Population genetic analyses reveal distinct geographical blooms of the jellyfish *Rhizostoma octopus* (Scyphozoa)

FERGAL GLYNN[1,2], JONATHAN D. R. HOUGHTON[1,2,3] and JIM PROVAN[1,2]*

[1]*School of Biological Sciences, Queen's University Belfast, 97 Lisburn Road, BT9 7BL, Belfast, UK*
[2]*Institute for Global Food Security, Queen's University Belfast, Belfast, UK*
[3]*Queen's Marine Laboratory, 12–13 The Strand, BT22 1PF, Portaferry, UK*

Understanding the spatial integrity and connectivity of jellyfish blooms is important for ecologists and coastal stakeholders alike. Previous studies have shown that the distribution of jellyfish blooms can display a marked consistency in space and time, suggesting that such patterns cannot be attributed to passive processes alone. In the present study, we used a combination of microsatellite markers and mitochondrial cytochrome oxidase I sequences to investigate genetic structuring of the scyphozoan jellyfish *Rhizostoma octopus* in the Irish and Celtic Seas. The mitochondrial data indicated far higher levels of population differentiation than the microsatellites: $\Phi_{ST[MT]} = 0.300$ vs. $\Phi_{ST[NUC]} = 0.013$. Simulation studies indicated that the low levels of nuclear differentiation were not the result of limited power because of low levels of polymorphism. These findings, supported by palaeodistribution modelling and mismatch distribution analysis, are consistent with expansion of *R. octopus* from a single, limited refugium after the Last Glacial Maximum, followed by subsequent isolation, and that the discrepancy between the mitochondrial and nuclear markers is a result of the nuclear loci taking longer to reach mutation–drift equilibrium following the expansion as a result of their four-fold larger effective population size. The populations studied are probably not well connected via gene flow, and thus genetically as well as geographically distinct, although our findings also highlight the need to use a combination of organellar and nuclear markers to enable a more complete understanding of population demography and structure, particularly for species with large effective population sizes. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **116**, 582–592.

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INTRODUCTION

The application of population genetics approaches has provided many insights into the levels and patterns of gene flow in marine organisms. The traditional view was that there were few barriers to population connectivity in the marine realm, particularly for organisms with planktonic or partially planktonic life cycles (Palumbi, 1994; Norris, 2000). Subsequent molecular studies on marine populations utilizing mitochondrial (mt)DNA, however, indicated that intraspecific genetic structuring does exist (Chow *et al.*, 1997; Zane *et al.*, 1998; Keeney *et al.*, 2005; Darling, Kucera & Wade, 2007). More recently, the development of microsatellite markers has offered further opportunities to study genetic structuring because theoretical studies have suggested that the use of multiple, multi-allelic

loci should offer greater power than mtDNA to detect population subdivision, particularly at low levels (Larsson *et al.*, 2009), and this has been largely borne out by empirical studies (Iacchei *et al.*, 2014; Godhe *et al.*, 2014; but see also Provan *et al.*, 2009). It has also been demonstrated, however, that population demographic changes such as those associated with the climatic fluctuations of the Pleistocene (approximately 2.58 Mya to 11 kya) can give rise to apparently contradictory signals of population subdivision across different markers (Lukoschek, Waycott & Keogh, 2008; Larmuseau *et al.*, 2010). Thus, depending on the demographic history of the populations under study, the use of both mtDNA and microsatellites may be required to gain a complete understanding of patterns of gene flow.

Within this context, there is international interest in the drivers, overall abundance and connectivity of jellyfish blooms (i.e. Phylum Cnidaria, Class

