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Multiple genetic stocks of longfin squid *Loligo pealeii* in the NW Atlantic: stocks segregate inshore in summer, but aggregate offshore in winter

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ABSTRACT: The longfin squid *Loligo pealeii* is distributed widely in the NW Atlantic and is the target of a major fishery. A previous electrophoretic study of *L. pealeii* was unable to prove genetic differentiation, and the fishery has been managed as a single unit stock. We tested for population structure using 5 microsatellite loci. In early summer (June), when the squids had migrated inshore to spawn, we distinguished 4 genetically distinct stocks between Delaware and Cape Cod (ca. 490 km); a 5th genetic stock occurred in Nova Scotia and a 6th in the northern Gulf of Mexico. One of the summer inshore stocks did not show genetic differentiation from 2 of the winter offshore populations. We suggest that squids from summer locations overwinter in offshore canyons and that winter offshore fishing may affect multiple stocks of the inshore fishery. In spring, squids may segregate by genetic stock as they undertake their inshore migration, indicating an underlying mechanism of subpopulation recognition.

KEY WORDS: Fisheries · Spawning migration · Microsatellites · Population structure · Population recognition · Null alleles

INTRODUCTION

The high dispersal capability of many marine organisms has typically been associated with low levels of genetic differentiation, especially when compared to terrestrial and freshwater species (Ward et al. 1994, Graves 1998, Waples 1998). Several recent studies using highly variable genetic markers have shown examples of marine organisms that do exhibit population substructure (for reviews see Bohonak 1999, Hellberg et al. 2002). The presence of distinct genetic units within commercially important species bears direct relevance to the management of fisheries.

Population genetic structure of cephalopods is scarcely known. Within the Class Cephalopoda (Phylum Mollusca), the squids (Order Teuthoidea) are one of the most speciose and numerically abundant groups, and play a central role in marine ecosystems (cf. Boyle & Boletzky 1996). Among the world's inshore fisheries, the loliginid squids (Family Loliginidae) are now one of the most commercially valuable species. These stocks must be managed judiciously due to recent high levels of exploitation. Most loliginid fisheries, including that for the longfin squid *Loligo pealeii* (Lesueur 1821), are managed as a single unit stock (NEFSC 1996). The reason for this is that most studies of genetic structuring in squid populations have suggested widespread genetic uniformity (e.g. Garthwaite et al. 1989, Reichow & Smith 2001, Shaw et al. 1999, 2004). In addition, offshore of the Atlantic coast of the USA, the lack of conspicuous physical oceanographic barriers to gene flow would suggest that separate genetic stocks are unlikely to exist.

Loligo pealeii has a life span <1 yr, due in large part to the very high growth rates that squids achieve (Hatfield et al. 2001), and to the relatively small sizes at which the species is capable of maturing under certain (yet unknown) conditions. Off southern New England (the main fishery), squids are found inshore in summer as they spawn, and offshore during winter in submarine canyons with stable temperatures (Summers 1983). There is evidence that they also undergo substantial north-south migrations on the order of 500 km or more (Macy & Brodziak 2001). Recent morphometric studies of size and age at maturity provide evidence

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that the link between inshore and offshore components of the stock may be more complicated than formerly believed: age structure remains nearly constant through winter and spring, and statolith back-aging suggests that spawning recruitment may occur year-round (Macy & Brodziak 2001).

An allozyme study of *Loligo pealeii* showed that only 1 locus was polymorphic enough to detect differentiation (Garthwaite et al. 1989). Based upon that single locus, those authors suggested that 3 separate populations of *L. pealeii* existed along the Atlantic coast of the USA at Virginia, Cape Cod, and Georges Bank. However, the *L. pealeii* fishery has continued to be managed as a single genetic stock, given the lack of convincing genetic data to the contrary. Because recent increases in value have attracted attention to the *L. pealeii* fishery, and due to refinement of molecular genetic techniques, we decided to use microsatellites (Buresch et al. 2001, Maxwell et al. 2000) to test for population substructure.

