1 & Table 1). With the exception of samples from Iceland, taken in spring 2003 (Table 1: Location 26), collections were made between June 2000 and July 2002. DNA was extracted from 2 to 3 mm^2 pieces of mantle or foot tissue using the HOTSHOT technique (Truett et al. 2000) for genotyping at 5 microsatellite loci isolated from *B. undatum* (Weetman et al. 2005). Approximately 50 to 100 ng DNA was used in each 15 µl PCR reaction, containing NH_4 buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.75 U *Taq* (all Bioline), 200 nM Cy5-labelled forward primer, 100 nM reverse primer and ddH_2O. Amplification was carried out on Hybaid Omni-

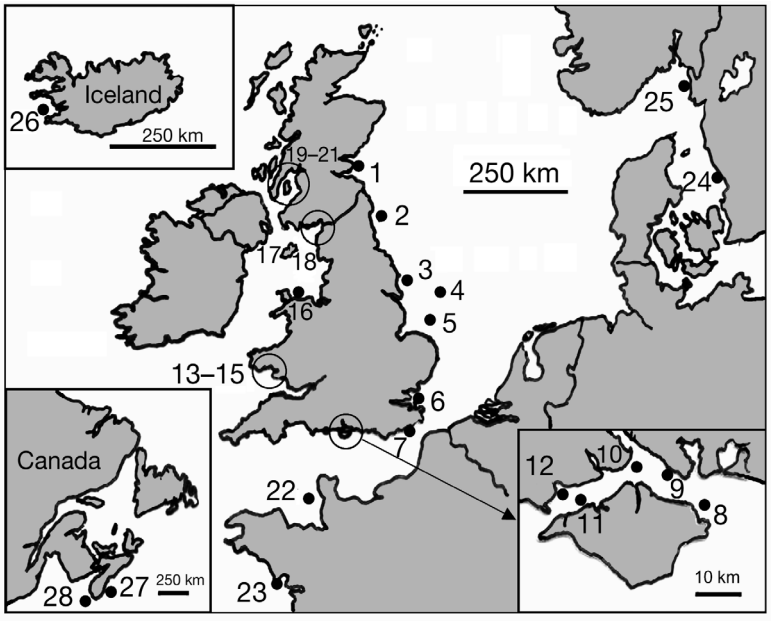

Fig. 1. *Buccinum undatum.* Sampling locations: numbers correspond to those in Table 1. Open rings: microgeographic groups of sample locations; from upper left downwards: Clyde Sea, Solway Firth, Carmarthen Bay and the Solent (enlarged in lower-right inset; other microgeographic areas enlarged in Fig. 5)

Table 1. *Buccinum undatum.* Sampling locations of (Great Britain, except where stated otherwise), collection date, sample size (N), expected heterozygosity (H_e) and allelic richness ($\hat{A}$)

Sample no. and location	Coordinates Lat.	Long.	Date (mo/yr)	N	*Bu*145 H_e	*Bu*145 $\hat{A}$	*Bu*119 H_e	*Bu*119 $\hat{A}$	*Bu*151 H_e	*Bu*151 $\hat{A}$	*Bu*122 H_e	*Bu*122 $\hat{A}$	*Bu*76 H_e	*Bu*76 $\hat{A}$	Mean H_e	Mean $\hat{A}$
1 St. Andrews Bay	56° 15′ N	02° 38′ W	01/01	48	0.77	6.5	0.68	4.0	0.92	16.2	0.92	16.1	0.59	4.0	0.77	9.3
2 Blyth	55° 21′ N	01° 29′ E	11/01	69	0.79	7.2	0.69	4.0	0.94	16.2	0.87	11.9	0.44	5.4	0.75	8.9
3 Bridlington (inshore)	54° 04′ N	00° 08′ E	08/01	93	0.78	7.6	0.64	3.9	0.95	17.7	0.90	15.3	0.23	3.1	0.70	9.5
4 (offshore)	53° 52′ N	01° 35′ E	10/00	60	0.78	6.1	0.68	4.4	0.94	16.6	0.90	15.3	0.41	4.0	0.74	9.3
5 Cromer Knoll	53° 18′ N	01° 18′ E	06/00	96	0.71	6.5	0.62	3.9	0.95	18.4	0.91	16.1	0.51	4.4	0.74	9.9
6 Whitstable Flats	51° 23′ N	01° 03′ E	08/00	93	0.68	6.6	0.64	3.9	0.95	17.9	0.89	14.8	0.28	4.1	0.68	9.5
7 Hastings Shingle Bank	50° 48′ N	00° 33′ E	08/01	89	0.77	7.3	0.67	4.6	0.94	17.5	0.92	16.2	0.44	3.9	0.75	9.9
8 Warner Shoal (Solent)	50° 43′ N	01° 05′ W	07/02	48	0.75	6.4	0.63	3.8	0.95	16.9	0.90	13.5	0.59	3.5	0.76	8.8
9 Browndown Bank (Solent)	50° 47′ N	01° 12′ W	07/02	48	0.79	6.0	0.65	3.5	0.94	16.1	0.91	15.2	0.66	3.9	0.79	8.9
10 Stanswood Bay (Solent)	50° 47′ N	01° 18′ W	06/01	52	0.72	4.9	0.65	4.1	0.94	17.2	0.87	13.2	0.62	3.0	0.76	8.5
11 Newtown (Solent)	50° 43′ N	01° 27′ W	07/02	46	0.73	4.5	0.63	3.5	0.94	15.8	0.91	13.7	0.60	3.5	0.76	8.2
12 Lymington (Solent)	50° 44′ N	01° 30′ W	07/02	47	0.74	5.4	0.60	4.0	0.92	16.4	0.92	14.8	0.67	4.5	0.77	9.0
13 Carmarthen Bay B (inner)	51° 39′ N	04° 29′ W	09/01	45	0.75	5.7	0.64	4.7	0.93	17.2	0.90	14.4	0.28	3.0	0.70	9.0
14 A (outer)	51° 35′ N	04° 32′ W	09/01	36	0.81	6.7	0.69	4.4	0.96	18.7	0.94	17.6	0.30	3.0	0.74	10.1
15 D (inner)	51° 41′ N	04° 34′ W	09/01	58	0.69	4.6	0.55	3.9	0.94	16.2	0.92	16.9	0.21	3.6	0.66	9.0
16 Bangor	53° 16′ N	04° 01′ W	11/00	44	0.72	5.7	0.60	3.5	0.94	16.6	0.89	14.0	0.41	3.8	0.71	8.7
17 Solway Firth B (outer)	54° 36′ N	03° 52′ W	09/01	48	0.72	4.9	0.66	3.9	0.94	16.9	0.91	14.6	0.34	3.7	0.71	8.8
18 A (inner)	54° 41′ N	03° 44′ W	09/01	45	0.53	4.5	0.59	3.8	0.94	17.1	0.87	11.1	0.55	5.1	0.69	8.3
19 Clyde Sea W (outer)	55° 42′ N	04° 59′ W	10/00	47	0.76	7.0	0.62	4.8	0.93	18.0	0.92	17.1	0.42	4.0	0.73	10.2
20 H (mid)	55° 47′ N	04° 56′ W	10/00	42	0.79	6.5	0.63	3.9	0.92	14.2	0.93	18.0	0.49	4.9	0.75	9.5
21 M (inner)	56° 04′ N	04° 53′ W	10/00	41	0.78	6.3	0.58	4.5	0.91	14.8	0.89	11.7	0.37	3.9	0.71	8.3
22 Jersey (UK Channel Islands)	49° 07′ N	02° 14′ W	03/02	67	0.79	6.3	0.67	4.2	0.95	17.7	0.91	16.5	0.37	5.1	0.74	10.0
23 Carnac (France)	47° 30′ N	03° 00′ W	06/01	91	0.75	6.1	0.61	4.2	0.95	18.0	0.91	14.7	0.30	4.2	0.70	9.4
24 Varberg (Kattegat, Sweden)	57° 15′ N	12° 10′ E	2000	39	0.77	5.9	0.72	4.9	0.92	14.1	0.89	15.5	0.26	3.6	0.71	8.8
25 Sannäs (Skaggerak, Sweden)	58° 47′ N	11° 11′ E	2000	45	0.73	6.1	0.71	5.0	0.88	13.0	0.90	12.6	0[a]	1.0	0.64	7.5
26 Reykjavik (Iceland)	64° 10′ N	22° 01′ W	04/03	36	0.73	5.6	0.61	4.9	0.84	13.2	0.81	9.7	0.57	4.8	0.71	7.6
27 Nova Scotia P (Canada)	43° 52′ N	64° 46′ W	12/01	31	0.07	2.8	0.20	2.0	0.89	15.5	0.91	13.5	0.49	2.0	0.51	7.2
28 C (Canada)	43° 42′ N	66° 06′ W	12/01	39	0.36	5.3	0.15	2.0	0.92	15.8	0.91	12.8	0.47	3.9	0.56	8.0