*Corresponding author. E-mail: j.provan@qub.ac.uk

Scyphozoa: Hamner & Dawson, 2009; Brotz *et al.*, 2012; Condon *et al.*, 2013). These blooms represent the concentration of many free swimming medusae in a particular area either through rapid population growth (a true bloom) or advection from another area (an apparent bloom: Graham, Pag & Hamner, 2001; Graham *et al.*, 2003). True blooms are associated typically with species displaying metagenic life histories comprising free-swimming and sexually reproducing medusae and benthic polyps that reproduce through asexual strobilation (Graham *et al.*, 2001; Richardson *et al.*, 2009; Gibbons & Richardson, 2013). In most cases, a given cohort of medusae will persist from spring through to autumn, whereas the asexually reproducing polyps can survive for many years (Thein, Ikeda & Uye, 2012). This inter-annual persistence of an asexually reproducing sessile life stage can lead to the regular re-occurrence of blooms in specific locations (Houghton *et al.*, 2006a, b; Lilley, Houghton & Hays, 2008), population structuring (Pitt & Kingsford, 2000), and eventual phylogenetic differentiation. As efforts to incorporate jellyfish more effectively into ecosystem and fisheries models gather momentum (Pauly *et al.*, 2008; Brotz *et al.*, 2012; Fleming *et al.*, 2015), such information is important when considering the temporal and spatial integrity of apparently isolated bloom events (Lee *et al.*, 2013).

The utility of population genetics to clarify the connectivity or discreteness of jellyfish blooms has been shown, with studies having revealed population structuring (Dawson, 2005), cryptic speciation (Dawson & Jacobs, 2001; Holland *et al.*, 2004), and even anthropogenic introductions (Dawson, Gupta & England, 2005). Almost all such studies of scyphozoan jellyfish population genetics have employed a limited number of markers, with the exception of Aglieri *et al.* (2014), with most studies relying mainly on the mitochondrial cytochrome oxidase I (COI) gene (Holland *et al.*, 2004), although some have additionally employed data from the nuclear ribosomal DNA cistron (Dawson & Jacobs, 2001; Stopar *et al.*, 2010). The development of microsatellite markers for several jellyfish species (Coughlan, Seymour & Cross, 2006; Peplow *et al.*, 2009; Reusch *et al.*, 2010; Bolte *et al.*, 2013; Meek *et al.*, 2013) potentially offers the opportunity to study fine-scale genetic structuring, although, to date, there has only been a single published study on scyphozoans (Aglieri *et al.*, 2014).

In the present study, we used a combination of recently developed microsatellite markers and COI sequences to investigate genetic structuring of *Rhizostoma octopus*, a scyphozoan jellyfish with a generally predictable and temporally stable geographical distribution, including regular but apparently discrete blooms of adult jellyfish in bays in the Irish Sea (Doyle *et al.*, 2006; Houghton *et al.*, 2006b). Previous genetic analyses within the genus have provided conflicting results, with Ramšak, Stopar & Malej (2012) reporting little partitioning of genetic diversity between blooms of *R. pulmo* in the Mediterranean Sea, whereas Lee *et al.* (2013) found notable population structure in *R. octopus* in the Irish Sea and from La Rochelle, France, although levels of differentiation were far less pronounced in the nuclear gene studied (calmodulin) compared to the mitochondrial COI gene. The use of microsatellites, with their potentially increased resolution, should allow us to determine not only whether any fine-scale structure exists in *R. octopus*, even in cases where such levels may be extremely low (Wirth & Bernatchez, 2001), but also whether there are any discrepancies between mtDNA and microsatellites, possibly resulting from demographic changes during the Pleistocene.

MATERIAL AND METHODS

SAMPLING AND DNA EXTRACTION

Samples were collected from four locations throughout the Irish and Celtic Seas (Fig. 1, Table 1) in August/September 2011. Genomic DNA was extracted using a modified version of the CTAB phenol/chloroform protocol described by Porebski, Bailey & Baum (1997), where extracted DNA that had been subjected to phenol and chloroform wash was stored in a 1 : 1 supernatant : isopropanol state at -20 °C until needed for polymerase chain reaction (PCR), and then pelleting and the alcohol wash were carried out before elution. Long-term storage of eluted DNA resulted in loss of high molecular weight (genomic) DNA and reduced amplification success.