MATERIALS AND METHODS

Sample collection. Squid samples were collected by trawl from 6 inshore areas: Nova Scotia (Stn 1), Cape Cod (Stn 2), Long Island Sound (Stn 3), New Jersey (Stn 4), Delaware (Stn 5), and northern Gulf of Mexico (Stn 6); 1 mid-shelf area: Georges Bank (Stn 7); and 3 offshore areas: Veatch Canyon (Stn 8), Hudson Canyon (Stn 9), and Washington Canyon (Stn 10) (Fig. 1). Inshore summer samples and the mid-shelf sample were collected during the 2001 spawning season from May to June. Winter offshore samples were collected from different years (Table 1). The northern Gulf of Mexico (Stn 6), Hudson Canyon (Stn 9), and Nova Scotia (Stn 1) samples were collected during winter 2000, prior to the inshore summer sampling. Washington (Stn 10) and Veatch (Stn 8) canyons were sampled in winter 2002, after the inshore summer samples.

DNA extraction, amplification and genotyping. Squids were frozen until DNA extraction. Genomic DNA was extracted from 20 to 40 mg of adult arm tip tissue by a variation of the phenol/SDS procedure (Sambrook et al. 1989). Five microsatellite primers (Lfor3, Lp2, Lp4, Lp5 and Lp12) (Maxwell et al. 2000) were applied to each sample. Microsatellites were amplified using the polymerase chain reaction (PCR) in a Perkin Elmer 3700 thermocycler under the following conditions: 300 s at 95°C, then 25 to 35 cycles of 30 s at 95°C, 30 s at 55°C, 30 s at 72°C, and a final elongation time of 120 s. Reaction mixes contained 1 µl of template DNA, 1.5 mM $MgCl_2$, 0.2 mM of each nucleotide, 0.1 mM of each primer (1 primer infrared labeled), 0.08 units of *Taq* polymerase (Promega) with the manufacturer's supplied Buffer B, and deionized water to a final volume of 10 µl. PCR products were separated on a 6% denaturing polyacrylamide gel, and analyzed on a LI-COR 4000 model sequencer. Fragment sizes were determined by comparison to commercial size standards (LI-COR) using GelPro Analyzer software (Media Cybernetics). Samples of alleles of known size were run on each gel as standards to assure correct allele assignment. Allele lengths that could not be determined the first time were repeatedly run until unequivocal assignment.

