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Genetic structure of the tall sea pen
Funiculina quadrangularis in NW Scottish sea lochs

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Abstract

The tall sea pen *Funiculina quadrangularis* has a patchy distribution around the UK, being found in sheltered waters below 20 m depth on the northwest coast of Scotland and Ireland. The limited distribution and sensitivity to bottom fishing activities make *F. quadrangularis* vulnerable to reduction in population numbers that may lead to genetic isolation and reduced diversity. Because of this vulnerability and low resilience to physical disturbance, the tall sea pen is recognised as a Species of Principle Importance under the Natural Environment and Rural Communities Act, 2006, UK. (http://www.legislation.gov.uk/ukpga/2006/16/contents) and is also on the Biodiversity Action Plan list of Priority Species for the UK. In the Mediterranean it is recognised as a sensitive and essential fish habitat because it forms habitat for several commercially important crustaceans. The aim of this study was to understand the current state of the genetic structure and gene flow of *F. quadrangularis* in areas of NW Scotland. We developed 10 microsatellite markers and used them to genotype 176 samples from four populations. Overall, our results suggest that there is high genetic diversity and high gene flow between colonies of *F. quadrangularis* in and among locations in Loch Linnhe and Loch Duich. As a result of the high rates of gene flow, genetic differentiation between sites was low. This may provide resilience to human impacts if distant populations have a high connectivity. However, care must be taken, as small but significant isolation by distance was found between the most geographically distant sites and only a small part of the species range was examined in this study. The genetic tools developed here will provide a foundation for wider studies of this vulnerable species.

Introduction

Cold water corals can form important habitats that may be classified as vulnerable marine ecosystems (FAO 2009). Although progress has been made to protect these habitats, there remain significant anthropogenic pressures (Rogers & Gianni 2010). Recovery potential is limited by dispersal ability, so a greater understanding of population connectivity is essential for marine conservation (Jones & Srinivasan 2007). Population genetic analysis is a critical tool that can reveal connectivity patterns and can be used as a proxy for dispersal potential (Weersing & Toonen 2009).

The tall sea pen *Funiculina quadrangularis* (Pallas, 1766) is a colonial cnidarian of the subclass Octocorallia, distinguished by the presence of polyps with eight tentacles (Hughes 1998). These polyps arise from a calcareous axial rod that is square in section and anchored by a peduncle at its base (Greathead *et al.* 2007). Often described as feather-like in appearance, individuals can exceed 2 m in length with up to a quarter of the axis embedded in the sediment (Greathead *et al.*

2007). *Funiculina quadrangularis* is adapted to sheltered mud habitats between 20 and 2000 m depth, and often forms dense forests (Hughes 1998). With an almost global distribution, *F. quadrangularis* has been found in sea lochs and open waters of the northwest coast of Scotland (Greathead *et al.* 2007), as well as coastal waters of the North Atlantic and Mediterranean (Ager & Wilding 2009), New Zealand (Manuel 1988), Japan (Fujita & Ohta 1988) and the Gulf of Mexico (Felder & Camp 2009).

Demersal fishing activities present the greatest threat to the survival of *F. quadrangularis* colonies and so may have had a significant influence on its distribution. In the Mediterranean Sea, large declines in numbers have been observed where trawling has occurred (Rogers & Gianni 2010). In the Mediterranean and possibly elsewhere in the NE Atlantic, *F. quadrangularis* is typical of essential habitat for the Norway lobster, *Nephrops norvegicus*, and the shrimp *Parapenaeus longirostris*, the target of major trawl and creel fisheries (European Commission 2006; Greathead *et al.* 2007). Bottom trawling may cause significant physical disturbance to *F. quadrangularis* (MacDonald *et al.* 1996), as it has a rigid axial rod and is unable to withdraw into the sediment, unlike other UK sea pen species (Hughes 1998). Therefore, *F. quadrangularis* may act as an indicator of the state of health of deep-sea mud habitats and is considered a Vulnerable Marine Ecosystem (VME) indicator species (Rogers & Gianni 2010).