[a]Locus monomorphic

gene thermal cyclers, using the locus-specific cycling conditions given in Weetman et al. (2005). Loci were screened on ALFExpress sequencers with Cy5-labelled marker products of known size in each gel lane to permit alleles to be sized using Fragment Manager 1.2 (Amersham-Pharmacia).

Data analysis. In addition to our routine data-checking procedures such as repetition of PCRs and mixture of individuals from different populations on the same ALFExpress gels, raw data were examined for scoring errors, and the possible occurrence of null alleles was assessed at each locus using MICRO-CHECKER (van Oosterhout et al. 2004). Weir & Cockerham's (1984) estimators of *F*-statistics, allelic richness $\hat{A}$ (allele numbers standardised by rarefaction to the smallest sample size included), Nei's (1978) unbiased expected heterozygosity (H_e), and tests for significant deviations from Hardy-Weinberg proportions, H-W (as either an excess or deficit of heterozygotes) were computed using FSTAT 2.9.3.2 (Goudet 1995). The exact tests in GENEPOP 3.4 (Raymond & Rousset 1995b) were used to assess linkage disequilibrium among loci within populations and allelic (genic) differentiation among populations (each test used 100 batches of 5000 iterations). Analysis of molecular variance (AMOVA) was computed using ARLEQUIN 2.0 (Schneider et al. 2000). Isolation by distance was examined via Mantel tests implemented by the POPTOOLS add-in for Microsoft© EXCEL© (written by Greg Wood, CSIRO; available from www.cse.csiro.au/poptools), comparing matrices of pairwise genetic and geographic distance (using 5000 permutations). The latter was measured as the shortest distance (by sea) between any pair of sampling locations, using the measuring tool in Microsoft© ENCARTA© 2000. Mantel tests in POPTOOLS were also applied to examine the consistency of pairwise F_{ST} results among loci. To test whether heterozygosity values for each locus in each population exceeded those expected at mutation-drift equilibrium under the infinite alleles model, (IAM), strict stepwise mutation model (SMM) or 2-phase model (TPM, with the proportion of single-step mutations set to 0.7) we used BOTTLENECK (Cornuet & Luikart 1996). All 3 mutation models were used because our previous research on a gastropod had shown that mutation patterns can be variable among loci and are not predictable by the repeat sequence of specific loci (Weetman et al. 2006). As recommended by Luikart & Cornuet (1998) for studies with few microsatellite loci, the Wilcoxon signed-ranks procedure was used. Although the power of these tests was low owing to the small number of loci and the presence of null alleles, they represent a conservative assessment of the occurrence of population bottlenecks and thus provide strong evidence in cases where significant results are found.

Patterns of genetic structure were investigated using the Bayesian group-level method of Corander et al. (2003), implemented in BAPS 3.1, the goal of which is to identify the optimal number and membership of relatively genetically homogeneous clusters of populations. Our experience with other data sets suggests that power to detect partitions is reduced with relatively few loci. Thus, we employ BAPS here as a statistical tool to aid interpretation of macrogeographic patterns of structure suggested by multidimensional scaling (MDS) (using SPSS 11.5), and not to attempt to identify panmictic units. Moreover, to examine the consistency of clustering results across loci we adopted a jackknifing-type approach by repeating the BAPS analysis omitting a different locus each time. Migration rates within microgeographic groupings of populations were estimated using BAYESASS 1.2 (Wilson & Rannala 2003). Although the sensitivity of BAYESASS results to the number of highly polymorphic loci employed has not yet been assessed (note that Wilson & Rannala 2003 simulated di-allelic loci), marker information was undoubtedly limited for this method. However, we employed this method primarily as a preliminary analysis to test whether a consistent pattern of directional migration could be detected across microgeographical sampling areas, rather than to obtain precise estimates of migration rates. At least 3 runs of BAYESASS were completed for each set of populations before data were gathered. This was necessary to (1) examine the appropriateness of the default chain length (3×10^6 iterations) and the burn-in period (1×10^6 iterations), and (2) determine appropriate 'delta' values for each parameter, which control the maximum amount a parameter may change during an iteration. In all cases, default parameters proved acceptable and were retained. Delta values were set to allow 40 to 60% of proposed changes to be accepted, as recommended by Wilson & Rannala (2003) (BAYESASS user guide, available from www.rannala.org/labpages/software.html). Following these preliminary runs, data were gathered from 5 runs for each set of populations.