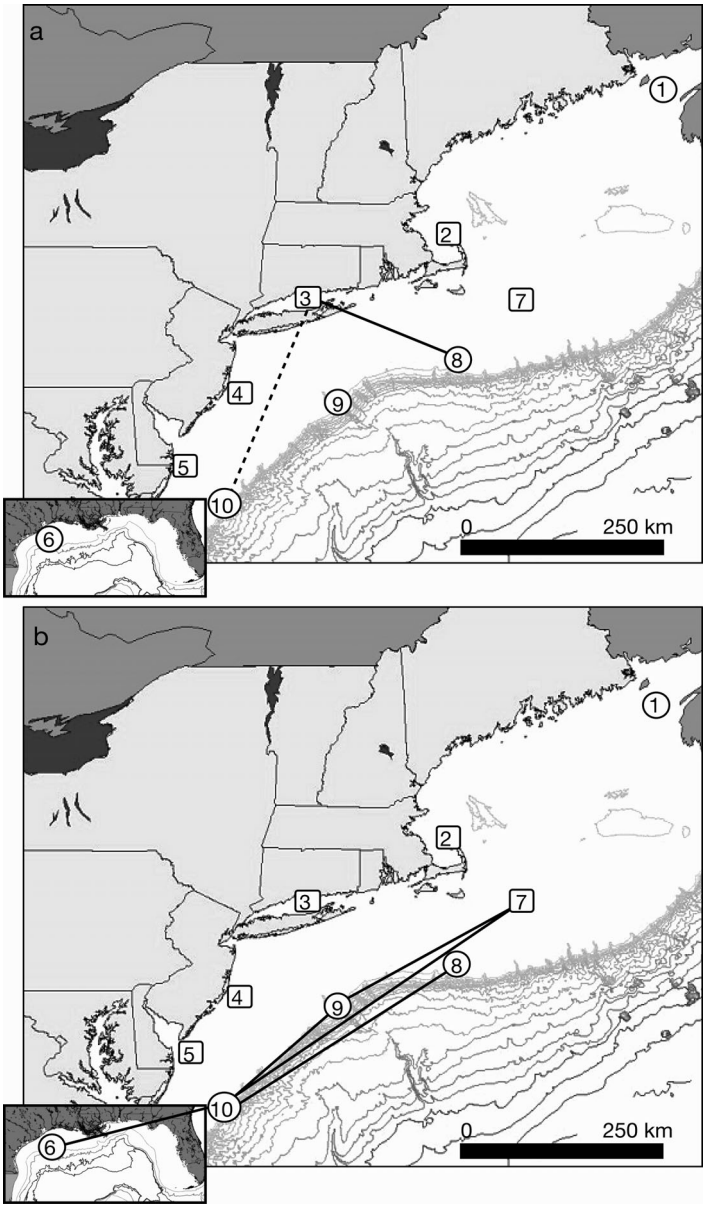

Fig. 1. *Loligo pealeii*. Sampling stations in the NW Atlantic. (a) Inshore–offshore connections; (b) offshore connections only. Rectangles: summer samples; circles: winter samples. Connecting lines: non-significant differences in allele frequencies (i.e. gene flow) between stocks; dotted line: non-significant F_{ST} value; inset: Gulf of Mexico sampling station

Table 1. *Loligo pealeii*. Sampling stations and collection details (compare with Fig. 1). In: inshore; Mid: mid-shelf; Off: offshore. W: winter; S: summer

Stn	Name	Latitude	Longitude	Sampling Size	Location	Period
1	Nova Scotia	44.0° N	66.8° W	45	In	W 2001–2002
2	Cape Cod	42.0° N	70.2° W	38	In	S 2001
3	Long Island	41.2° N	72.5° W	93	In	S 2001
4	New Jersey	39.4° N	74.3° W	76	In	S 2001
5	Delaware	38.6° N	75.0° W	61	In	S 2001
6	Gulf of Mexico	29.3° N	94.8° W	45	In	W 2000–2001
7	Georges Bank	41.5° N	68.5° W	42	Mid	S 2001
8	Veatch Canyon	40.2° N	69.6° W	34	Off	W 2001–2002
9	Hudson Canyon	39.5° N	72.0° W	30	Off	W 2000–2001
10	Washington Canyon	37.4° N	74.5° W	20	Off	W 2001–2002

Data analysis. Observed and expected heterozygosities were calculated for each locus, and genotypes were tested for linkage disequilibrium to determine independence of loci using FSTAT version 2.93 updated from Goudet (1995). Genotype and allele frequencies of the microsatellite loci were used to estimate variances in gene frequencies within and among sampling stations. Within subpopulations, deviations from Hardy-Weinberg equilibrium were estimated by F_{IS} values calculated with FSTAT version 2.93 (Goudet 1995). To determine the degree of genetic divergence among squids from different sampling stations, pairwise F_{ST} values were calculated (Goudet 1995). When F_{IS} values indicated that random mating could not be assumed within populations, statistical significance for F_{ST} values was estimated using an exact *G*-test by randomising genotypes among samples (for details see Goudet 1995).

Mantel matrix correlations (Mantel 1967) were used to compare geographic and genetic distance. We calculated Cavalli-Sforza & Edwards chord distances (Cavalli-Sforza & Edwards 1967) to reconstruct evolutionary relationships among our samples. This Euclidean distance was the most efficient measure out of 7 distances compared in reconstructing trees when heterozygosities were high (Takezaki & Nei 1996). Calculations of these genetic distances were performed by GENETIX (Belkhir et al. 1997). Geographic distance was defined as the straight-line distance between the centres of different collection areas.

Due to high F_{IS} values observed for most populations, we tested all loci for the probability of containing null alleles using the program MICRO-CHECKER (van Oosterhout et al. 2004). Four loci (Lfor3, Lp 2, Lp 5 and Lp 12) showed null alleles in 1 to several populations. To balance for false assignment of homozygotes, we set the second allele of such individuals to an arbitrary number (smallest allele size minus 1 repeat). That is, if the smallest allele was 103, we named the (probably) falsely assigned second allele '100' because we used trinucleotide repeat loci. This introduced a conservative bias because null alleles may consist of several size classes themselves, in which size-homoplasy tends to reduce the 'true' population divergence. We used these corrected alleles for calculating genetic divergence among populations (F_{ST} values) and for the Mantel test. Since R_{ST} values are calculated using similar alleles, they cannot be calculated after correcting for null alleles.

RESULTS

All 5 microsatellite loci were highly polymorphic, ranging from 15 to 37 different alleles per locus; loci Lp2 and Lp4 exhibited the greatest number of alleles (Table 2). No significant linkage disequilibrium was found among loci. Observed levels of heterozygosity ranged from 66 to 88%. After correction for null alleles, all populations were in Hardy-Weinberg equilibrium; F_{IS} values corrected and not corrected for null alleles are shown in Table 3. The mean F_{STcorr} value of 0.036 (uncorrected F_{ST} value = 0.039) for all loci and all 10 samples revealed significant genetic substructuring among all populations of squids (Table 4).

Each of the 4 inshore (Stns 2 to 5) and the mid-shelf (Stn 7) summer samples along the Atlantic coast of the USA (Fig. 1) were genetically distinct from one another; pairwise F_{STcorr} values ranged from 0.0115 between Georges Bank (Stn 7) and Long Island (Stn 5) to 0.052 between New Jersey (Stn 4) and Cape Cod (Stn 2) (Table 4). The overall F_{STcorr} value among all these inshore samples was 0.033. One of the summer inshore genetic stocks (Long Island, Stn 3) did not show significant differentiation from the winter offshore populations in Veatch (Stn 8) and Washington (Stn 10) canyons, as indicated by statistically non-significant F_{STcorr} values in Table 4 and by connecting lines in Fig. 1.

Table 2. *Loligo pealeii*. Number of alleles per locus and sample station; (additional MICRO-CHECKER corrected alleles (van Oosterhout et al. 2004) not included

Locus	Stn 1	Stn 2	Stn 3	Stn 4	Stn 5	Stn 6	Stn 7	Stn 8	Stn 9	Stn 10	ALL
Lfor3	12	10	14	13	11	13	14	11	11	10	18
Lp2	10	19	14	17	10	10	12	10	9	8	31
Lp4	20	14	23	24	23	20	22	20	20	15	37
Lp5	8	5	9	6	5	6	7	4	7	5	16
Lp12	7	6	5	6	7	5	6	6	5	7	15

Table 3. *Loligo pealeii*. F_{IS} values per locus and sample station before (number above) and after (number below) correction for null alleles using MICRO-CHECKER (van Oosterhout et al. 2004). Superscripted numbers next to corrected F_{IS} values indicate how many falsely assigned homozygous alleles were corrected. ⋆: indicative adjusted nominal level (5%) was 0.001; ns: non-significant; overall corrected F_{IS}: significance of all corrected F_{IS} values

Locus	Stn 1	Stn 2	Stn 3	Stn 4	Stn 5	Stn 6	Stn 7	Stn 8	Stn 9	Stn 10
Lfor3	0.216⋆	0.063	0.221⋆	0.018	0.105	0.297⋆	0.196⋆	0.483⋆	0.184	0.551⋆
	0.081^{6}	0.063	0.073^{14}	0.018	0.105	0.125^{7}	0.099^{3}	0.223^{6}	0.184	0.275
Lp2	0.394⋆	−0.036	0.073	0.043	−0.079	0.052	0.324⋆	0.066	0.123	0.114
	0.015^{16}	−0.036	0.073	0.043	−0.079	0.052	0.063^{10}	0.066	0.123	0.114
Lp4	−0.083	0.043	0.057	−0.009	0.069	−0.002	0.070	0.091	−0.034	0.309⋆
	−0.083	0.043	0.057	−0.009	0.069	−0.002	0.070	0.091	−0.034	0.212^{2}
Lp5	0.385⋆	−0.037	0.273⋆	−0.006	−0.132	0.178	0.282⋆	0.264⋆	0.287⋆	0.450⋆
	0.040^{13}	−0.037	-0.001^{22}	−0.006	−0.132	0.178	0.025^{10}	0.046^{7}	0.105^{5}	0.057
Lp12	0.165	0.094	0.120	0.158⋆	0.238⋆	0.400⋆	0.172	0.355⋆	0.069	−0.394
	0.165	0.094	0.120	0.038^{10}	0.028^{12}	-0.011^{15}	0.172	0.019^{10}	0.069	−0.394
All	0.202	0.027	0.145	0.037	0.039	0.169	0.203	0.248	0.125	0.235
	0.036	0.027	0.063	0.018	0.005	0.066	0.083	0.093	0.089	0.100
Overall corrected F_{IS}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 4. *Loligo pealeii*. Genetic substructuring among populations. Pairwise F_{ST} values (below diagonal) and geographic distances in km (above diagonal). Two F_{ST} values are shown: before (number above) and after (number below; F_{STcorr}) correction for null alleles. Numbers in **bold** indicate non-significant genetic difference. Statistical significance adjusted for multiple comparisons = 0.001

	Stn 1	Stn 2	Stn 3	Stn 4	Stn 5	Stn 6	Stn 7	Stn 8	Stn 9	Stn 10
Stn 1		478	527	772	956	2966	311	979	661	481
	0									
Stn 2	0.0974		90	316	492	2488	296	206	979	630
	0.0858	0								
Stn 3	0.0687	0.0345		246	428	2448	294	269	125	456
	0.0484	0.0384	0							
Stn 4	0.0909	0.0526	0.0208		184	2219	511	231	335	346
	0.0808	0.0520	0.0236	0						
Stn 5	0.0917	0.0392	0.0186	0.0597		2039	693	555	231	170
	0.0810	0.0377	0.0197	0.0518	0					
Stn 6	0.0947	0.0665	0.0228	0.0380	0.0575		2730	2592	2372	2086
	0.0836	0.0679	0.0250	0.0294	0.0509	0				
Stn 7	0.0712	0.0263	0.0105	0.0257	0.0293	**0.0108**		172	528	688
	0.0467	0.0296	0.0115	0.0284	0.0318	0.0185	0			
Stn 8	0.0830	0.0468	**0.0021**	0.0256	**0.0134**	**0.0310**	0.0226		231	527
	0.0663	0.0530	**0.0065**	0.0252	0.0154	0.0236	0.0260	0		
Stn 9	0.0610	0.0336	0.0135	0.0307	0.0477	**0.0102**	0.0277	0.0277		231
	0.0434	0.0356	0.0144	0.0333	0.0465	0.0188	**0.0075**	0.0314	0	
Stn 10	0.0795	**0.0137**	0.0236	0.0403	0.0361	**0.0373**	**0.0079**	**0.0203**	**0.0155**	
	0.0503	0.0217	**0.0153**	0.0369	0.0361	**0.0305**	**0.0060**	**0.0180**	**0.0078**	0

The Nova Scotia (Stn 1) samples were distinct from all other population samples, as indicated by high F_{STcorr} values between that population and all others (F_{STcorr} values ranging from 0.0434 to 0.0858; Table 4). Based on F_{STcorr} values, Gulf of Mexico samples (Stn 6) were distinct from all inshore spawning populations (Stns 2 to 5; Fig. 1), the mid-shelf Georges Bank (Stn 7) and the Nova Scotia population as well as offshore Veatch (Stn 8) and Hudson (Stn 9) canyons. However, the Gulf of Mexico samples were not distinctly different from the offshore Washington Canyon (Stn 10). The latter result might have been dependent on the small sample size of the Washington Canyon population (n = 30) because the calculated F_{STcorr} value was high (0.0305) although not significant.

A Mantel test showed no significant correlation between genetic and geographic distances among the 4 inshore summer samples (Stns 2 to 5; Fig. 1) (correla-

tion coefficient = 0.18, ns) or among all 10 sampling stations (correlation coefficient = 0.15, ns). These results demonstrated that genetic differences could not be explained by isolation by distance (Slatkin 1993).