Distribution estimates in Scottish waters demonstrated that *F. quadrangularis* has the lowest abundance and most restricted distribution of the three species of sea pens found around Scotland (Greathead *et al.* 2007). The species is considered to be nationally rare and of high conservation significance (Hughes 1998). Although not protected by law, *F. quadrangularis* is a subject of the 'Mud in Deep Water Habitat Action Plan' of the UK Biodiversity Action Plan (UK Biodiversity Group 1999) because of its fragmented distribution, sensitivity to physical disturbance, and association with high biodiversity (Hughes 1998). It is also listed as a species 'of principle importance for the purpose of conserving biodiversity' under section 41 (England) and section 42 (Wales) of the Natural Environment and Rural Communities Act 2006. Indeed, *F. quadrangularis* has been associated with the brittle star *Asteronyx lovenii* (Fujita & Ohta 1988) and *F. quadrangularis* colonies have been observed as nurseries for fish larvae (Baillon *et al.* 2012). The habitat occupied by sea pen and burrowing megafauna communities has also been listed by OSPAR as threatened and/or declining (OSPAR 2004) and has been considered an essential and sensitive fish habitat in the Mediterranean Sea (European Commission 2006).

However, very little is known about the life history of *F. quadrangularis*. The species is gonochoric with annual broadcast spawning of gametes and its large egg size indicates possible lecithotrophy (Edwards & Moore 2009). However, nothing is known about larval dispersal and gene flow between colonies; such studies are vital in order to understand the ability of this species to endure natural or anthropogenic changes to the environment, particularly disturbance resulting from fishing activities. One way to infer population connectivity is to assess the genetic distinctiveness of populations. Microsatellite markers can be useful tools for detecting gene flow between populations and have been used successfully for deep-sea coral species, particularly the reef-forming hexacorallia *Lophelia pertusa* (Le Goff-Vitry *et al.* 2004; Morrison *et al.* 2011; Dahl *et al.* 2012), and some octocorallia (Costantini *et al.* 2007; Mokhtar-Jamaï *et al.* 2013). High levels of gene flow between colonies would imply that loss of some colonies would not result in overall loss of genetic diversity of the species and that areas with colonies lost to trawling activities potentially could be recolonised in the future.

Objectives

The purpose of this study was to determine the genetic diversity and connectivity of *F. quadrangularis* colonies in sea lochs of NW Scotland. Highly polymorphic microsatellite markers were developed and used to investigate the genetic diversity, gene flow and genetic structure among colonies at four locations in two sea lochs. To our knowledge this is the first study on the connectivity of sea pen populations using microsatellite markers.

Methods

Sample collection

Tissue samples from 176 specimens of *F. quadrangularis* were collected in August 2010 from four locations in the sea lochs of Northwest Scotland. Sampled colonies were as geographically distant as possible within each loch and represent four presumed populations: two in Loch Duich and two in Loch Linnhe (Fig. 1). All specimens sampled were between 18 and 35 m depth and collection was undertaken using SCUBA. A 2–4 cm tip of each sea pen was cut off with scissors and placed in a polythene collecting bag for retrieval. The living sea pen was left otherwise undisturbed. Tissue samples were placed into 97% ethanol immediately upon retrieval to the surface.