To determine the significance of single test results, sequential Bonferroni correction (Rice 1989) was applied to account for inflation of the Type I error rate. However, where several independent tests were made of a single hypothesis (for example results from different loci for a test of deviation from H-W or population differentiation), we applied the Bernoulli equation: $p = [N!/(N-K)!K!] \cdot \alpha^K (1-\alpha)^{N-K}$, where N is the number of tests and K the number below the designated Type I error rate, α (e.g. Moran 2003). Following the terminology of Kinnison et al. (2002), we refer to this as the 'binomial likelihood method' (LM). The LM technique is perhaps particularly useful for small numbers of marker loci since, unlike Fisher's combination of prob-

abilities, it is not influenced by single extremely low p-values and always requires repetition of individually significant results across tests to yield an overall $p < 0.05$.

RESULTS

Within-population structure and properties of loci

There was no evidence for linkage disequilibrium between loci or within populations, with only 8 of 280 tests showing $p < 0.05$ (LM $p = 0.25$ for any pair of loci), and no individual tests were significant following sequential Bonferroni correction. Significant departures from Hardy-Weinberg proportions were observed in all populations for *Bu*145 and in 23 populations for *Bu*76 (LM $p \ll 0.001$ for each locus over all populations). *Bu*122 showed no significant deviations from H-W, whereas both *Bu*151 and *Bu*119 showed 2 cases of homozygote excess, and *Bu*119 showed 2 of homozygote deficit (LM for each locus over all populations, $p = 0.25$). Application of LM suggested that only the Hastings sample showed a significant deviation from H-W across *Bu*119, *Bu*151 and *Bu*122, which resulted from 2 marginally significant tests ($0.02 < p < 0.05$). Therefore, within-population structure could not explain the overall highly significant excess of homozygotes at *Bu*145 and *Bu*76, which suggested the presence of null alleles at these loci. Using MICRO-CHECKER, we implemented Brookfield's (1996) method ('Brookfield 1' in MICRO-CHECKER) to estimate null-allele frequencies for *Bu*145 (mean null frequency ± SD = 0.183 ± 0.063) and *Bu*76 (0.086 ± 0.061) and generated a data set that included the null recoded as a new allele. Unless stated otherwise, all subsequent analyses are based on this corrected data set. Whilst in some cases the effect of ignoring null alleles by using the uncorrected data would have been quite substantial (for example, via inflation of average F_{ST}), differentiation attributable to the recoded null alleles themselves was very small, with their average F_{ST} levels much lower than the average across all alleles for each locus (data not shown). Table 1 shows genetic-diversity statistics for each population for the data set, with nulls recoded at *Bu*145 and *Bu*76.

Macrogeographic genetic structure and diversity

Based on all the loci, cluster analysis (using BAPS 3.1) suggested 5 groupings of populations, with a (near maximum) posterior probability for the best clustering solution of 1.00. In addition, membership of the designated clusters was strongly supported for all populations, with a minimum Bayes factor (which estimates how many times worse the solution becomes) of 9×10^6 for movement of any population to a cluster other than that to which it was optimally allocated. Statistical results from BAPS were consistent with visual exploration of the data using MDS of $F_{ST}/(1-F_{ST})$, shown in Fig. 2. Clusters comprised a highly divergent grouping of the 2 populations from Nova Scotia (Canada), relatively high divergence of a single-population 'cluster' from Reykjavik (Iceland), and 3 less distinct clusters from within the European continental shelf area: Sännas (Swedish Skaggerak); the Solent (UK); and all other sampling locations (Fig. 2). This clustering solution proved wholly robust to the removal of any of *Bu*145, *Bu*119, *Bu*151 or *Bu*122 (overall minimum Bayes factor for movement of any population from optimal cluster = 2×10^5). However, removal of Locus *Bu*76 resulted in collapse of the partition between the UK Solent and the European continental shelf, although the other 3 clusters remained identical, and support for membership of clusters was very strong (minimum Bayes factor for movement of any population from optimal cluster = 2×10^{17}). An MDS plot suggested a similarly strong effect, with Solent locations falling well within the range of Shelf locations when *Bu*76 was removed (not shown). Examination of allele frequencies at *Bu*76 showed that the high differentiation of the Solent populations at this locus alone was due to a major upward shift in the frequency of 1 of the 2 most frequent alleles (223 bp allele: range 0.40 to 0.56 across Solent populations compared to 0 to 0.21 across all other European populations) and an allied downward shift in the other most common allele (227 bp allele: range 0.28 to 0.39 in Solent vs. 0.60 to 1.00 in all other European populations).

Genetic diversity varied considerably for sampling locations allocated to the different clusters, with allelic richness much lower for Nova Scotia, Reykjavik and

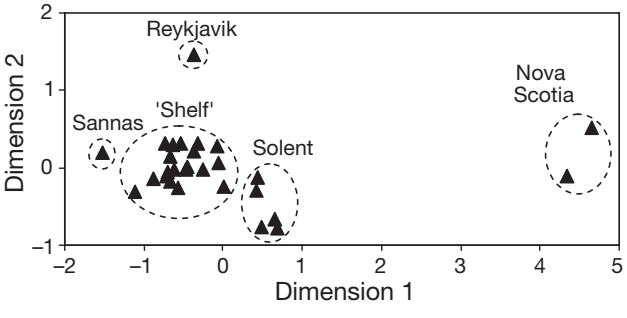

Fig. 2. *Buccinum undatum.* Multidimensional scaling (MDS) plot of all sampling locations based on $F_{ST}/(1-F_{ST})$; plot stress = 0.076. Dashed circles: groups identified by cluster analysis. Note that partitioning of the Solent from the Shelf cluster is not well supported across loci (see 'Results')

Table 2. *Buccinum undatum.* BOTTLENECK test results showing number of loci for which observed heterozygosity (H_{obs}) exceeded that expected (H_{eq}) under 3 microsatellite mutation models (IAM: infinite alleleles model; TPM: 2-phase model; SMM: stepwise mutation model). Wilcoxon test (1-tailed for $H_{obs} > H_{eq}$): *p < 0.05