We found null alleles in all loci and in all but one population (Stn 2) (Table 3). Non-corrected and corrected F_{ST} values are provided in Table 4; 33 out of 45 pairwise comparisons remained statistically significant after correction. On average, F_{STcorr} values were 0.004 (± 0.0089 SD) higher after correction than before. Five pairwise comparisons remained 'non-significant'; these F_{STcorr} values decreased, on average, by 0.0016 (± 0.0046 SD). Two F_{STcorr} values became non-significant after correction (Stns 3–10 and 7–9) and decreased, on average, by 0.0135 (± 0.0057 SD). Five F_{STcorr} values became significant after correction (Stns 6–7, 6–8, 6–9, 5–8, and 2–10); these F_{STcorr} values increased, on average, by 0.0027 (± 0.006).

DISCUSSION

Loligo pealeii migrates seasonally north of Cape Hatteras (North Carolina). In late autumn, squids move from their inshore spawning grounds to more thermally stable waters in canyons along the edge of the continental shelf (Summers 1983). Our microsatellite data provided strong genetic evidence for multiple stocks of *L. pealeii* along the Atlantic coast of the USA. At least 4 distinct spawning populations occur from Cape Cod to Delaware, a geographic area that spans a length and breadth of approximately 700 and 100 km, respectively, along the continental shelf. These results support the contention of Garthwaite et al. (1989) that multiple stocks exist in the *L. pealeii* fishery.

Precise molecular genetic markers such as microsatellite loci have only recently been developed for cephalopods (cf. Shaw 2003), and their application to cephalopod population structure has given mixed results. We found high levels of variability at all microsatellite loci, a trend similar to that seen in other cephalopods (Adcock et al. 1999, Reichow & Smith 1999, Shaw et al. 1999, Naud et al. 2004). This was in contrast to low levels of variability previously detected in *Loligo pealeii* with allozymes (Garthwaite et al. 1989). Genetic differentiation among population samples has been demonstrated in the Order Octopoda (*Octopus vulgaris*, Murphy et al. 2002) and Order Sepiodea (*Sepia officinalis*, Perez-Losada et al. 2002; *Sepiella maindroni*, Zheng et al. 2001). However, a population genetic study of *S. officinalis* in the Adriatic Sea revealed a panmictic population (Garoia et al. 2004), while populations in the Atlantic and Mediterranean around the Iberian peninsula were genetically differentiated (Perez-Losada et al. 2002).

In the Teuthoidea, studies of the squids *Illex argentinus* (Adcock et al. 1999), *Loligo vulgaris* (Garoia et al. 2004) and *L. opalescens* (Reichow & Smith 2001) failed to detect any genetically well-defined stocks. Based on 6 highly variable DNA microsatellite loci, Shaw et al. (2004) did not find genetic substructure in *L. gahi* on a geographic scale comparable to *L. pealeii*, but squid from the Peruvian coast were different from those off the Falkland Islands. In a similar fashion, Shaw et al. (1999) found only subtle substructure among populations of *L. forbesi* throughout most of its range, but observed more significant substructure at the isolated Azore Islands. All of the studies that did detect population structure were conducted in locations with marked oceanographic/geographic barriers to gene flow, while studies that reported homogeneity were from areas without conspicuous physical or hydrographic barriers. Our results for *Loligo pealeii* are strikingly different to those of previous studies, particularly to those conducted in areas without well-defined barriers. Several researchers have suggested that overlapping size cohorts and extended spawning periods seen in loliginids would tend to lead to genetic mixing (e.g. Macy & Brodziak 2001, Reichow & Smith 2001, Shaw et al. 2004). However, while *L. pealeii* exhibits patterns of cohort overlap and extension of spawning period similar to those of other loliginids, it nevertheless demonstrates genetic substructure. Improved information on species-specific migration and behavioral patterns — combined with data on maturity stage, size, and age — might begin to explain these differences in the genetic structure of cephalopods.

The recently developed program MICRO-CHECKER (van Oosterhout et al. 2004) indicated the presence of null alleles in all but 1 population and in up to all 5 loci (Table 3). The existence of null alleles in the same genetic markers has also been suggested by Shaw et al. (1999). We changed the homozygotes that were probably falsely assigned into heterozygotes, by introducing a new artificial smallest allele as the second allele. The null allele is likely to be an ancient primer site mutation that prevents the amplification of 1 allele during the PCR process. Over evolutionary time, the repeat-core associated with this flanking region has very likely accumulated stepwise mutations. Adding 1 artificial allele is therefore a conservative method to adjust for null alleles. One may question how the introduction of an additional allele influences the results, especially in cases where the artificial allele was only introduced in one but not in the other population. In response, we argue that because a new class of alleles (the null allele class vs. the 'visible' allele class) leads to a higher variation with which to work, the apparent F_{ST} value is actually increased. However, differences in apparent null allele frequency do reflect 'true' differences among popula-

tions caused by drift. Adding the identified null allele uncovers some of the drift effects and population differentiation. Therefore, we consider the use of F_{STcorr} values and the resulting population genetic structure as more reliable than using data uncorrected for null alleles. Looking at the changes in F_{STcorr} values in more detail, it becomes obvious that in 38 out of 45 cases the correction did not change the statistical significance of the pairwise comparisons. In 2 cases, F_{STcorr} values became statistically non-significant (Stns 3–10 and 7–9) and, in 5 cases, F_{STcorr} values became statistically significant after correction. While there is no doubt about the existence of genetically different inshore stocks, these 5 changes in- volved the connection of Gulf of Mexico to midshelf (Stn 7) and offshore populations (Stns 8 and 9), and in 3 cases inshore-to-offshore relationships (Stns 2–10 and 5–9). All of these values need to be interpreted cautiously. The non-significant F_{STcorr} value between inshore Long Island (Stn 3) and offshore Washington Canyon (Stn 10) is high (0.0305), and might be based solely on a sample size at Washington Canyon that was too small (n = 30) to detect genetic relatedness with statistical significance.

Given the lack of conspicuous physical oceanographic barriers to gene flow over the wide geographic range of *Loligo pealeii* (southern Caribbean to Nova Scotia) (Summers 1983), we did not expect to find population substructure within the southern New England/ Mid-Atlantic region. To what can we attribute this genetic differentiation? Our isolation-by-distance analysis showed no clear pattern of genetic differentiation based on geographic distances. One explanation is that animals are homing to their natal spawning grounds, a phenomenon demonstrated in some fish species (e.g. Cury 1994, Thorrold et al. 2001). An alternative explanation is that animals are travelling together in distinct spawning groups that do not show fidelity to particular sites. These groups may come together (either in mixed groups or in close proximity to each other) in offshore canyons to overwinter, and then re-form before migrating back to spawning sites in the spring. This type of migratory event would require an active behavioural process to differentiate and prefer 'own' over other subpopulations. Recognition and preference of own shoal members is well known in fish (Krause et al. 2000) but has not previously been shown in molluscs. If this type of recognition could be demonstrated, it would offer new insights into the biology of squid.

We sampled 3 offshore over-wintering stations along the shelf and found that squids at 2 offshore canyons (Stns 8 and 10, both sampled during winter of 2001–2002) did not show significant differentiation from an inshore summer population (Stn 3). We considered only the very low and non-significant F_{STcorr} value (0.0065) between Stns 3 and 8 to be a reliable indication for a connection between an inshore and an offshore sample. However, other offshore samples were genetically different from all the other inshore samples, while showing little differentiation to each other (Fig. 1b). There are several possible explanations for this phenomenon. (1) Sample sizes from the offshore canyons were small (n = 20 to 34), especially compared to some inshore samples. These small sample sizes may have produced non-significant results when comparing offshore populations. Perhaps larger sample sizes from each canyon would have revealed differentiation between offshore stations. (2) Offshore canyons represented a population that is distinct from the inshore populations. However, this does not seem likely, given the known migratory movements of *Loligo pealeii*. Instead, since most offshore canyons were differentiated from most inshore stations, this raises the question of where these offshore populations migrate in summer. It is possible that these offshore samples represent additional inshore stocks and that a finer sampling schedule would show these populations inshore in summer. Additionally, the inshore populations that we found may migrate to other winter canyons that we did not sample. It is remarkable that despite such long-distance migrations, squids apparently do not interbreed with other stocks.