Microsatellite analysis

Sections of 1 cm from the outer polyps were removed from each tissue sample and DNA was extracted using the
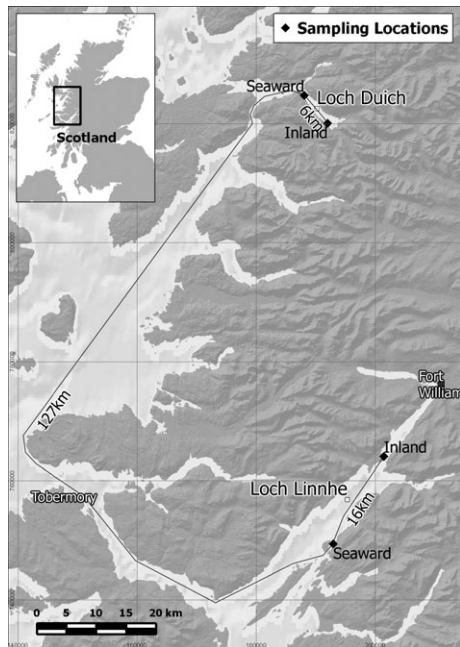
Fig. 1. *Funiculina quadrangularis* collection sites in Loch Linnhe and Loch Duich in NW Scotland.

DNeasy Tissue kit (QIAGEN Ltd. West Sussex, UK), following the manufacturer's instructions but modified for a digestion time of 2 days. DNA samples from each of the four collection sites were submitted to Ecogenics GmbH (http://www.ecogenics.ch; Zürich, Switzerland) for enriched genomic library construction and microsatellite primer development. Thirty-five potential primer pairs were produced by Ecogenics and these were tested for amplification on a subset of DNA samples by optimising PCR conditions and visualising products on an agarose gel.

Successful reactions for microsatellite loci that yielded fragments of appropriate size were repeated using fluorescently labelled oligonucleotides. Reactions were performed in 6-μl volumes containing 1 μl of 10–30 ng·μl^{-1} genomic DNA, 3.5 μl PCR Multiplex Mix (QIAGEN Ltd.) and 1.5 μl primer mix containing forward and reverse primers with concentrations of 0.2 pM. Thermal cycling conditions were as follows: an initial enzyme activation at 95 °C for 15 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C (first 15 cycles) and 54 °C (final 15 cycles) for 90 s, extension at 72 °C for 60 s, and a final extension at 60 °C for 30 min. To separate fragments, fluorescently labelled PCR products were run on an ABI3100 sequencer (Applied Biosystems, Warrington, UK) in 11-μl volumes containing 1 μl of 1:10 diluted PCR product, 9.8 μl formamide and 0.2 μl Gene-Scan 500 LIZ Size Standard (Applied Biosystems). Results were analysed for allelic variation using the software GENEMAPPER 4.1 (Applied Biosystems). Peaks were

scored for fragment size and verified by manual inspection, resulting in a matrix of allele pairs for each sample and locus.

Statistical analysis

Descriptive statistics were calculated individually for all loci as well as combined for each of the four locations and averaged over all locations. Genetic diversity was assessed by looking at observed (H_o) and expected (H_e) heterozygosity and the number of private alleles using the program GENALEX 6.5b3 (Peakall & Smouse 2006). To correct for the effect of sample size, allelic richness and private allelic richness were calculated using the software HP-RARE (Kalinowski 2005), which adjusts all population sample sizes to equal that of the smallest population and allows a better estimate of the number of alleles per population. To determine whether there was significant inbreeding or outbreeding at any of the collection sites, FSTAT 2.9.3.2 (Goudet 2001) was used to calculate Weir & Cockerham's (1984) F_{IS}, the inbreeding coefficient. Deviations from Hardy–Weinberg equilibrium (HWE), calculated as heterozygote deficiency, were determined using GENEPOP 4.0.10 (Raymond & Rousset 1995) using a Markov chain (MC) algorithm (Guo & Thompson 1992). The presence of null alleles at each locus was assessed using MICROCHECKER 2.3.3 (Van Oosterhout *et al.* 2004), and linkage disequilibrium tests between loci over all locations were conducted using FSTAT 2.9.3.2 (Goudet 2001). POWSIM (Ryman & Palm 2006) was used to determine the statistical power of the microsatellite loci to detect significance at various levels of differentiation, quantified as F_{ST}. The program estimates α (type I) error, the probability of rejecting the null hypothesis (H_o = genetic homogeneity) when it is true, and β (type II) error, the probability of rejecting the null hypothesis when it is false, and reports the proportion of significant values as assessed by Fisher's exact tests and chi-squared tests. To determine the statistical power, it was necessary to calculate the effective population size, which was estimated using the expected and observed linkage disequilibrium between individuals with the program NE-ESTIMATOR (Ovenden *et al.* 2007).