Population	No. of loci with $H_{obs} > H_{eq}$		
	IAM	TPM	SMM
1 St. Andrews Bay	5*	4*	1
2 Blyth	4	2	2
3 Bridlington (inshore)	4	2	2
4 Bridlington (offshore)	4*	3	2
5 Cromer Knoll	4*	2	2
6 Whitstable Flats	3	2	1
7 Hastings Shingle Bank	3	2	2
8 Warner Shoal (Solent)	5*	5*	2
9 Browndown Bank (Solent)	5*	4*	3
10 Stanswood Bay (Solent)	5*	4	3
11 Newtown (Solent)	5*	5*	5*
12 Lymington (Solent)	5*	5*	0
13 Carmarthen Bay B	4*	3	1
14 A	4*	4	3
15 D	4	3	1
16 Bangor	4*	4	2
17 Solway Firth B	4*	4	3
18 Solway Firth A	3	3	1
19 Clyde Sea W	4*	0	0
20 H	4*	4	2
21 M	4*	3	1
22 Jersey (UK Channel Islands)	4	3	3
23 Carnac (France)	4	3	1
24 Varberg (Kattegat, Sweden)	3	3	3
25 Sannäs (Skaggerak, Sweden)[a]	4*	2	1
26 Reykjavik (Iceland)	3	1	0
27 Nova Scotia P (Canada)	3	2	2
28 C (Canada)	3	2	2

[a]Test based on only 4 loci, because *Bu76* monomorphic

Sannäs than for the Shelf grouping; H_e was also particularly low in the Nova Scotia populations (Table 1). To investigate a possible influence on variation in genetic diversity among populations we tested for evidence of recent population bottlenecks (Table 2). We used data uncorrected for null alleles for this analysis, since the effect of using data containing recoded null alleles is unclear, whereas null allele-related homozygote excesses (at 2 of the loci) would simply reduce power to detect bottlenecks. This is because the genetic signal used by the BOTTLENECK algorithm arises from a relative excess of heterozygosity compared to numbers of alleles. Although 16 of the populations (all from the European continental shelf) showed a significant bottleneck result under the (least conservative) IAM, these results must be regarded with caution because of the poor correspondence of this model to microsatellite evolution. Under the most conservative mutation model, the SMM, only Newtown (Solent) showed significant evidence of a bottleneck. However, significant results with the most realistic of the 3 mutation models, the TPM, were obtained for 4 of 5 populations from the Solent, and also for St. Andrews (Table 2).

Population differentiation and isolation by distance within the European Shelf cluster

Genetic differentiation within the groupings identified by cluster analysis was much lower than that among clusters (Fig. 2), but was still pronounced (within vs. among dashed boxes in Table 3). In the Shelf cluster, which contained 19 sampling locations with an average pairwise distance of 910 km (range 7 to 2131 km), 74% of pairwise tests of differentiation were significant. For comparison with other studies not using the LM technique, the number of significant pairwise tests using Fisher's method to combine probabilities was 90.1%. There was a weak tendency for neighbouring samples to show similar levels of differentiation but, as Fig. 3 suggests, isolation by distance was not significant (Mantel test: $F_{ST}/(1-F_{ST})$ vs. ln km, p = 0.076; or p = 0.16 if distance were natural log-transformed). At a smaller scale, isolation by distance was significant (Mantel test $r = 0.60$, $p < 0.05$) for the chain of British North Sea coast sampling-locations (1 to 6 in Fig. 1 & Table 1), and the relationship quite smooth (Fig. 4), excepting 1 rather divergent point (from St. Andrews vs. Bridlington inshore). The mean global F_{ST} within the Shelf grouping was 0.014 (± 0.004 SE), and Fig. 3 suggests that this was influenced by relatively high divergence of a few sample sites, which were either geo-

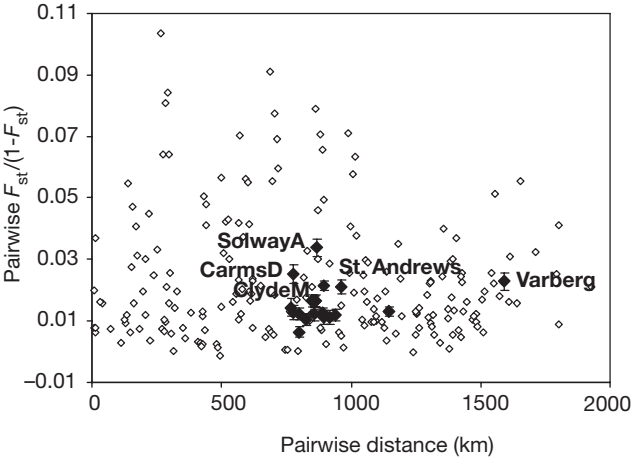

Fig. 3. *Buccinum undatum.* (◇) $F_{ST}/(1-F_{ST})$ plotted against distance for sampling locations within Shelf grouping identified by cluster analysis; indication of the relative level of differentiation of locations) (◆) average $F_{ST}/(1-F_{ST})$ values (± SE) of all pairwise comparisons (within cluster) involving each location, plotted against average pairwise distance; data points falling outside main visual grouping of average $F_{ST}/(1-F_{ST})$ values are labelled

Table 3. *Buccinum undatum*. Pairwise genetic differentiation between populations. Below diagonal: F_{ST}; above diagonal: number of loci (out of 5) for which tests of allelic differentiation yielded $p < 0.05$. Location numbers as in Fig. 1 and Table 1, but order of sample locations differs to correspond with groupings determined by cluster analysis (shown within dashed boxes). Note that partitioning of first 2 clusters from each other ('Shelf' and Solent) was wholly dependent on 1 locus (*Bu*76); removal of this locus reduces pairwise F_{ST} values by an average of 53% in UK Solent (Locations 8 to 12) and an average of 29% in all other locations. Maximum probabilities associated with number of loci individually <0.05 are: 0: p = 0.774; 1: p = 0.224; 2: p = 0.021; 3: p = 0.001: 4 or 5: $p \ll 0.001$ (binomial likelihood method, LM). Values corresponding to significant LM probabilities are in boldface