MICROSATELLITE GENOTYPING

Microsatellites were developed from *R. pulmo* sequences deposited in GenBank (accession numbers are provided in Table 2). Forward primers included a 19-bp M13 tail (CACGACGTTGTAAAACGAC) and reverse primers included a 7-bp tail (GTGTCTT). PCR was carried out in a total volume of 10 μL containing 100 ng of genomic DNA, 10 pmol of 6-FAM- or HEX-labelled M13 primer, 1 pmol of tailed forward primer, 10 pmol of reverse primer, $1 \times$ PCR reaction buffer, 200 μM each dNTP, 2.5 mM MgCl$_2$ and 0.25 U of GoTaq Flexi DNA polymerase (Promega). PCR was carried out on a MWG Primus thermal cycler using the parameters: initial denaturation at 94 °C for 5 min followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s (55 °C for RpMS-4), extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. Genotyping was carried out on an AB3730xl capillary genotyping

Figure 1. Locations of sites sampled in the present study. CB, Carmarthen Bay; TB, Tremadoc Bay; SF, Solway Firth; CS, Celtic Sea. The inset map shows western Europe, highlighting the area of the present study.

system (Life Technologies). Allele sizes were scored using LIZ size standards and were checked by comparison with previously sized control samples.

MTDNA SEQUENCING

A 639-bp region of the *R. octopus* mtDNA COI gene was amplified using the primers Ro-COI-F 5′-CA-

ACAAATTCTAAGATATTGGAAC-3′ and Ro-COI-R 5′-GGGTCGAAGGAAGATGTATTA-3′. PCR was carried out on a MWG Primus thermal cycler using the parameters: initial denaturation at 94 °C for 3 min followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR was carried out in a total volume of 20 μL containing 200 ng of genomic DNA, 10 pmol of each primer, 1 × PCR reaction buffer, 200 μM each dNTP, 2.5 mM $MgCl_2$, and 0.5 U of GoTaq Flexi DNA polymerase (Promega). Five microlitres of PCR product were resolved on 1.5% agarose gels and visualized by ethidium bromide staining, and the remaining 15 μL were EXO-SAP purified and sequenced in both directions using the BigDye sequencing kit, version 3.1 (Applied Biosystems) and run on an AB 3730XL DNA analyser (Life Technologies).

STATISTICAL ANALYSIS

Tests for linkage disequilibrium between pairs of microsatellite loci in each population were carried out in FSTAT, version 2.9.3.2 (Goudet, 2002). Levels of polymorphism measured as observed (H_O) and expected (H_E) heterozygosity averaged over loci for nuclear microsatellites, and as haplotype (H) and nucleotide (π) diversity for mtDNA, were calculated using ARLEQUIN, version 3.5.1.2 (Excoffier & Lischer, 2010). Inbreeding coefficients (F_{IS}) were estimated using FSTAT. Levels of interpopulation differentiation were estimated from allele (microsatellite) and haplotype (mtDNA) frequencies using Φ-statistics, which give an analogue of *F*-statistics (Weir & Cockerham, 1984) calculated within the analysis of molecular variance (AMOVA) framework (Excoffier, Smouse & Quattro, 1992), also using AR-LEQUIN Software package. Population-pairwise Φ_{ST} values were also calculated using ARLEQUIN. Significance of values was tested using 1000 permutations. A median-joining network showing the

Table 1. *Rhizostoma octopus* populations studied and summary diversity statistics

Population	Latitude (N)	Longitude (W)	Nuclear				Mitochondrial			
			N	H_O	H_E	F_{IS}	N	h	H	π
Carmarthern Bay	51.745	4.447	24	0.777	0.852	0.090**	24	15	0.920	0.004
Tremadoc Bay	52.728	4.066	23	0.765	0.824	0.074[NS]	22	3	0.178	0.001
Solway Firth	54.958	3.217	24	0.658	0.805	0.188**	19	10	0.854	0.006
Celtic Sea	51.783	6.650	15	0.717	0.808	0.117*	14	5	0.659	0.003

N, number of individuals studied; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; h, number of haplotypes detected; H, gene diversity; π, nucleotide diversity.
Significance of F_{IS}: *$P < 0.05$; **$P < 0.01$; NS, nonsignificant.