Overall genetic distance and pairwise genetic distances were assessed by calculating Weir & Cockerham's (1984) estimator F_{ST} using the computer software FSTAT 2.9.3.2 (Goudet 2001). GENEPOP 4.0.10 (Raymond & Rousset 1995) was used to test for allelic and genotypic differentiation between sites (*G* test), and IBDWS (Jensen *et al.* 2005) was used to test whether genetic differentiation could be a result of isolation by geographic distance. Geographic distance was measured as the minimum nautical distance between sites. A principal coordinate

analysis (PCoA) used a matrix of pairwise F_{ST} values as input. Two analyses of molecular variance (AMOVA) were performed based on F_{ST} values using 1000 randomisation replicates to test for significant variation between all sites and both lochs. Both PCoA and AMOVA were performed with the program GENALEX 6.5b3 (Peakall & Smouse 2006).

Results

Microsatellite marker development and variation

Twenty-eight primer pairs were tested for microsatellite amplification; of these, 15 produced products of correct size and were tested using fluorescently labelled primers in multiplex PCR reactions. Ten loci were selected for genotyping; the remaining five were removed from the analysis as a result of significant stuttering, allelic dropout, or non-specific binding that prevented accurate scoring of allele sizes.

All of the selected loci displayed polymorphisms, ranging from four to 40 alleles per locus (Table 1). In total, our 10 loci revealed 182 alleles. No duplicate multilocus genotypes were found; each sample presented a unique genotype. Linkage disequilibrium was not present for any pair of loci after Bonferroni corrections for multiple comparisons (Rice 1989). However, analysis of microsatellite errors revealed evidence of null alleles at two loci (FqM13 and FqM37) and therefore these loci were removed from subsequent analyses.

Overall effective population size (N_e) was estimated at 5301.4 (95%CI: 1536.8 – infinity) when samples were combined from all locations. The statistical power of the microsatellites to differentiate at low F_{ST} values revealed that the probability of α (type I) error ($F_{ST} = 0$) was 3.4–5.2% and β (type II) error was 99% when the average F_{ST} value was 0.0019.

Genetic diversity and Hardy–Weinberg equilibrium (HWE)

Expected heterozygosity ranged from 0.791 to 0.814 (Table 2). The mean number of alleles per locus ranged from 12.9 to 15.6, and the mean number of private alleles per locus ranged from 0.5 to 1.1. Calculations of allelic richness and private allelic richness, to control for variation in sample size, ranged from 4.89 to 5.00, and 1.18 to 1.32, respectively.

Significant deviations from HWE were observed for five of the eight loci for at least one site; however, after Bonferroni corrections, deviations from HWE were observed at only two loci for at least one site (Table 2). Overall, significant heterozygote deficiencies were identified at the Loch Duich seaward site and both Loch Linnhe sites; however, only the Loch Linnhe sites showed significant heterozygote deficiency after Bonferroni corrections (P = 0.05/32 = 0.00156). Significant positive F_{IS} values were found for the Loch Linnhe inland site and for the average of all sites, which indicated small yet significant levels of inbreeding that could explain deviations from HWE (Table 2). Values for inbreeding (F_{IS}) ranged from −0.177 to 0.144, and averaged over all loci values for each site ranged from −0.004 to 0.047 (Table 2).