Location		StA	Bly	BrI	BrO	Cro	Whi	Has	CaB	CaA	CaD	Ban	SoB	SoA	ClW	ClH	ClM	Jer	Car	Var	War	Bro	Sta	New	Lym	San	Rey	NoP	NoC
1	StA		**2**	**4**	**2**	**2**	**3**	**3**	**2**	**3**	**4**	**4**	**3**	**3**	1	**3**	**3**	1	**3**	**3**	**4**	**3**	**3**	**4**	**4**	**4**	**5**	**4**	**5**
2	Bly	0.009		**2**	**3**	**2**	**3**	**3**	1	**2**	**4**	**2**	**3**	**3**	**3**	**2**	**3**	1	**3**	1	**3**	**3**	**2**	**2**	**3**	**4**	**5**	**4**	**4**
3	BrI	0.025	0.007		1	**2**	1	1	0	**2**	1	1	1	**3**	**3**	**2**	**4**	**2**	**3**	**4**	**3**	**2**	1	1	**2**	**4**	**5**	**5**	**5**
4	BrO	0.013	0.006	0.003		1	1	1	1	0	**3**	**3**	**2**	**4**	**3**	**2**	**4**	0	**3**	**2**	**2**	**2**	1	1	**3**	**4**	**5**	**5**	**5**
5	Cro	0.011	0.014	0.011	0.007		1	**2**	**2**	**3**	**3**	1	1	1	**4**	**4**	**4**	1	**3**	**4**	**3**	**3**	**3**	**3**	**3**	**5**	**5**	**5**	**5**
6	Whi	0.021	0.016	0.008	0.010	0.004		**3**	1	**2**	**3**	**3**	**2**	**2**	**3**	**4**	**5**	**2**	**2**	**4**	**3**	**3**	**2**	**3**	**4**	**5**	**5**	**5**	**5**
7	Has	0.017	0.007	0.002	0.003	0.004	0.012		**2**	1	**3**	1	0	**2**	**3**	**3**	**4**	0	**4**	**2**	**2**	1	**3**	1	**3**	**4**	**5**	**5**	**5**
13	CaB	0.025	0.007	0.000	0.008	0.019	0.024	0.006		0	0	1	**2**	**2**	**2**	**3**	**4**	1	1	**4**	**3**	**3**	**2**	**2**	**3**	**5**	**5**	**4**	**5**
14	CaA	0.022	0.005	0.005	0.004	0.016	0.017	0.006	0.008		**2**	**2**	1	**4**	**3**	**2**	**4**	0	**2**	**2**	**4**	1	**3**	**2**	**2**	**4**	**5**	**5**	**5**
15	CaD	0.049	0.025	0.012	0.025	0.028	0.032	0.018	0.006	0.019		0	**2**	**2**	**4**	**4**	**4**	**2**	**2**	**4**	**4**	**3**	**4**	**2**	**5**	**4**	**5**	**5**	**5**
16	Ban	0.023	0.012	0.006	0.013	0.005	0.014	0.005	0.007	0.010	0.007		1	**2**	**3**	**4**	**4**	0	1	**4**	**3**	**2**	**2**	**2**	**3**	**4**	**5**	**5**	**5**
17	SoB	0.024	0.006	0.003	0.006	0.010	0.017	0.001	0.003	0.003	0.013	0.006		**2**	**4**	**4**	**5**	1	1	**3**	**3**	**3**	**3**	**2**	**4**	**4**	**5**	**5**	**4**
18	SoA	0.029	0.034	0.038	0.036	0.010	0.019	0.029	0.048	0.040	0.046	0.016	0.036		**4**	**4**	**4**	**3**	**2**	**4**	**4**	**3**	**4**	**3**	**5**	**5**	**5**	**5**	**4**
19	ClW	0.006	0.010	0.013	0.011	0.011	0.014	0.013	0.018	0.023	0.040	0.021	0.019	0.029		1	**2**	**2**	**3**	**3**	**3**	**4**	**3**	**4**	**3**	**5**	**4**	**4**	**5**
20	ClH	0.015	0.010	0.014	0.008	0.017	0.029	0.008	0.011	0.012	0.020	0.016	0.012	0.043	0.008		**3**	**3**	**3**	**3**	**3**	**3**	**4**	**3**	**3**	**5**	**5**	**5**	**5**
21	ClM	0.025	0.019	0.023	0.024	0.022	0.024	0.022	0.019	0.019	0.023	0.019	0.024	0.032	0.016	0.016		**3**	1	**4**	**5**	**4**	**4**	**5**	**4**	**5**	**5**	**5**	**5**
22	Jer	0.010	0.001	0.000	0.001	0.005	0.007	0.000	0.001	0.000	0.014	0.001	0.002	0.028	0.010	0.008	0.012		1	1	**2**	1	1	**3**	**2**	**5**	**5**	**4**	**5**
23	Car	0.016	0.012	0.009	0.011	0.010	0.007	0.013	0.016	0.008	0.023	0.008	0.018	0.022	0.013	0.016	0.008	0.002		**3**	**3**	**2**	**3**	**3**	**4**	**5**	**5**	**5**	**5**
24	Var	0.029	0.012	0.016	0.010	0.021	0.019	0.017	0.025	0.009	0.040	0.031	0.015	0.053	0.018	0.022	0.030	0.011	0.021		**3**	**2**	**3**	**4**	**3**	**4**	**5**	**5**	**5**
8	War	0.066	0.073	0.084	0.066	0.054	0.094	0.052	0.076	0.076	0.097	0.065	0.065	0.064	0.071	0.063	0.090	0.071	0.092	0.097		**2**	1	1	0	**5**	**5**	**5**	**4**
9	Bro	0.039	0.044	0.053	0.036	0.031	0.060	0.027	0.050	0.040	0.071	0.041	0.043	0.047	0.053	0.043	0.061	0.038	0.056	0.062	0.010		0	0	**2**	**5**	**4**	**5**	**4**
10	Sta	0.055	0.066	0.072	0.053	0.041	0.075	0.045	0.071	0.068	0.094	0.056	0.063	0.051	0.068	0.069	0.088	0.059	0.080	0.086	0.007	0.001		0	**2**	**5**	**5**	**5**	**5**
11	New	0.060	0.062	0.065	0.052	0.041	0.078	0.039	0.058	0.059	0.075	0.048	0.051	0.055	0.068	0.060	0.078	0.055	0.078	0.083	0.005	0.001	0.000		**2**	**4**	**5**	**4**	**4**
12	Lym	0.037	0.047	0.056	0.040	0.029	0.060	0.030	0.052	0.051	0.076	0.045	0.043	0.040	0.042	0.038	0.061	0.044	0.059	0.066	0.004	0.008	0.013	0.013		**5**	**4**	**5**	**5**
25	San	0.059	0.033	0.034	0.041	0.053	0.048	0.037	0.034	0.032	0.051	0.047	0.025	0.087	0.046	0.042	0.066	0.034	0.051	0.037	0.126	0.109	0.131	0.118	0.094		**5**	**5**	**5**
26	Rey	0.057	0.061	0.076	0.060	0.069	0.080	0.063	0.082	0.084	0.107	0.091	0.073	0.095	0.064	0.063	0.084	0.073	0.083	0.069	0.110	0.090	0.113	0.117	0.073	0.106		**5**	**5**
27	NoP	0.256	0.284	0.311	0.292	0.255	0.295	0.272	0.328	0.318	0.353	0.302	0.313	0.257	0.275	0.306	0.302	0.292	0.304	0.307	0.235	0.241	0.231	0.244	0.236	0.361	0.297		**2**
28	NoC	0.245	0.265	0.289	0.272	0.239	0.281	0.247	0.303	0.290	0.326	0.276	0.286	0.242	0.260	0.283	0.288	0.272	0.286	0.290	0.201	0.210	0.205	0.211	0.209	0.333	0.281	0.020	