Table 2. *Rhizostoma octopus* microsatellite primers

Locus	Repeat	Primers (5′- to 3′)	Size range (bp)	GenBank
Rp-MS1	$(GCACGCACACAC)_7$	F: CCCTCATACGTTATGTCATGG R: CAGCAGTTCTGACAAGTATTTATTATTC	148–205	DQ093644
Rp-MS3	$(TGX)_{14}$	F: TTTGGTCGTGTCCTGTTTGA R: CGCCAAGAGCAGAATCAATA	141–212	DQ075948
Rp-MS4	(ACTACAC) complex	F: CCAACTAATAGAAACTAATCTAGACTAAAC R: AAAGTATGATTACGTGAAACGA	398–467	DQ075951
Rp-MS5	(TACAC) complex	F: AAAATTTGCTCTTATTTGATTCTCG R: GATGAAAATCGTGGAAGCTG	237–362	DQ075950

Forward tailed with CACGACGTTGTAAAACGAC.
Reverse tailed with GTGTCTT.

relationships between the mtDNA haplotypes was constructed using the NETWORK, version 4.5.1.6 (http//www.fluxus-engineering.com). In addition, tests for population expansion based on Tajima's D and Fu's F_S and a mismatch distribution analysis, which identifies characteristic 'waves' in the shape of the distribution resulting from expansion (Rogers & Harpending, 1992), were carried out in ARLEQUIN.

To identify possible spatial patterns of gene flow, BAPS, version 5 (Corander, Waldmann & Sillanpää, 2003) was used to identify clusters of genetically similar populations using a Bayesian approach. Ten replicates were run for all possible values of the maximum number of clusters (K) up to $K = 4$, the number of populations sampled in the study, with a burn-in period of 10 000 iterations followed by 50 000 iterations. Multiple independent runs always gave the same outcome. To further identify possible spatial patterns of gene flow, a principal coordinate analysis (PCoA) was carried out in GENALEX, version 6.1 (Peakall & Smouse, 2006). Inter-individual genetic distances were calculated as described in Smouse & Peakall, 1999, and the PCoA was carried out using the standard covariance approach.

Because of the genetic homogeneity revealed by the microsatellite loci studied, and to compare the relative power of microsatellites and the mtDNA to detect low levels of population differentiation, simulations were carried out using POWSIM, version 4.0 (Ryman & Palm, 2006). Simulations were carried out for an effective population size of $N_e = 1000$ to yield F_{ST} values of 0.0050, 0.0075, 0.0100, 0.0125, 0.0150, 0.0175 and 0.0200. Although *R. octopus* may have a larger effective population size, this is not relevant to the analysis because N_e only determines the time necessary to reach the target F_{ST}. Thus, the use of larger values of N_e is unjustified as the difference between, say, $N_e = 1000$ and 10 000 (and higher) is not important at values of F_{ST} as small as those

tested in the simulation (N. Ryman, pers. Comm.). In all cases, 1000 replicates were run and the power of the analysis was indicated by the proportion of tests that were significant at $P < 0.05$ using the observed allele frequencies for both the four microsatellite loci and the single mtDNA COI region studied [for $F_{ST} = 0$, this corresponds to the Type I (α) error]. For the mtDNA, sample sizes were adjusted as recommended by Larsson *et al.* (2009).

PALAEODISTRIBUTION MODELLING

Palaeodistribution modelling was carried out to determine the potential suitable range for *R. octopus* at the Last Glacial Maximum (LGM; approximately 21 kya) using the maximum entropy approach implemented in MAXENT, version 3.3.3 (Phillips, Anderson & Schapire, 2006). Species occurrence data between 1950 and 2000 were downloaded from the Global Biodiversity Information Facility data portal (http://www.gbif.org) and from the Ocean Biogeographic Information System (http://www.iobis.org), and supplemented with our own population data from the present study (117 spatially unique occurrences in total). Current-day bioclimatic data (MARSPEC; Sbrocco & Barber, 2013) were obtained at 5-min resolution and models were generated using cross-validation of 10 replicate runs under the default MAXENT parameters. Model performance was assessed based on the area under the receiver operating characteristic curve (AUC). Models were projected onto reconstructed bioclimatic data for the LGM (ensemble of five models: CNRM, ECBILTCLIO, FGOALS, HadCM and MIROC-322; Sbrocco, 2014). To identify potential areas where the model may have extrapolated beyond current climatic conditions that could lead to unreliable predictions, we carried out a multivariate environmental similarity surfaces (MESS) analysis (Elith, Kearney & Phillips, 2010) in MAXENT.