The Nova Scotia samples (Stn 1) were genetically distinct from all inshore and offshore samples. Pairwise F_{ST} values were much higher between Nova Scotia and all other groups than any other pairwise samples. Oceanic currents in this area may create a barrier to gene flow and cause a high degree of separation. At the opposite geographic location sampled — the northern Gulf of Mexico — samples were distinct from all but the offshore population at Washington Canyon (Stn 10). However, these data need to be considered cautiously because the results changed after correcting for null alleles. One explanation of the non-significant difference to Washington Canyon (Stn 10) might be based on the small sample size. Alternatively, it may indicate that squid paralarvae from the Gulf of Mexico make their way during the winter to offshore areas along the Atlantic coast by travelling first in the Gulf of Mexico gyre and then entering the Gulf Stream, a phenomenon that has been observed for some fish species (R. Scheltema pers. comm.). Mariculture trials on *Loligo opalescens* indicate that squid paralarvae may stay in the plankton for up to 1 to 2 mo before they are large enough to school and to control their position in the water column (Yang et al. 1986).

Comparison of our findings with population genetic studies on fish species shows that genetically differentiated populations can arise and persist in the absence of physical barriers or great distance. In most cases, the type of genetic markers used plays an important role in

the detection of genetic substructuring. Some early reports of broad genetic uniformity based on allozymes have been contradicted by subsequent analysis using mtDNA and the most sensitive markers for small scale and short-term gene flow — DNA microsatellites (Bohonak 1999, Hellberg et al. 2002).

Microsatellites have indicated significant genetic divergence in red drum *Sciaenops ocellatus* across the northern Gulf of Mexico (mean F_{ST} = 0.003) at a scale from 150 to 2000 km (Gold & Turner 2002). The cleaner gobi *Elacatinus evelynae*, with a pelagic stage of 21 d, showed strong genetic differentiation (F_{ST} = 70%) based on the mtDNA Cytochrome *b* gene (Taylor et al. 2003). These results show that strong phylogeographic structure can develop in the Caribbean Sea between marine populations separated by as little as 23 km for species that have potential for long-distance larval dispersal. Genetic differentiation between populations of apogonids at adjacent reefs (10 to 80 km) of the Great Barrier Reef has also been found by G. Gerlach (unpubl. data) using DNA microsatellite markers. A recent study of Atlantic cod *Gadus morhua*, a species located in a similar geographic region as *Loligo pealeii*, also indicated the existence of genetically differentiated populations. A weak, but consistent, differentiation was detected (using 10 microsatellite loci) among fish from 6 capture sites that spanned a distance of 300 km (Knutsen et al. 2003). The average F_{ST} value for all microsatellite loci was small (0.0023), but was statistically highly significant, even though cod populations intermingled inshore during summer and autumn feeding migrations. Similar genetic sub-structure has been observed in other cod studies of cod (Ruzzante et al. 1996, 1998, 2000), herring *Clupea harengus* (McPherson et al. 2001), European sea bass *Dicentrarchus labrax* (Naciri et al. 1999), European hake *Merluccius merluccius* (Lundy et al. 2000), and Pacific ocean perch *Sebastes alutus* (Withler et al. 2001).

Overall, these studies (in addition to our preliminary data) demonstrate that microsatellite markers are capable of distinguishing genetic differentiation down to a scale of tens of kilometers in at least some species with limited dispersal. However, the degree of genetic differentiation that we observed in populations of *Loligo pealeii* was, on average, higher than what was found in these other studies.

The implications of population structure in *Loligo pealeii* for current fisheries management are 2-fold. (1) Separate inshore spawning groups should not be treated as a single unit stock. (2) The intensive offshore fishery may be impacting the inshore fishery as well as the genetic composition of discrete spawning stocks. If squid are travelling in discrete, related groups when migrating from offshore canyons to inshore spawning sites (whether showing spawning site fidelity or not), then targeted, intensive winter fishing in specific offshore canyons could potentially impact or even eliminate specific local summer stocks. Fishery managers must consider these potential interactions when planning for separate stock quotas. There is much to learn about the migratory and reproductive strategies of mobile loliginid squids, which play a central role in most coastal marine ecosystems.

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