Genetic distance and structure

Overall F_{ST} value was 0.005 (95%CI: 0.001–0.009). Pairwise F_{ST} values ranged from 0.0001 to 0.0072, with values significantly different from zero observed between the Loch Duich sites and the Loch Linnhe inland site (Table 3). However, only genetic distances between the Loch Duich inland site and the Loch Linnhe inland site showed values significantly different from zero after Bonferroni corrections (P = 0.05/6 = 0.00833; Table 3). Genotypic and genic differentiation were calculated to determine how genotype and allele frequencies differ

Table 1. Microsatellite markers developed for *Funiculina quadrangularis*.

Locus	Primer sequences (5′-3′)		Motif	Number of alleles	Allele size range (bp)
	Forward	Reverse			
FqM09	TCTTTGACACACACGCAC	ACCGAGTTACACTGATGAGC	(CA)	21	114–158
FqM10	AACGACAGGCTTTACCTGGG	ACGTCTATTATCGTGGCCAACTC	(GT)	24	124–190
FqM11	TACCCTCTTTTGTCCCAGGC	GAACACAGGGACGTCATGC	(GT)	27	208–262
FqM13	GTACGGACCTCCCCACATAC	TATACATCGGCACACACACG	(GT)	16	82–118
FqM14	TGCTTTTCTTCGCTATGTTGC	TCTCCGTAAGTCAATAGTACACAC	(AC)	23	180–229
FqM16	TAGACGCAAACTGCACACAC	CTCGACAGCTCAACACTCAAC	(CA)	40	97–217
FqM37	GGATTGCATGAAGACGCGG	TGTCTGTGTGTGTACATGCG	(CA)	9	216–238
FqM38	AAACTTTTCCGACTCAACCG	GGTTTCGGAAGATTGTGGGC	(GT)	13	134–174
FqM39	AGTAGGGTGGTCTCGCTAAC	TGAGTTGGCATCATTCTCGC	(CA)	5	164–172
FqM54	CGTTGACGACGAAAGCTC	TCTCCGTAAGTCAATAGTACACAC	(CAAA)	4	150–162

Table 2. Descriptive statistics of microsatellite loci from *Funiculina quadrangularis* in two NW Scottish sea lochs, including number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosity and inbreeding coefficient (F_{IS}) for each locus and sampling location.

Locus	Loch Duich inland (n = 48)				Loch Duich seaward (n = 48)				Loch Linnhe inland (n = 53)				Loch Linnhe seaward (n = 27)				Averaged over all sites			
	N_a	H_o	H_e	F_{IS}	N_a	H_o	H_e	F_{IS}	N_a	H_o	H_e	F_{IS}	N_a	H_o	H_e	F_{IS}	N_a	H_o	H_e	F_{IS}
FqM09	14	0.958	0.896	−0.07	16	**0.896**	0.907	0.013	17	0.849	0.897	0.054	13	0.815	0.86	0.053	15	**0.88**	0.879	0.008
FqM10	21	**0.833**	0.896	0.071	19	**0.896**	0.912	0.018	18	0.849	0.873	0.027	15	1	0.894	**−0.121**	18.3	**0.895**	0.883	0.016
FqM11	23	0.875	0.931	0.061	19	**0.875**	0.911	0.04	23	**0.811***	0.947	**0.144***	15	**0.778***	0.9	0.138	20	**0.835***	0.911	**0.096***
FqM14	16	**0.833**	0.889	0.064	20	0.958	0.918	−0.044	21	**0.811***	0.918	**0.117**	15	0.815	0.871	0.065	18	**0.854**	0.888	**0.054**
FqM16	27	0.917	0.905	−0.013	21	0.833	0.896	0.07	30	0.981	0.937	−0.048	28	0.889	0.955	0.07	26.5	0.905	0.912	0.021
FqM38	9	0.875	0.745	**−0.177**	10	0.688	0.709	0.031	7	0.717	0.709	−0.011	9	**0.741**	0.751	0.014	8.8	0.755	0.72	−0.042
FqM39	4	0.646	0.678	0.048	4	0.625	0.606	−0.032	5	0.585	0.612	0.045	4	0.556	0.593	0.065	4.3	0.603	0.615	0.039
FqM54	4	0.666	0.637	−0.048	4	0.583	0.543	−0.076	4	0.547	0.544	−0.005	4	0.556	0.621	0.108	4	0.588	0.579	−0.02
FqM13#	10	0.604	0.759	**0.206**	12	0.75	0.817	0.083	11	**0.717**	0.816	**0.122**	8	0.814	0.797	−0.022	–	–	–	–
FqM37#	6	**0.396***	0.671	**0.412***	6	**0.625**	0.718	0.13	8	0.623	0.661	0.058	6	0.704	0.636	−0.109	–	–	–	–
All	13.4	**0.76**	0.792	0.051	13.1	**0.773***	0.785	0.026	14.4	**0.749***	0.784	**0.054**	11.7	**0.767**	0.773	0.028	–	–	–	–
All unlinked loci	14.8	0.826	0.814	−0.004	14.1	0.794	0.792	0.008	15.6	**0.769***	0.797	**0.045**	12.9	**0.769***	0.791	0.047	14.4	**0.789***	0.798	**0.025**