RESULTS

POPULATION GENETIC ANALYSES

No evidence of linkage disequilibrium was detected between any of the four nuclear microsatellite loci analyzed. Between 13 (Rp-MS1) and 25 (Rp-MS5) alleles were detected, with a total of 73 (mean = 18.25 per locus). Within-population levels of observed (H_O) and expected (H_E) heterozygosity ranged from 0.658 (Solway Firth) to 0.777 (Carmarthern Bay; mean = 0.729) and from 0.805 (Tremadoc Bay) to 0.852 (Carmarthen Bay; mean = 0.822), respectively (Table 1). Levels of F_{IS} were significantly different from zero in three of the four populations, and ranged from 0.074 (Tremadoc Bay) to 0.188 (Solway Firth; mean = 0.075). Summary statistics by locus are given in the Supporting information (Table S1).

A total of 27 mitochondrial COI haplotypes were identified (Fig. 2). Nineteen of these were found in a single individual, and three of the remaining eight, including the two most common haplotypes, were found in more than one population. Within populations, between three (Tremadoc Bay) and 15 (Carmarthen Bay) haplotypes were detected (mean = 8.25). Levels of haplotype (H) and nucleotide (π) diversity ranged from 0.178 (Tremadoc Bay) to 0.920 (Carmarthen Bay), and from 0.001 (Tremadoc Bay) to 0.006 (Solway Firth) respectively (Table 1).
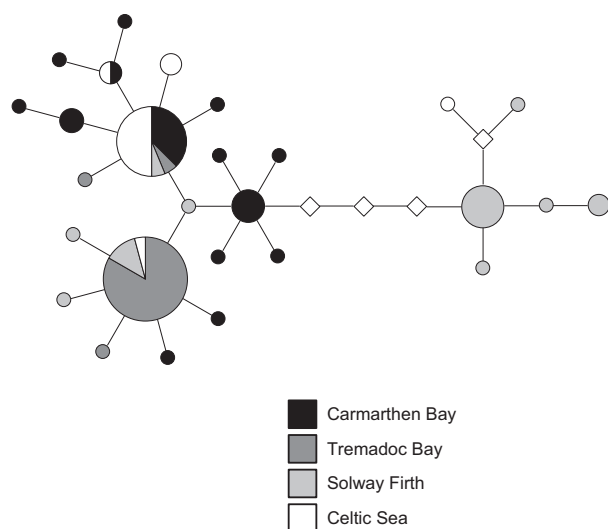

Figure 2. Median-joining network showing the relationships between the 27 haplotypes detected by sequencing the mitochondrial DNA cytochrome oxidase I (COI) region. Circle sizes are approximately proportional to haplotype frequency: the smallest circle represents a single individual; the largest circle represents 24 individuals. Each connection represents a single mutation and small open diamonds represent missing intermediate haplotypes.

AMOVA revealed a small but significant overall differentiation based on nuclear microsatellites ($\Phi_{ST[NUC]}$ = 0.013; P < 0.001), as well as a much higher level based on the mtDNA COI ($\Phi_{ST[MT]}$ = 0.300; P < 0.001) (Table 3). Population-pairwise Φ_{ST} values ranged from zero (three pairs) to 0.046 (Tremadoc Bay/Celtic Sea) for nuclear microsatellites, and from zero (Carmarthen Bay/Celtic Sea) to 0.579 (Tremadoc Bay/Celtic Sea) for the mtDNA COI (Table 4). The BAPS analysis indicated that all the individuals analyzed were grouped into a single genetic cluster (100% probability). This was reflected in the PCoA, which showed no evidence of geographical structuring of individual multilocus genotypes (Fig. 3). The values for both Tajima's D and Fu's F_S were significantly negative (−1.434; P = 0.049) and −16.077; P < 0.0001, respectively), consistent with sudden population expansion. The mismatch distribution analysis (see Supporting information, Fig. S1), which resulted in a Harpending's raggedness index of 0.045 (P = 0.297), also did not reject the sudden expansion model.

The simulation studies suggested that the nuclear microsatellite data were able to detect F_{ST} values of as low as 0.0100 at least 90% of the time, and 0.0125 at least 98% of the time (Fig. 4). The mtDNA COI locus had much lower power (only 9% and 16% for the same two values) and could only detect F_{ST} = 0.05 in 88% of the simulations. At the lowest values of F_{ST} ($\leq$ 0.01) used in the simulations, the power of the nuclear microsatellite loci was generally five- to 10-fold that of the mtDNA COI locus.