Table 4. *Buccinum undatum.* (a) Mantel test probabilities of no correlation between pairwise F_{ST} matrices for each locus pairing (probabilities of significant correlation are in bold). (b) Global F_{ST}, mean allelic richness and mean expected heterozygosity with standard errors for each locus. Data are from the Shelf grouping identified by cluster analysis

	*Bu*145	*Bu*119	*Bu*151	*Bu*122	*Bu*76
(a) **Pairwise**					
*Bu*145					
*Bu*119	**0.001**				
*Bu*151	**0.042**	0.099			
*Bu*122	**0.004**	**0.006**	**0.000**		
*Bu*76	**0.002**	**0.004**	0.065	**0.001**	
(b) **Global**					
F_{ST}	0.024	0.012	0.004	0.011	0.028
SE	0.007	0.004	0.002	0.003	0.007
$\hat{A}$	6.6	4.2	17.3	15.8	4.7
SE	0.20	0.09	0.30	0.44	0.16
H_e	0.74	0.64	0.94	0.90	0.38
SE	0.014	0.010	0.003	0.004	0.025

Table 5. *Buccinum undatum.* Recent, per generation, immigration rate estimates (mean of 5 runs) from BAYESASS (see Fig. 5), with standard deviation of estimates across runs given below each mean. Boldface values along diagonal = proportion of non-immigrant individuals

From	**Into**		
	W	H	M
Clyde W	**0.71**	0.06	0.03
	0.00	0.00	0.01
Clyde H	0.10	**0.74**	0.04
	0.01	0.01	0.01
Clyde M	0.20	0.20	**0.93**
	0.01	0.01	0.01
	B	A	
Solway B	**0.74**	0.20	
	0.00	0.02	
Solway A	0.26	**0.80**	
	0.00	0.02	
	A	B	D
Carmathen A	**0.72**	0.03	0.04
	0.00	0.01	0.00
Carmathen B	0.11	**0.73**	0.11
	0.02	0.04	0.05
Carmathen D	0.17	0.24	**0.84**
	0.03	0.05	0.04

graphically peripheral (Varberg, St. Andrews) or from the most inshore locations (Solway A, Carms arthen Bay D, Clyde Sea M). Agreement among loci was quite good in terms of relative levels of differentiation among populations (Table 4a), but there was considerable variation among loci in the absolute level of F_{ST}, with lowest and highest F_{ST} values corresponding to the most and least polymorphic loci, respectively (Table 4b).

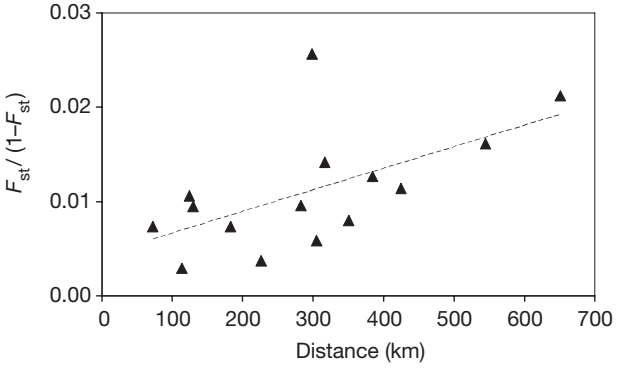

Fig. 4. *Buccinum undatum.* $F_{ST}/(1-F_{ST})$ plotted against natural logarithm of distance for sampling locations along British North Sea coast (Locations 1 to 6 in Fig. 1). Dashed line fitted by linear regression

Microgeographic structure and migration analysis

Along the British west coast, 3 localities contained multiple samples (Carmarthen Bay, Solway Firth, Clyde Sea). The distance between any pairing of sample sites within each of these areas ranged from approximately 7 to 40 km, whereas distances among the areas was between 200 and 620 km. Despite this hierarchy in pairwise distance, AMOVA showed that only 0.58% of genetic variation was partitioned among areas ($p = 0.084$), with 1.75% among sites within areas ($p < 0.001$), suggesting that local, rather than regional processes, might be more important in determining structure for these sampling locations. Furthermore, within each of these areas, more-inshore locations (where inshore refers to distance from the open sea) tended to show both lower diversity and greater differentiation (Table 1 & Fig. 3). To investigate a possible influence on the local distribution of genetic diversity, we estimated contemporary migration rates for the populations from the Solway Firth, Clyde Sea and Carmarthen Bay to test the *a posteriori* hypothesis that migration might be influenced by the situation of populations along gradients of more inshore to offshore locations. Full results are given in Table 5, although we caution against literal interpretation of the immigration rate estimates, since only the 3 loci free of null alleles (*Bu*119, *Bu*151, *Bu*122) could be used for this analysis (see 'Materials and methods'). Nevertheless, patterns of asymmetry in immigration rates were consistent across the 3 areas, with all 8 paired immigration rate comparisons showing higher inshore than offshore immigration (Fig. 5). With the exception of 1 comparison that might represent a Type II error, patterns of among population differentiation corresponded with bias in immigration rates (Fig. 5).

DISCUSSION

Macrogeographic patterns of genetic structure

Our study included sampling locations spanning a substantial portion of *Buccinum undatum*'s broad geographical distribution, and we used a combination of cluster analysis and multidimensional scaling to identify higher-level genetic structure. For populations from Nova Scotia and Iceland, which were identified as distinct genetic clusters, high differentiation was expected because of their large geographic separation from all other sampling locations. These distant populations also exhibited markedly lower genetic diversity and, although the low number of loci available for BOTTLENECK means a conclusion must be quite cautious, the Canadian and Icelandic samples showed no evidence of a bottleneck even under the least conservative mutation model (IAM). Indeed for the Canadian samples, expected heterozygosity (H_e) *and* allelic richness ($\hat{A}$) were exceptionally low, whereas bottlenecks would be expected to cause elevated H_e:$\hat{A}$. Thus, it is possible that the low genetic diversity in the Icelandic and (especially) the Canadian locations relative to our other samples might arise from less recent reductions in effective population size (N_e). For example, low mtDNA diversity has been found in W Atlantic (North American) populations of rocky-shore taxa such as the intertidal whelk *Nucella* sp., and is thought to reflect recolonisation from the European shelf following the Pleistocene glacial maximum approximately 11 000 yr ago (Wares & Cunningham 2001). Whether similar post-Pleistocene patterns of recolonisation might also apply to *B. undatum* is currently under investigation.