Bold values are significant (P < 0.05). *Indicates values that remained significant after Bonferroni corrections for multiple comparisons (P = 0.05/32 = 0.00156). The two loci marked with #show significant linkage and are excluded from overall calculations.

Table 3. Pairwise F_{ST} values calculated using eight microsatellite loci for *Funiculina quadrangularis* collection sites in two NW Scottish sea lochs. Bold values indicate that genetic differentiation between sites are significantly different from zero (P < 0.05).

Collection site	Loch Duich inland	Loch Duich seaward	Loch Linnhe inland
Loch Duich seaward	0.0059	–	–
Loch Linnhe inland	**0.0072***	0.0042	–
Loch Linnhe seaward	0.0070	0.0001	0.0002

*Indicates values that remained significant after Bonferroni corrections for multiple comparisons (α = 0.05/6 = 0.00833).

between locations. Genotypic differentiation was significant (P < 0.05) for comparisons between the Loch Linnhe inland site and the Loch Duich sites; values for comparison between the Loch Duich inland site and the Loch Linnhe inland site showed significant genotypic differentiation after Bonferroni corrections for multiple comparisons (P = 0.05/6 = 0.00833). Comparisons between the Loch Linnhe inland site and the Loch Duich sites also showed significant genetic differentiation after Bonferroni corrections (P = 0.05/6 = 0.00833).

Minimum nautical distance between sampling sites ranged from 6.9 to 144 km. Results from the Mantel test for matrix correlation between genetic distance and geographic distance showed that there was a significantly positive relationship (Z = 2.5606; P = 0.0104; Fig. 2), although the results were not significant after Bonferroni corrections (P = 0.05/6 = 0.00833).
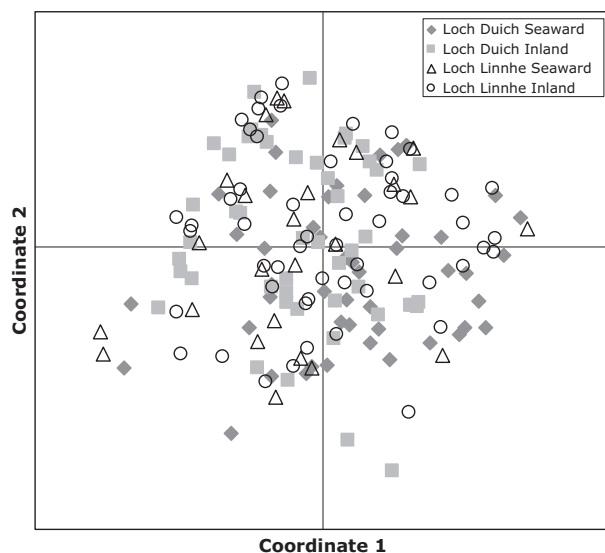
A principal coordinate analysis was performed using an F_{ST} distance matrix based on pairwise comparisons of individual colonies. A scatterplot of individual colonies based on the first two principal coordinates, which explained 43.98% of the total variance, is shown in Fig. 3. The analysis of molecular variance (AMOVA) based on sampling sites showed that less that 0.5% of variation is observed among populations, whereas 97% of variation is observed within individuals. Although very little variation is shown between populations, the observed F_{ST} values were significantly lower than the randomisation replicates (F_{ST} = 0.005, P = 0.016). A similar pattern is found when differences between loch is considered; <0.2% of variation is observed between lochs but this structure is significant compared with the randomisation replicates (F_{ST} = 0.005, P = 0.006).

Discussion

High genetic diversity was present at all four locations, which is similar to patterns reported for other octocorals
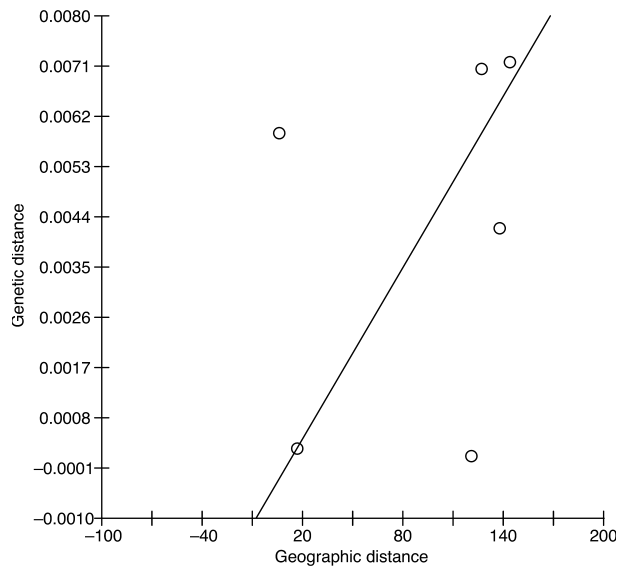
Fig. 2. Graph of isolation by distance comparing genetic distance (pairwise F_{ST} values) with geographic distance (kilometres) between location pairs.

Fig. 3. Plot of samples positioned by the first two axes of the principal coordinate analysis.

(Gutierrez-Rodriguez & Lasker 2004; Costantini *et al.* 2007) and hexacorals (Maier *et al.* 2005). Significant heterozygote deficiencies were found in samples from both Loch Linnhe locations, which have also been documented in shallow-water corals (Ayre & Hughes 2000; Ridgway *et al.* 2001) and deep-sea corals (Le Goff & Rogers 2002). Our results point to little population structure in our data. Mean and pairwise genetic differentiation was very low between populations, the PCoA shows no population

structure and population variation accounted for <0.5% of the observed variation. All of these results may suggest high gene flow between sampling locations, in agreement with patterns reported for scleractinian corals (Ayre *et al.* 1997; Ridgway *et al.* 2001). However, there are two results which indicate some level of population structure that should not be overlooked. We report a pattern of isolation by distance and the significance of the AMOVA that showed lower between-population F_{ST} values than expected.

This potential disparity of results may be explained by the limited sampling of this study. *Funiculina quadrangularis* is a near globally distributed species, but we have only sampled four relatively nearby populations from similar depth range and environment. We might expect the pattern of population structure to become more apparent by sampling larger special scales and greater depth ranges to gain a better understanding of the genetic structure of the entire population. This study lays the foundation for a wider study by demonstrating the efficacy of these markers at detecting population variations for this important species.

Factors promoting population structure in Funiculina quadrangularis

The patterns observed may result from a number of factors including dispersal ability, currents and historical factors.

Although habitat availability is a limit on distributions, it may be that factors promoting or limiting connectivity such as currents and circulation may be more important in explaining the observed population genetic patterns. The circulation around upper (inland) Loch Linnhe demonstrates complicated dynamics (Allen & Simpson 1998), which is typical of the patterns shown in the inner Hebrides (Ellett & Edwards 1983). This may inhibit larval dispersal and could account for the significant difference seen between the inner Loch Linnhe and inner Loch Duich sites. Larval retention is, to some extent, expected in confined coastal populations (reviewed in Cowen & Sponaugle 2009), so our fjord populations may not be typical of the entire range. For example, Le Goff-Vitry *et al.* (2004) showed that the fjordal populations of the cold-water coral *Lophelia pertusa* were atypical of the population as a whole. The larval behaviour of *F. quadrangularis* may contribute to the low F_{ST} values found in this study. *Funiculina quadrangularis* is gonochoric, with a sex ratio of 1:1 and annual broadcast spawning of gametes with females producing 500–2000 oocytes per cm (Edwards & Moore 2009). The production of lecithotrophic larvae has been suggested because of the large oocyte size (>800 μm) and spawning during winter

months, when there is low plankton density (Edwards & Moore 2009). Therefore, larvae may be able to spend relatively long periods of time in the water column, leading to greater dispersal ability and increased potential gene flow between colonies.

We did observe a limited pattern of isolation by distance, and sampling locations within lochs showed less divergence than between lochs, with the exception of the comparison between the seaward locations. This is likely to be a result of the greater influence of tidal currents at the more exposed sites and limited larval dispersal potential because of the physical isolation of the areas farther inland. Significant isolation by distance of corals in other studies has been attributed to spatially restricted larval dispersal (Hellberg 1995; Maier *et al.* 2005). Additionally, historical factors related to the possible recent origin of loch populations might explain the genetic patterns observed. During the last glacial maximum (LGM; 15,000–25,000 years ago), sea levels were reduced by about 120 m and Scotland was below the ice cap (Siegert & Dowdeswell 2004). Rapid range expansion following deglaciation could have resulted in low genetic differentiation of *F. quadrangularis* colonies around Scotland. Current genetic patterns of Atlantic cod in the North Atlantic date back to the LGM (Pampoulie *et al.* 2008). Therefore, the low genetic differentiation and isolation by distance found in the current study could be explained by rapid colonisation of sea lochs after the LGM and reduced gene flow between the inland sites thereafter.

Conservation

Habitat requirements for *F. quadrangularis* are such that the patchiness of suitable habitat may influence dispersal and recruitment rates in certain areas. The fragmented distribution in UK waters may demonstrate that this habitat type is rare (Greathead *et al.* 2007). Limited habitat may lead to increased inbreeding at sites that are surrounded by less suitable habitat. This may occur in Loch Linnhe, as the larger size of the sea loch may generate higher wave and tide energy and therefore fewer sheltered areas for colonization by *F. quadrangularis* in comparison to Loch Duich. Indeed, genetic differentiation between the Loch Linnhe sites was much less than between the two Loch Duich sites, although neither were significantly different from zero.

The results of this study indicate that high genetic diversity and low genetic differentiation characterize *F. quadrangularis* colonies in lochs of NW Scotland. It has been reported that *F. quadrangularis* is highly susceptible to damage from trawling (MacDonald *et al.* 1996; European Commission 2006; Rogers & Gianni 2010). The

low genetic differentiation observed for *F. quadrangularis* in NW Scotland may suggest high rates of gene flow and therefore a potential to recolonise areas lost to disturbance, provided the original disturbance has ceased and the underlying environment and substrata are within species' tolerance. However, the presence of unique genotypes for every sample supports the absence of asexual reproduction as suggested by Edwards & Moore (2009). This characteristic may increase the vulnerability of the species and should be considered in conservation efforts. Care must be taken not to overinterpret these results, as they characterise the genetic structure of *F. quadrangularis* at a local level and may not represent that of the species as a whole. The markers developed herein will provide the foundation for further studies to determine the genetic structure of *F. quadrangularis* in wider NW Scotland, Europe and other parts of the world.

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