PALAEODISTRIBUTION MODELLING

For all models, AUC values were high (mean AUC = 0.995). The modelled current-day distribution was a largely accurate prediction of the current range of *R. octopus*, highlighting that the coastal areas of northwestern Europe as most suitable (Fig. 5A). The palaeodistribution model indicated extensive suitable habitat in the Mediterranean at the LGM but very little in the north-east Atlantic, with the only suitable habitat being limited to a small area in the Bay of Biscay adjacent to the palaeocoastline (Fig. 5B). The MESS analysis did not indicate any areas in the model where extrapolation beyond current climatic conditions had occurred.

DISCUSSION

Although the results from the two sets of markers in the present study revealed differing levels of population structuring, they can be interpreted as being generally consistent with population expansion fol-

**Table 3.** Analysis of molecular variance

Source of variation	Nuclear				Mitochondrial			
	d.f.	Sum of squares	Variance	%	d.f.	Sum of squares	Variance	%
Among populations	3	6.666	0.019	1.33***	3	9.153	0.140	30.02***
Within populations	168	236.979	1.411	98.67	75	24.417	0.326	69.98

***$P < 0.001$.

Table 4. Population-pairwise Φ_{ST} values

	Carmarthen Bay	Tremadoc Bay	Solway Firth	Celtic Sea
Carmarthen Bay	–	0.437	0.100	*0.068*
Tremadoc Bay	*−0.005*	–	0.410	0.579
Solway Firth	*−0.011*	0.039	–	0.206
Celtic Sea	0.029	0.046	*−0.006*	–

Lower diagonal matrix: nuclear. Upper diagonal matrix: mitochondrial
Values not significantly different from zero are shown in italics.
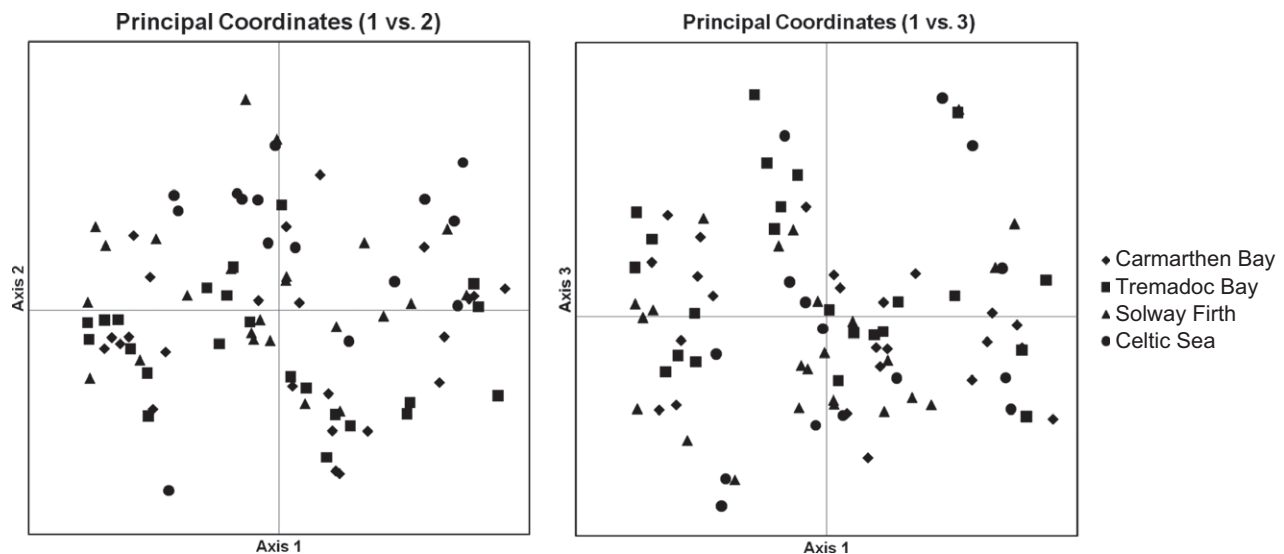
Figure 3. Results of the principal coordinate analysis. The first three axes accounted for 23.51%, 21.54%, and 17.44%, respectively, of the total variation (62.49%).