The other 3 genetic clusters comprised populations from the European continental shelf. Whilst clustering of the Swedish Skaggerak sample from Sannäs was supported across loci, support for distinctness of the UK Solent from the major Shelf cluster was underpinned by a large shift in the frequencies of just 2 alleles at Locus *Bu*76, which was evident in all 5 Solent samples. Although unlikely, we cannot rule out selection operating on *Bu*76 in the Solent. However, such marked, yet genomically-restricted, allelic variation is consistent with the action of a short-lived population bottleneck producing substantial, but fleeting, potential for heightened genetic drift. This explanation is supported by strong evidence for population bottlenecks in 4 of the 5 samples from the Solent. In addition to being highly differentiated, the Sannäs population exhibited the lowest $\hat{A}$ and H_e of any sample from the European continental shelf, consistent with low whelk density in this area (Valentinsson et al. 1999), which might be isolated by the very deep water of the Norwegian Trench and Skaggerak. Indeed, in Valentinsson's

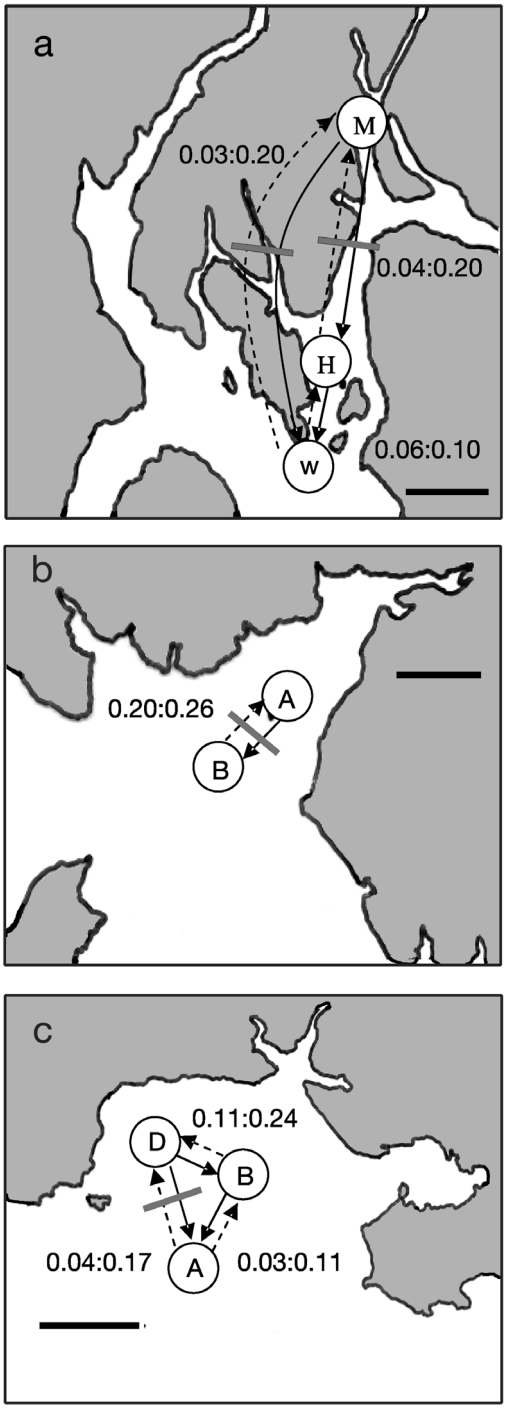

Fig. 5. *Buccinum undatum.* Recent migration rates in 3 British west coast areas: (a) Clyde Sea, (b) Solway Firth and (c) Carmarthen Bay; all scale bars = 10 km. Ratios for each pair of sites show immigration-rate estimates for directions indicated by dashed arrow:continuous arrow. Values are means of 5 runs of BAYESASS; patterns of asymmetry were consistent in every run for each area. Grey bars across arrows indicate populations between which tests for differentiation were significant – note that for Carmarthen Bay A vs. B, 2 (of 5) loci showed $0.05 < p < 0.06$, suggesting possible Type II error

(2002b) RAPD study of *Buccinum undatum*, (primarily from the Swedish Skaggerak), populations 1 to 2 km apart exhibited as much differentiation as those separated by 20 to 200 km — a pattern consistent with local isolation of small populations (see Hellberg et al. 2002).

Structure within the European Shelf: widespread differentiation, but low F_{ST}

There was widespread differentiation among the samples of the Shelf cluster, with the majority of pairwise tests significant. Yet the global F_{ST} was only 0.014, which is very low for a marine invertebrate with limited potential for larval dispersal (reviewed by Colson & Hughes 2004), particularly since most inter-sample distances far exceeded a plausible dispersal distance for individual *Buccinum undatum*. Whilst we found good agreement among loci in the relative levels of differentiation among populations, global F_{ST} values varied between 0.004 and 0.028 for the most and least polymorphic loci, respectively. As reported recently in other microsatellite-based studies (Knutsen et al. 2003, O'Reilly et al. 2004) this suggests that absolute levels of F_{ST} can be biased downward by high polymorphism, potentially creating a problem when comparing our results with previous studies of low-dispersing marine invertebrates, since most used allozymes (see Colson & Hughes 2004). Nevertheless, 2 of our loci (Bu 119 and Bu 76) showed low polymorphism for microsatellites (see Table 4), yet the associated single-locus F_{ST} values were still only 0.012 and 0.028, respectively. Thus, whilst marker polymorphism probably created some downward bias to estimates of differentiation in our study, it seems an inadequate explanation for the low F_{ST} we detected.

For F_{ST} to reflect contemporary, rather than historical gene flow, and thus to correlate with dispersal potential, populations should be close to migration-drift (m-d) equilibrium (e.g. Whitlock & McCauley 1999). Evidence for m-d equilibrium may be provided by isolation by distance (IBD) — an increase in F_{ST} with increasing distance — as the importance of homogenising gene flow to genetic drift gradually declines (Hutchison & Templeton 1999). Although not detectable among all 19 sampling locations comprising the Shelf cluster, IBD was significant along the British North Sea coast. Since this area must have been colonised following the Pleistocene, and population sizes must be large to support substantial fisheries, this suggests that substantial migration will have been required for populations to approach m-d equilibrium (Crow & Aoki 1984). Indeed global F_{ST} for the North Sea coast was low (0.010), and primarily attributable to the comparisons involving the northern sample sites Blyth and St. Andrews (see Table 3). With pairwise distances spanning 70 to 650 km, this suggests that distance-limited dispersal, rather than historical connectivity, can be an important factor in determining the contemporary genetic structure of *Buccinum undatum* at moderate geographic scales. Long-distance migration by *B. undatum* cannot be ruled out, but is unlikely to be common (Hancock 1963, Himmelmann 1988, Himmelmann & Hamel 1993); thus, substantial migration probably occurs via semi-continuity of population distributions.

Is asymmetric migration a determinant of microgeographic structure?

Genetic variation was partitioned among sample sites within the 3 British west-coast sampling areas (the Clyde Sea, Solway Firth and Carmarthen Bay) rather than among the areas. Whilst this could be partly attributable to lack of migration-drift equilibrium, another explanation is that some of the populations within these areas might be small and isolated as a result of contemporary migration processes. Within each area, the most inshore populations showed the lowest genetic diversity and greatest differentiation, consistent with relatively small N_e (see Frankham 1996). Immigration rates (m) proved to be consistently asymmetric, with all pairwise comparisons showing greater m into offshore, than inshore populations. Although estimators of contemporary migration, such as BAYESASS give results that represent both non-reproductive dispersal and genetically effective migration, our estimates of m may appear too high to be compatible with the estimates of pairwise F_{ST} we obtained. However, in asymmetric systems F_{ST} does not necessarily result from the average of N_e and m, but rather may reflect primarily the immigration:drift balance of subpopulations with lower N_e and m (Wakeley 2001, Wilkinson-Herbots & Ettridge 2004). Nevertheless, we cannot rule out the possibility that our estimates of m might be inflated as a result of using a small number of loci and the existence of unsampled 'ghost' populations (Slatkin 2005) that donate migrants to the sampled populations. Ghost populations seem a plausible explanation for results in the Solway, where m estimates were high and only moderately asymmetric, but F_{ST} was the highest for any of the pairwise comparisons in microgeographic areas. Although the role of ghost populations has not been explored for recent migrant analyses, it will be important for future studies on contemporary migration processes to conduct more extensive local-scale sampling. Nevertheless, the consistency of the pattern of offshore migration in each surveyed areas supports its biological reality, even if absolute values of m are inflated.

Why might migration in these areas be asymmetric? Firstly, at local scales whelk habitat will usually be 2-dimensional, but may become less so for populations situated within inlets, estuaries, bays, etc., giving fewer potential neighbouring recipient populations into which emigration can occur. Secondly, adaptation to local estuarine conditions could explain a lack of migration in general, but a strong outbound migration bias only if (1) adaptation to abiotic or biotic features of more estuarine conditions does not incur especially severe fitness costs for immigrants into more maritime locations, or (2) increased competition inshore raises emigration. These are clearly not mutually exclusive explanations and require further investigation with a larger number of loci and more sample sites, but asymmetric migration seems a plausible candidate explanation for the reduced diversity and elevated differentiation of inlet populations. Moreover, a reduction in the homogenising effect of immigrant gene flow, but more substantial emigration, could mean that inlet populations serve as sources of ecologically relevant genetic diversity for more seaward locations.

Are bottlenecks anthropogenically induced?

The Solent area showed the strongest evidence for recent population bottlenecks in our data set. Although the small number of loci and null alleles may reduce the power of the tests we applied, they are likely to make them more conservative, and so the detection of significant heterozygosity excess indicates strongly the occurrence of recent bottlenecks. Though speculative at present, we suggest that pollution from tributyl tin (TBT), which can cause imposex in *Buccinum undatum* (ten Hallers-Tjabbes et al. 1994, Strand & Jakobsen 2002), might be involved. From collections of *B. undatum* made during the mid-1990s, the Solent showed the most severe symptoms of imposex of 26 European locations (Nicholson & Evans 1997) that covered a similar sampling area to our own, although unfortunately did not include St. Andrews Bay, where we also found a significant bottleneck. The timescale of TBT-use would place a maximum probable impact on *B. undatum* of about 30 yr ago. Barring unrealistically small *Ne*, this is within the timescale that a genetic bottleneck signal can be detected by the BOTTLENECK software (ca. 0.2 to 4.0 N_e generations: Luikart & Cornuet 1998). However, evidence of overfishing of *B. undatum* in the Solent has been reported (Nicholson & Evans 1997), and Colson & Hughes (2004) did not detect evidence for genetic bottlenecks in British *Nucella lapillus* populations known to have been decimated by TBT pollution. Since the timescale of TBT impact must have been similar, a possible explanation for this difference in bottleneck signals between whelk species is the greater potential for *N. lapillus* to recover because it is not commercially exploited. Targeted sampling of areas differing in TBT pollution and whelk-fishing activity is required to test this hypothesis.

Conclusion and implications for management

In summary, our results show that *Buccinum undatum* exhibits widespread population structure, but high differentiation only across very large geographical scales. Low F_{ST} levels across most of the European continental shelf appear at odds with the limited potential for dispersal of *B. undatum*, and are unlikely to be attributable solely to either high marker polymorphism or historical connectivity of populations. We suggest that semi-continuity of populations may permit exchange of migrants, despite low individual vagility. Whilst genetic data can help to identify population distributions (see review by Rousset 2001), failure to consider the continuity of species' distributions may be an important reason why predictions of genetic differentiation from the life histories of marine invertebrates are often inaccurate. As a result of their relatively greater genetic isolation and possibly also reduced input of migrants, more careful management needs to be applied to populations in enclosed or physically isolated areas (e.g. the Clyde Sea). Fine-scale sampling is required in future studies of *B. undatum* or other marine gastropods to investigate whether biased migration is a common or inlet-specific feature and to test the role of distance-limited dispersal as a determinant of genetic population-structure at local scales. Wherever possible, we have specifically examined the consistency of results across loci because of the relatively small number of markers in our study — a direct result of the great difficulty in developing microsatellites for *B. undatum* (see Weetman et al. 2005). However, for some analyses our study had low precision and/or power, and although challenging, screening of additional molecular markers is important for future research. Nevertheless, our study represents one of the largest population genetic investigations of a marine invertebrate to date, and by highlighting multiple possible determinants of genetic differentiation (distance, history, continuity of distributions, marker polymorphism, bottlenecks and biased dispersal) should serve to generate hypotheses for future studies.

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