lowing the LGM and subsequent divergence, with limited gene flow between the regions under investigation. Our findings are broadly comparable with those obtained in a previous study on *R. octopus* (Lee *et al.*, 2013), and highlight an emerging trend from the currently limited number of microsatellite-based population genetic analyses in gelatinous zooplankton (Bolte *et al.*, 2013; Aglieri *et al.*, 2014), namely that blooms can readily be traced to relatively isolated, self-sustaining populations. From an ecological perspective, such information is insightful given that scyphozoa have often been viewed as transient components of marine food webs, with very little spatial integrity or trophic relevance (Doyle *et al.*, 2006; Houghton *et al.*, 2007). The growing body of evidence to show that jellyfish blooms can persist in large numbers in particular locations over time (through processes in addition to advection) promotes the much needed inclusion of such species in ecosystem models (Pauly *et al.*, 2008; Doyle *et al.*, 2014).
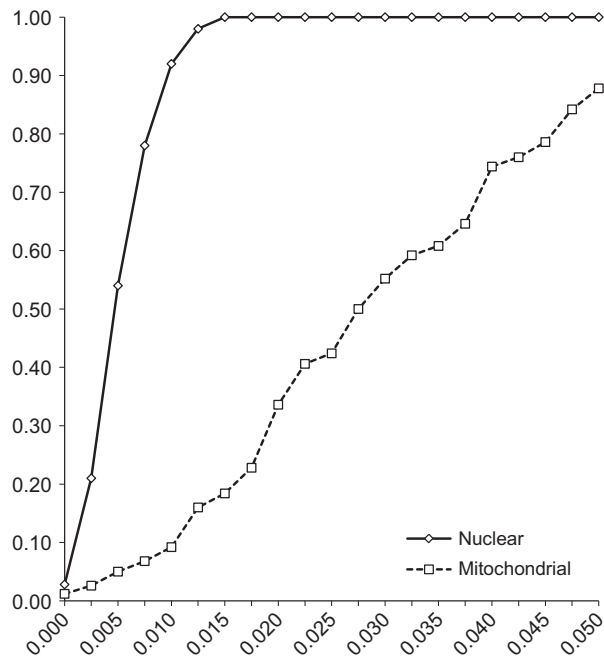
Figure 4. Results of the POWSIM analysis. The *y*-axis represents the power of the markers to successfully recover the value of F_{ST} indicated on the *x*-axis, expressed as the proportion of 1000 simulations (for details, see text). For $F_{ST} = 0$, this is the Type I (α) error value.

Figure 5. Results of the species distribution modelling. A, current-day model. B, palaeodistribution model for the Last Glacial Maximum (LGM; approximately 21 kya). Darker blue areas indicate those more suitable for *Rhizostoma octopus*. Small yellow circles in (A) indicate occurrence data used to generate the models.

Discrepancies between the levels of genetic structuring revealed by nuclear and organellar markers have been reported in a wide range of species (Karl *et al.*, 2012). These can be the result of a variety of processes, including sex-biased dispersal (Cano, Mäkinen & Merilä, 2008), homoplasy at microsatellite loci (Estoup, Jarne & Cornuet, 2002), selection (de Innocentiis *et al.*, 2001) or differences in effective population size (Palumbi, Cipriano & Hare, 2001). The observed disparity between levels of population differentiation revealed by nuclear and mitochondrial markers in the present study, which differ by more than an order of magnitude ($\Phi_{ST[NUC]} = 0.013$ vs. $\Phi_{ST[MT]} = 0.30$), can be explained most readily by the last of these. For diploid species, such as *R. octopus*, the effective population size of the haploid mitochondrial genome is half that of the diploid nuclear genome. In addition, in idealized populations of dioecious taxa with even sex ratios, the effective population size of the mitochondrial genome could be assumed to be 0.25 of the effective population size of the nuclear genome, leading to differences in the time required for reciprocal monophyly via lineage sorting (Maynard Smith, 1987; Palumbi *et al.*, 2001; Hudson & Coyne, 2002). A lack of resolving power as a result of insufficient polymorphism in the microsatellites is not supported by the simulation analyses,
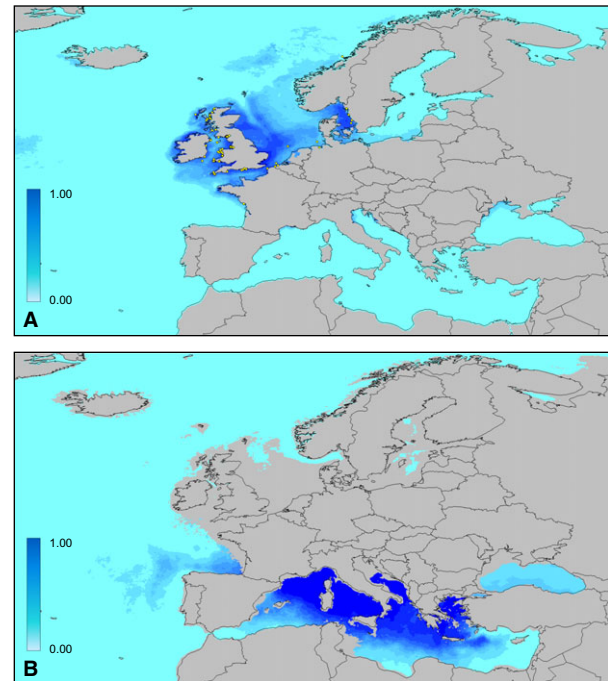
which indicated that the microsatellites had far greater power than mtDNA over a range of simulated F_{ST} values based on the empirical allele frequencies.

Differences in F_{ST} and its equivalents between nuclear and mitochondrial markers can be further exaggerated where populations have undergone recent expansion. In such circumstances, nuclear loci will take longer to reach mutation–drift equilibrium. This has been suggested previously for other marine species with large effective population sizes (Lukoschek *et al.*, 2008; Larmuseau *et al.*, 2010). The results of the palaeodistribution modelling indicate an extremely restricted area of suitable habitat for *R. octopus* in the northeast North Atlantic during the LGM compared to its current distribution. The model did suggest the presence of suitable habitat in the Mediterranean but, although this area was not isolated from the Atlantic despite the drop in sea levels during the glacial period, the Strait of Gibraltar represents a major biogeographical barrier to a range of marine species (Baus, Darrock & Bruford, 2005; Paternello, Volckaert & Castilho, 2007). Furthermore, climate-induced range shifts and contractions such as those that occurred during the Pleistocene

are believed to result from population extirpation, rather than migration (Dalén *et al.*, 2007; Bennett & Provan, 2008; Provan & Bennett, 2008). Our findings, including the significant negative values for both Tajima's D and Fu's F_S and the mismatch distribution analysis, are consistent with expansion of *R. octopus* from a single, limited refugium after the LGM, followed by subsequent isolation, as indicated by the mtDNA and the nuclear F_{IS} values, which suggest inbreeding within three of the four populations. Many northern North Atlantic marine species survived the LGM in a range of refugia (Provan, 2013) and the low levels of nuclear genetic differentiation observed in *R. octopus* are consistent with high historical gene flow, suggesting an extended period of genetic connectivity consistent with LGM survival of populations in the same area. Population isolation following the expansion would give rise to the observed discordance between mtDNA and microsatellites.

Despite the discrepancies observed between mtDNA and microsatellites, the case for using multiple, unlinked nuclear loci for genetic studies on scyphozoa is strong. As a basic tool, the mitochondrial COI marker allows a great deal of information to be gathered and comparisons to be made with many other scyphozoan species for which population data sets exist (Holland *et al.*, 2004; Dawson, 2005; Prieto, Armani & Macías, 2013). The additional potential power of microsatellites, as indicated by the simulation studies, could be useful in fine-scale analyses of population structure in other species that appear to have little geographically-based population structuring such as the congener, *R. pulmo* (Ramšak *et al.*, 2012). With the recent publication of a study of *Pelagia noctiluca* genetics employing microsatellite markers (Aglieri *et al.*, 2014) considered together with the findings of the present study, we foresee a shift in scyophozoan studies toward including panels of unlinked, high-resolution nuclear markers. As in the present study, a combination of organellar and nuclear markers may be necessary to provide a more complete understanding of population demography and structure, particularly for species with large effective population sizes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Results of the mismatch distribution analysis.

Table S1. Summary statistics by locus. N, number of individuals studied; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient.