Marine Biology (2002) 140: 103–115
DOI 10.1007/s002270100680

S.W. Herke · D.W. Foltz

Phylogeography of two squid (*Loligo pealei* and *L. plei*) in the Gulf of Mexico and northwestern Atlantic Ocean

Received: 8 February 2001 / Accepted: 12 July 2001 / Published online: 29 September 2001

Abstract The loliginid squids *Loligo pealei* LeSueur and *L. plei* Blaineville (both recently proposed for reclassification as *Doryteuthis*) are commercially important, similar in appearance, and sympatric throughout much of the northwestern Atlantic Ocean, the Gulf of Mexico, and the Caribbean Sea. To investigate possible cryptic speciation and population structure, we examined samples (collected from 1995 to 1997) of both species for restriction fragment length polymorphisms (RFLPs) in PCR products of the mitochondrial gene cytochrome *c* oxidase (subunit I). RFLP haplotypes were further characterized by direct sequencing. In North American waters, cryptic speciation was rejected by the far greater nucleotide sequence divergence between species (~14%) versus within species (<1%). Each species displayed about a dozen RFLP haplotypes, but only three of their respective haplotypes were found among 90% of *L. pealei* specimens ($n = 356$) and 97% of *L. plei* specimens ($n = 431$). For *L. pealei*, a genetic break existed between the northern Gulf of Mexico and the Atlantic Ocean; among sample units within each population, gene flow was consistent with panmixia. The phylogeography of *L. pealei* is likely a consequence of the eastward currents of the Florida Straits, the elevated temperatures of those surface waters, and the restriction of this species to the continental shelf. For *L. plei*, a genetic break existed between longitudes 88°W and 89°W, with the northwestern Gulf of Mexico and the northeastern Gulf–Atlantic Ocean comprising separate populations; among sample units within each population, gene flow fit an isolation-by-distance model. If the genetic break found for *L. plei* represents resident populations separated by nearshore physical parameters (e.g. effects of the Mississippi River and the sediment boundary at longitude 88°W), the lack of structure within the Gulf for *L. pealei* might be due to its distribution farther from shore. However, the two populations of *L. plei* probably represent annual recolonization from the southwestern Gulf of Mexico and from the eastern Caribbean Sea, whereas the populations of *L. pealei* probably are permanent residents within their respective regions.

Communicated by J.P. Grassle, New Brunswick

Supplementary materials: Additional documentary material has been deposited in electronic form at and can be obtained from http://dx.doi.org/10.1007/s002270100680.

S.W. Herke (✉) · D.W. Foltz
Department of Biological Sciences,
202 Life Sciences Building,
Louisiana State University, Baton Rouge,
LA 70803-1715, USA

E-mail: sherke@unix1.sncc.lsu.edu
Tel.: +1-225-5782601
Fax: +1-225-5782597

Introduction

Longfin squid (*Loligo pealei*) and arrow squid (*L. plei*) are commercially important, similar in appearance, and sympatric throughout much of the northwestern Atlantic Ocean, the Gulf of Mexico, and the Caribbean Sea (Roper et al. 1984; Sánchez et al. 1996). For both of these neritic squids, although maximum size and age at maturity vary with latitude (Cohen 1976; Arocha and Urosa 1991; Brodziak and Macy 1996), specimens from widely separated regions do not show dramatic differences in morphology. As such, each species appears to comprise a single, panmictic population. However, squid are composed mostly of soft tissues, the measurement of which is difficult to standardize among researchers. Also, squid growth patterns are highly responsive to environmental variables (Cohen 1976; Pierce et al. 1994; Shaw et al. 1999). Thus, morphological characters are often insufficient for delimiting intraspecific population structure and for differentiating species within the genus *Loligo* (Cephalopoda: suborder Myopsida). In contrast, recent allozyme and microsatellite analyses have revealed distinct multiple populations within *L. forbesi* (Brierley et al.

1995; Shaw et al. 1999), subspecies within *L. vulgaris* (Augustyn and Grant 1988), and cryptic sibling species within *Photololigo* (Yeatman and Benzie 1994). Similarly, molecular studies have questioned the validity of other cephalopod species that were originally defined by morphology. For instance, a recent mitochondrial DNA study indicated that *Octopus vulgaris*, previously believed to range throughout much of the temperate to tropical oceans, actually comprises a cryptic species complex (Söller et al. 2000). Molecular characters have also been used for higher-level phylogenetic systematics of squid. In particular, based on a recent phylogenetic analysis of the squid family Loliginidae (involving morphology, allozymes, and mitochondrial DNA sequences), all American species of *Loligo* may soon be reclassified as *Doryteuthis* (Anderson 2000a).

The coastal waters of eastern North America are inhabited by four species of *Loligo*, which are all similar in appearance. Small specimens (i.e. <10 cm mantle length) are especially difficult to identify to species; furthermore, the degree of similarity increases in regions of sympatry (Sánchez et al. 1996; Vecchione et al. 1998). To avoid misidentifications, many surveys have classified specimens only to the genus level. Confusion over the identity of captured specimens is minimized somewhat by the apparent restriction of *L. ocula* and *L. roperi* to the Caribbean Islands (Cohen 1976), but specimens taken south of Cape Hatteras could be either *L. pealei* (longfin squid) or *L. plei* (arrow squid). Longfin squid is found from about 46°N (Nova Scotia) to 10°N (Orinoco Delta, Venezuela), with a biomass center of distribution off the northeastern coast of the United States. On the other hand, the range of arrow squid extends from about 35°N (Cape Hatteras) to 35°S (northern Argentina), with a center of distribution in the Caribbean Sea (Voss et al. 1973; Cohen 1976; Roper et al. 1984; Arocha and Urosa 1991). With morphology as the only guide, the existence of cryptic species and population structure would be easy to overlook in these loliginids.

Within North American waters, the extensive ranges of the longfin squid (~5,000 km) and of the arrow squid (~3,500 km) encompass Cape Hatteras, Cape Canaveral, the Florida peninsula, and the western panhandle of Florida. These locations are boundaries at which environmental and biological factors change, partitioning the coastal marine environment into biogeographic provinces with markedly different species compositions (Briggs 1995). For species with ranges spanning more than one biogeographic province, Avise (1992, 1996) proposed that there should be concordance between recognized biogeographical boundaries and phylogeographic boundaries (i.e. abrupt geographic partitions of intraspecific genotypes). This hypothesis envisions that the factors creating biogeographic boundaries will also affect population genetic structure by natural selection or by reducing gene flow. For instance, the American oyster (*Crassostrea virginica*), the horseshoe crab (*Limulus polyphemus*), and the black sea bass (*Centropristis striata*) share a phylogeographic pattern in which populations in the Gulf of Mexico are genetically divergent from those in the northwestern Atlantic Ocean (Avise 1992, 1996). Given this concordance across phylogenetically divergent taxa, Avise proposed that shared geological events (during the Pleistocene and Pliocene epochs) were largely responsible for the observed patterns. For any particular species, our ability to detect population genetic structure (e.g. as defined by Wright's F_{st} values) emanating from those ancient events depends largely upon the life history characteristics of the organism.

Most of the known biology of longfin squid and arrow squid would predict low levels of genetic differentiation (e.g. low F_{st} values) across their respective ranges. First, they have a high dispersal potential, because the adults are capable of long migrations (O'Dor and Webber 1986) and both species have a paralarval stage (i.e. an initial free-living planktonic stage, differing in both morphology and vertical distribution from older juveniles). Second, spawning by both species occurs all along the coast in nearshore waters rather than in localized breeding areas. Third, they are demersal spawners that typically lay eggs in large communal masses, and spawning aggregations can contain hundreds of thousands of adults (Hanlon 1998). Also, spawning by both longfin and arrow squid occurs throughout most of the year, with peak spawning during summer and autumn (Summers 1983; Hanlon and Messenger 1996; Brodziak 1998). Finally, both species will attach egg masses to any hard substrate or even anchor them in sand (Vecchione 1988). Thus, potential spawning grounds for squid have always existed around the entire Florida peninsula, which could allow for gene flow between Atlantic and Gulf populations [in contrast to estuarine-dependent organisms for which contiguous nursery habitats may have been sharply reduced by low sea levels during the Pleistocene (Avise 1996)].

However, other factors suggest the potential for population structure or even cryptic speciation. In particular, by spanning multiple biogeographic provinces, the overall ranges of both species encompass several distinct habitats. Also, gene flow in the temperate longfin squid might be inhibited by the warm surface waters and strong eastward currents around the southern tip of Florida. Finally, compared to other species for which Atlantic versus Gulf distributions have been studied, cephalopods have short generation times (O'Dor and Webber 1986). Statolith-based aging techniques have verified that the maximum lifespan of a longfin squid is about 1 year (Brodziak 1998), and arrow squid are believed to have similar maximum lifespans. These short lifespans are thought to be responsible for episodic population expansions and collapses in cephalopods (O'Dor and Coelho 1993), a process that can lead to population structure if the collapses create small, isolated populations.

In other migratory, pelagic species indigenous to the northwest Atlantic Ocean and the Gulf of Mexico, the influence of the biogeographic boundaries appears to be limited. For instance, putative Atlantic and Gulf popu-

lations of king mackerel (*Scomboromorus cavalla*) and greater amberjack (*Seriola dumerili*) have extremely low F_{st} values, and mark-and-recapture data are consistent with present-day gene flow between the two populations of each species (Gold and Richardson 1998a,b). Similarly, morphological analyses suggest that longfin and arrow squid are continuously distributed throughout their ranges, although longfin squid may be uncommon along the peninsular coast of Florida (Cohen 1976). Nevertheless, as noted above, there may be reason to expect population structure within both species. Further, one or both nominal species might harbor cryptic sibling species that are parapatrically distributed across a biogeographic boundary. Hence, the following null hypotheses were defined for the present study covering the northwestern Atlantic Ocean and the northern Gulf of Mexico.

- H_0-1: Neither nominal species is composed of cryptic species within the study area.
- H_0-2: Gene flow within both species is consistent with a model of panmixia (i.e. $F_{st} = 0$ across the study area).
- H_0-3: Population structure differing from panmixia is concordant with the classic Gulf of Mexico–Atlantic Ocean phylogeographic pattern observed for other marine taxa in the region.

Preliminary surveys indicated that these two species harbored sufficient polymorphisms in the mitochondrial gene CO-I (cytochrome *c* oxidase, subunit I) to permit population-level studies. Nucleotide variation was detected primarily by endonuclease digestion of PCR products, although DNA sequences were ascertained for representatives of each restriction haplotype. Population structure was determined by geographically associated changes in the frequencies of restriction haplotypes, and the cryptic species hypothesis was evaluated by comparing CO-I nucleotide divergences between and within the described species. Finally, the observed phylogeographic patterns are discussed in terms of oceanographic features as well as the life history characteristics and ranges of both species.

Materials and methods

Details of capture sites and processing of *Loligo pealei* LeSueur and *L. plei* Blainville samples are available as electronic supplementary material at http://dx.doi.org/10.1007/s002270100680. From 1995 to 1997, specimens were collected by otter trawls (bottom-water) from most of the range of each species within North American waters (see "Results" for details). Specimens supplied by the National Marine Fisheries Service and by the South Carolina Department of Natural Resources were from fishery surveys that used randomized block designs to determine trawl stations, whereas specimens supplied by commercial fishermen were bycatch from the Dry Tortugas pink shrimp fishery. Mantle lengths for longfin (*L. pealei*) specimens ($n = 356$) ranged from 35 to 460 mm (mean ± SD: 153 ± 63 mm); lengths for arrow squid (*L. plei*) specimens ($n = 431$) ranged from 15 to 275 mm (89 ± 47 mm). Species identifications were validated by the inclusion of specimens collected from the northern Gulf of Mexico during 1993 and identified by Sánchez et al. (1996). Genomic DNA was extracted from 10 to 20 mg of frozen mantle tissue by a phenol-chloroform procedure (Herke 1999), ethanol-precipitated, dried, and resuspended in 50 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). A 709-bp fragment of the mitochondrial CO-I gene was amplified by PCR with primers designed by Folmer et al. (1994). The 50 µl reactions contained 180 µM each dNTP, 1.9 mM $MgCl_2$, 15 pmol of each primer, 1× rec-*Tbr* buffer, 1 U rec-*Tbr* polymerase (Amresco, Solon, Ohio), and 1.0 µl of DNA extract. After a 5 min hot-start (90°C), samples underwent 43 cycles of 95°C (30 s), 50°C (30 s), and 72°C (30 s).

RFLP (restriction fragment length polymorphism) analyses were the primary means of detecting nucleotide variation in the PCR products. Restriction digests were done according to the manufacturer's protocols, and fragments were separated in either 2% or 3% agarose 3:1 gels (Amresco) stained with ethidium bromide. Following a preliminary survey with 23 restriction enzymes (Herke 1999), promising endonucleases were used on at least 40 samples to check for polymorphic restriction sites. For each species, we then chose enzymes that generated unambiguous, intraspecific RFLPs: *Bst*NI, *Hae*III, and *Hin*fI for longfin squid; *Ase*I and *Hsp*92II for arrow squid. To ensure accurate species identifications, all samples were also digested with *Msp*I, which had highly conserved digest patterns (two restriction sites in longfin squid and one site in arrow squid). Digestion profiles of all enzymes were combined such that each specimen was assigned to a composite PCR-RFLP haplotype.

Representatives of each haplotype were sequenced in both directions ($n = 26$ longfin squid; $n = 33$ arrow squid). For haplotypes represented by more than one specimen, PCR products from at least two squid were sequenced, and the first two specimens for analysis were selected from the most geographically separated sample sites. PCR products were purified with QIAquick columns (QIAGEN, Valencia, Calif.), and sequencing reactions were performed with the ABI-Prism Dye-Terminator kit as previously described (Herke 1999). Labeled extension products were analyzed with an automated DNA sequencer (Applied BioSystems model 377A) at the Museum of Natural Science, Louisiana State University. CO-I sequences were compared in Sequencher 4.0 (Gene Codes, Ann Arbor, Mich.), trimmed to 658 bp (by excision of primer locations), and aligned in CLUSTAL W ver. 1.7 (Thompson et al. 1994). Transition/transversion ratios and percent sequence divergences were calculated in MEGA ver. 1.01 (Kumar et al. 1993). For each species, graphs of minimum-spanning networks were produced from calculations by the program MINSPNET (Excoffier 1993), which used uncorrected distance matrices calculated in MEGA.

Specimens for molecular analysis were selected from a wide array of the available trawl stations. After specimens within each species were grouped by capture locations to the nearest 0.5° of latitude and longitude, they were clustered into seven sample units within the Gulf of Mexico and seven units within the Atlantic Ocean. For each species, the haplotype frequencies across the sampling range were analyzed visually by bar graphs for apparent genetic breaks; this analysis indicated two populations for longfin squid (Gulf of Mexico versus Atlantic Ocean) and two populations for arrow squid (west versus east of the Mississippi River). For arrow squid (the more inshore species), sampling was intensified near Mobile Bay to better define the apparent genetic break, and haplotype frequencies were tested for homogeneity across relevant sample units by a χ^2-test. Subsequent statistical analysis was done by the program AMOVA (analysis of molecular variance in Arlequin 1.1; Schneider et al. 1997). Statistical significance in AMOVA is calculated as a nonparametric permutation test, even though the data are actually randomly recombined at each level of the analysis (i.e. it is a combinatorial test). Simulation analyses by Herke (1999) showed that the statistical power of AMOVA is limited when only two major populations (i.e. groups) are postulated, unless the two groups contain many sample units. To allow for uncorrected significance values of $P < 0.01$ in AMOVA, each species was redivided into five larger sample units per putative population {[10!÷(5!×5!)] = 252 paired combinations of five sample units, of which symmetry considerations rendered only 126 unique combinations}. The divisions were based primarily on maintaining similar sample sizes within units and partially on the presence of major

geographic features (e.g. large rivers, bays, and undersea canyons). For all analyses, corrected table-wide significance values for each species were subsequently calculated through a sequential Bonferroni procedure (Rice 1989).

Gene flow patterns within each species were based on all of the original haplotypes (no collapsing into composite haplotypes) and illustrated through neighbor-joining trees of the coancestry coefficients for sample units. However, for both bar graph and AMOVA analyses, multiple haplotypes within each species obscured rather than clarified population structure. Appropriate reductions in the number of haplotypes were based on preliminary bar graphs and the minimum-spanning networks. For bar graphs, the rarest haplotypes were grouped with the most common member of their cluster, and low-frequency haplotypes were aggregated with those displaying the same distributional pattern. In AMOVA analyses, statistical power was increased by assigning all specimens within a cluster to a single haplotype (i.e. haplotypes A and B in longfin squid; haplotypes A, B, and C in arrow squid). Such reductions are useful in AMOVA analyses because, even for populations without haplotypes in common, the apparent degree of differentiation is reduced by the existence of numerous haplotypes per population (Charlesworth 1998; Herke 1999). Typically, AMOVA fixation indices are reported as Φ_{st} values because the genetic distances among haplotypes are considered. Our analysis used only two composite haplotypes for longfin squid and distances were extremely small among the three composite haplotypes of arrow squid, so fixation indices are reported as standard F_{st} values.

Results

All restriction enzyme digests produced fragments that summed to the size of the PCR product from the mtDNA CO-I gene. Further, for *Loligo pealei* and *L. plei*, the RFLP analyses revealed three common haplotypes (A, B, and C), along with many rare haplotypes (Table 1).

Sequence comparisons

Unique CO-I sequences have been deposited in GenBank for 21 *L. pealei* (accession nos. AF207909–207926 and AY039621–039623) and 23 *L. plei* (accession nos. AF207927–207947 and AY039619–039620). Within the two consensus sequences (658 bp, excluding primer positions) that were generated from the common haplotypes, there were 105 variable sites between longfin and arrow squid: 81 sites with fixed differences and 24 sites involving polymorphisms within one or both species (Fig. 1). One interspecific difference at a first codon position resulted in an inferred amino acid replacement (leucine for methionine); all other differences were silent substitutions in the first or third codon positions. Uncorrected nucleotide divergence between longfin squid and arrow squid ranged from 13.7% to 15.0%, and the interspecific transition/transversion ratio ranged from 1.49 to 1.72.

For longfin squid, all nucleotide differences were silent substitutions in the third codon position. Among all haplotypes, there were 20 transitions and 4 transversions at the 21 variable sites; the RFLP analysis detected nearly 40% of the variable nucleotides detected by sequencing (Table 2). The minimum-spanning network documented two clusters that centered on the numerically dominant haplotypes A or B (Fig. 2). Nucleotide divergence between clusters was about 1%; within clusters, most haplotypes differed from their nearest neighbor by a single transition (~0.15% sequence divergence).

For arrow squid, all nucleotide differences were silent substitutions in the first or third codon positions. Among all haplotypes, there were 22 transitions and no transversions at the 22 variable sites; the RFLP analysis detected over 35% of the variable nucleotide sites (Table 2). The minimum-spanning network depicted three closely related clusters centered on haplotypes A, B, and C (Fig. 2). Nucleotide divergence between haplotypes was generally <0.5%.

For both species, most haplotypes were present in the Atlantic Ocean as well as in the Gulf of Mexico.

Table 1 *Loligo pealei*, *L. plei*. Frequencies of PCR-RFLP haplotypes for both species, based on composite restriction digests of a 709-bp fragment of the mtDNA CO-I gene. For each enzyme, the presence (+) and absence (–) of restriction sites is indicated; sites are listed in the same order as in Fig. 1

Loligo pealei ($n=356$)						*Loligo plei* ($n=431$)				
Haplotype		Restriction enzymes				Haplotype		Restriction enzymes		
Name	(%)	*Bst*NI	*Hae*III	*Hin*fI	*Msp*I	Name	(%)	*Ase*I	*Hsp*92II	*Msp*I
A	69.9	+ – –	+ + +	–	+ +	A	53.9	– + –	– +	– + –
B	14.0	+ – –	+ + +	+	+ +	B	21.3	– + –	– –	– + –
C	6.2	+ – –	+ – +	–	– +	C	22.0	– + +	– +	– + –
D	2.5	+ – +	+ + +	–	+ +	D	0.5	– – –	– +	– + –
E	2.5	+ + +	+ + +	–	+ +	E	0.2	– + –	+ +	– + –
F	2.0	+ – –	+ – +	–	+ +	F	0.5	– + –	– +	+ + –
G	0.6	+ – –	+ + –	–	+ +	G	0.2	+ + –	– –	– + –
H	0.8	+ – –	+ – +	+	+ +	H	0.5	– + +	– –	– + –
I	0.3	+ – –	– + +	+	+ +	I	0.5	+ + –	– +	– + –
J	0.3	+ – –	+ + –	+	+ +	J	0.2	– + +	– +	– + +
K	0.3	+ – +	+ + +	+	+ +	K	0.2	+ + +	– +	– + –
L	0.3	+ + –	+ + +	–	+ +	Sum	100.0			
M	0.3	+ – –	+ + +	–	+ –					
Sum	100.0									

Fig. 1 *Loligo pealei*, *L. plei*. Consensus nucleotide sequences and restriction sites found among the common PCR-RFLP haplotypes in the mtDNA gene CO-I. *Dashes* represent bases of the 25-mer L-COI primer (Folmer et al. 1994); *dots* represent nucleotide identity with *L. pealei*; and, IUB-IUPAC degenerate codes represent intraspecific nucleotide variations. A *box* indicates the one site of an inferred amino acid replacement (leucine for methionine). Recognition sequences for *L. pealei* endonucleases (*above data*) are CCWGG (*Bst*NI), GGCC (*Hae*III), GANTC (*Hin*fI), and CCGG (*Msp*I). Recognition sequences for *L. plei* endonucleases (*below data*) are ATTAAT (*Ase*I), CATG (*Hsp*92II), and CCGG (*Msp*I). Endonuclease designations *enclosed by parentheses* indicate restriction sites found only in less common haplotypes (see Table 1)

Restriction sites, which previously had been inferred from digest patterns, were confirmed by directly sequencing PCR products from representative individuals (Table 2). Multiple individuals within each PCR-RFLP haplotype usually showed at least one nucleotide difference, and, in several cases, the shared absence of a restriction site was homoplasious (i.e. the restriction sequence was altered either at different positions or with different bases). As a result, three rare PCR-RFLP haplotypes were homoplasious (Fig. 2): haplotype G in longfin squid and haplotypes D and H in arrow squid.

Phylogeography

For both species, the bar graphs revealed a distinct shift in haplotype frequencies approximately in the middle of their respective ranges off North America. The shift for longfin squid was between units 5 and 6 at the Florida peninsula, reflecting one population in the Atlantic Ocean and one in the Gulf of Mexico (Fig. 3). In contrast, the shift for arrow squid was near the Mississippi River (Fig. 4), forming a western population (i.e. northwestern Gulf of Mexico) and an eastern population (i.e. northeastern Gulf of Mexico and Atlantic Ocean). Within populations, coancestry coefficients showed that gene flow among longfin squid sample units was consistent with panmixia, while gene flow among arrow squid sample units fit an isolation-by-distance model (Fig. 5). The AMOVAs supported the population structuring noted above for both species. Within each population, there was no discernible genetic structure for either longfin squid ($P > 0.52$) or for arrow squid ($P > 0.29$). Between populations, both species had significant F_{st} values ($P < 0.008$; AMOVA permutation value): $F_{st} = 0.112$ for longfin squid and $F_{st} = 0.295$ for arrow squid. F_{st} values remained significant for both species ($P < 0.02$) even after being adjusted for multiple tests (i.e. three hierarchical levels within each species).

Atlantic samples of longfin squid were differentiated from Gulf of Mexico samples primarily by the frequencies of the less common haplotypes. For instance, nearly 80% of haplotype B occurred in the Gulf of Mexico, while about 90% of the haplotypes C, E, and F occurred in the Atlantic Ocean. The strength and exact placement of the phylogeographic break around the

Table 2 *Loligo pealei*, *L. plei*. Variable nucleotide sites in mtDNA sequences (cytochrome *c* oxidase, subunit I; see Fig. 1) representing PCR-RFLP haplotypes of two species [*subscripts* identify multiple representatives within an RFLP haplotype (see Fig. 2); *dots* denote identity with first sequence; *asterisks* indicate that the nucleotide site polymorphism created a restriction site polymorphism detectable by one or more enzymes (Table 1)]

Haplotype	Nucleotide position (*Loligo pealei*)																					
	4 8	1 2	1 0	1 6	2 0	2 1	2 6	2 7	3 9	3 1	3 2	3 5	3 6	3 7	3 7	3 9	4 1	5 2	5 3	5 4	5 9	6 7	6 0
A_1	T	C	T	T	G	T	G	A	G	C	T	C	C	G	A	A	T	C	C	T	T	
A_2	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
B_1	·	T	·	·	·	C	A	·	·	·	·	·	·	·	C	·	·	T	·	·	C	
B_2	·	T	·	·	·	C	A	·	·	·	C	·	·	·	C	·	·	T	·	·	C	
B_3	·	T	·	·	·	C	A	·	·	·	·	T	·	·	C	·	·	T	·	·	·	
B_4	·	T	·	C	·	C	A	·	·	·	·	·	·	·	C	·	·	T	·	·	·	
B_5	·	T	·	·	·	C	A	G	·	·	·	·	·	·	C	·	·	T	·	·	·	
	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	
C																						
D	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	
E	·	·	·	·	·	·	T	·	·	·	·	·	·	·	G	G	·	·	·	·	·	
F	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	
G_1	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	
G_2	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	G	·	·	
H_1	·	T	·	·	·	C	A	·	A	·	·	·	·	·	C	·	·	T	·	·	C	
H_2	C	T	·	·	·	C	A	·	A	·	·	·	·	·	C	·	·	T	·	·	C	
H_3	·	T	·	·	·	C	A	·	A	·	·	·	·	·	C	·	·	T	·	C	C	
I	·	T	·	·	A	C	A	·	·	·	·	·	·	·	C	·	C	T	·	·	·	
J	·	T	·	·	·	C	A	·	·	·	·	·	·	A	C	·	·	T	·	·	C	
K	·	T	·	·	·	C	A	·	·	·	·	·	·	·	C	G	·	T	·	·	·	
L	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	
M	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	
					*	*			*	*			*	*	*	*						

Haplotype	Nucleotide position (*Loligo plei*)																																	
	5 7	1 2	1 3	2 8	2 3	2 6 4	2 8	3 6	4 0	4 1	4 3	4 3	4 4	4 5	4 5	4 6	4 7	4 8	4 8	5 0	5 1	5 8	6 0
A_1	G	A	C	A	A	T	C	A	T	G	A	T	G	C	G	G	T	G	A	G	G	C
A_2	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·
A_3	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
B_1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·
B_2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	A	·	·
B_3	·	·	·	·	·	·	·	·	·	·	·	·	A	·	A	·	C	·	·	·	·	·
B_4	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	A	·
B_5	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
C_1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·
C_2	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	A	·	·	·	·
C_3	·	·	·	·	·	·	·	·	·	·	G	·	A	·	·	·	·	A	·	·	·	·
C_4	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	A	G	·	·	·
D_1	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·
D_2	·	G	·	·	·	·	·	·	·	A	·	C	·	·	·	·	·	·	·	·	·	T
E	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
F_1	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
F_2	·	·	·	G	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
G	A	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	A	·	·	·
H_1	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	A	·	·	·	·	·
H_2	·	·	·	·	·	·	·	·	·	·	·	A	T	·	·	·	A	·	·	·	·	·
I_1	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
I_2	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
J	·	·	·	·	G	·	·	·	C	·	G	·	·	·	·	A	·	A	·	·	·	·
K	A	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	A	·	·	·	·
	*		*	*					*			*		*	*			*				

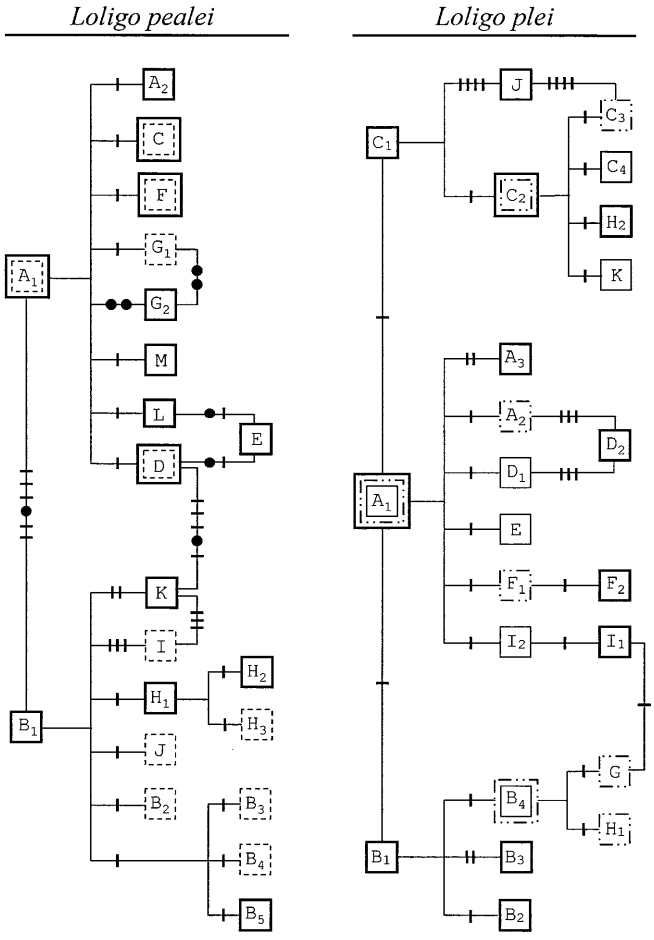

Fig. 2 *Loligo pealei*, *L. plei*. Minimum-spanning networks of CO-I nucleotide sequences of *L. pealei* (haplotypes A–M) and *L. plei* (A–K). *Each square* represents a DNA sequence as defined in Table 2; *concentric squares* indicate sequences found in squid from multiple geographic locations: northwestern Atlantic Ocean (*thick unbroken line*); northern Gulf of Mexico (*dashed line*); Gulf of Mexico, east of the Mississippi River (*thin unbroken line*); and, Gulf of Mexico, west of the river (*dot-dashed line*); *cross-bars* indicate inferred transitions; *solid circles* indicate inferred transversions. Haplotypes A and B within *L. pealei* and haplotypes A, B, and C within *L. plei* are best candidates for most recent common ancestor within each cluster; these haplotypes are geographically widespread and show the most connections to other haplotypes (Crandall and Templeton 1993). See Table 1 for definitions of haplotype symbols

Florida peninsula was not determined because few longfin squid specimens were available from that area. In contrast to longfin squid, arrow squid exhibited an abrupt phylogeographic break within the Gulf of Mexico between longitudes 88°W and 89°W. West of the Mississippi River, the average frequency of haplotype A was about 30% across all sample units, whereas haplotype B slowly decreased from 70% near Mexico to 40% at the river and haplotype C gradually increased from 0% to 25% at the river. East of the Mississippi River to Cape Hatteras, haplotype B was virtually absent from all sample units; average haplotype frequencies were approximately 70% A, 25% C, and 5% rare types, except for sample unit 6 in which about 10% (nine squid) of the specimens were haplotype B.

Within sample unit 6 for arrow squid, the initial haplotype frequencies were indicative of a sharp phylogeographic break along the gulf coasts of Alabama and Florida. Haplotype C was predominantly found in the eastern half of the unit, and haplotype B was almost entirely restricted to the western half. Further, the three representative DNA sequences of haplotype B (i.e. from 30°N; 88°W; south of Mobile Bay, Alabama) were identical to those of three haplotype B specimens captured in the western sample units 1, 3, and 5 (all three Atlantic Ocean specimens with this haplotype had unique DNA sequences). To pinpoint the genetic break within sample unit 6, a total of 86 squid were drawn equally from two subunits (Fig. 4): 6-West with five trawl stations south of Mobile Bay and 6-East with four trawl stations south of Pensacola Bay. All of these stations had been sampled between 6 and 10 November 1995, and similar haplotype frequencies were found at stations within subunits. Even so, the haplotype frequencies were significantly different between 6-West and 6-East, between 6-West and the western sample unit 5, and between 6-East and the eastern sample unit 7 (Fig. 6). These comparisons suggested that the region straddled by 6-West and 6-East was either a mixing zone between two populations or possibly a secondary genetic breakpoint. We chose to place all of sample unit 6 within the eastern population because its composite haplotype frequencies were significantly different from those of the western sample unit 5, but were not significantly different from those of the eastern sample unit 7. The Bonferroni corrections for multiple tests ($P < 0.05$) did not eliminate any of the significant differences above, except for the comparison of the 6-East subunit with the eastern sample unit 7 (which was reduced to nearly significant).

Discussion

Anderson (2000b) used CO-I as part of his phylogenetic analysis of the squid family Loliginidae. Based on CO-I sequences that he deposited in GenBank, the average uncorrected divergence in CO-I between species currently classified as *Loligo* is about 18% (range: 11–22%). The single exception in Anderson's data was the roughly 6% sequence divergence between two nominal species (*L. vulgaris* and *L. reynaudii*), which Augustyn and Grant (1988) had previously concluded were of subspecific status (based on morphological, meristic, and allozymic characters). In the present study, the minimal divergence (0.15–1.4%) of intraspecific sequences for CO-I indicates that neither longfin nor arrow squid harbor cryptic species within North American waters. The CO-I sequence data also imply recent origins for the common RFLP haplotypes within each species and a deep evolutionary split between the two species. Despite the large divergence between interspecific DNA sequences, longfin squid were distinguished from arrow squid by only one predicted amino acid replacement.

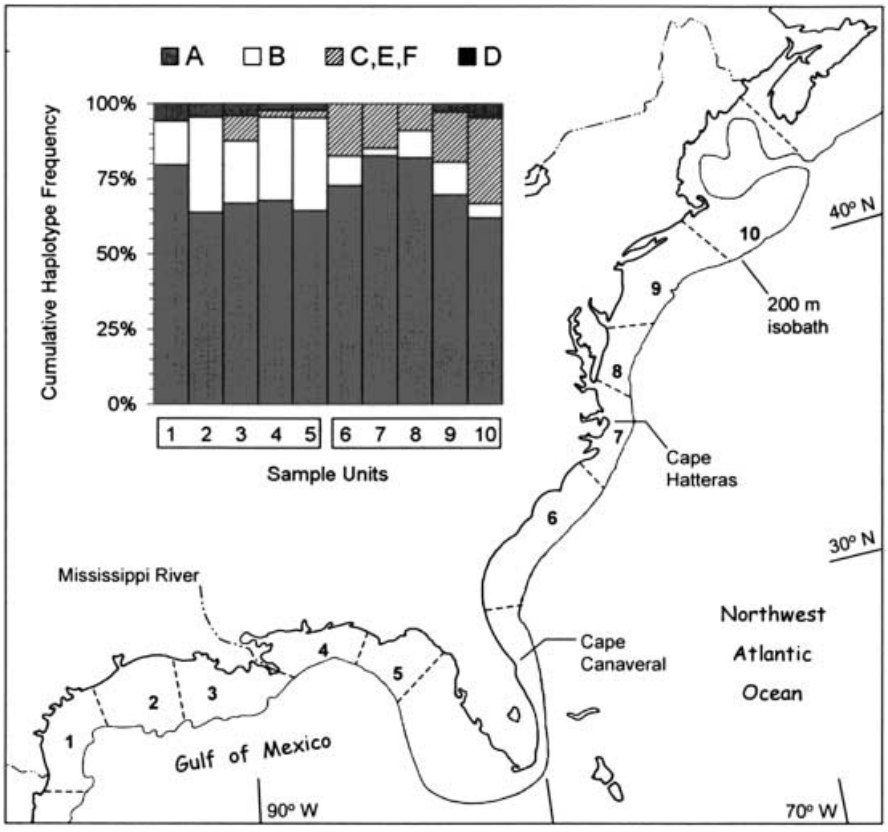

Fig. 3 *Loligo pealei*. PCR-RFLP haplotype frequencies among sample units for the mtDNA CO-I gene. Sample units are separated by *dashed lines* on the map, with the number of specimens for units 1–10 (respectively) being 34, 22, 24, 43, 42, 40, 40, 33, 36, and 42. For each unit, its identification number marks the mean latitude and longitude of all trawl stations (weighted by the number of specimens per station). The bar graph combines rare haplotypes (10 individuals) with either haplotype A (G, L, M) or haplotype B (H–K). *L. pealei* is reported to have a continuous distribution around the Florida peninsula (Roper et al. 1984); however, between units 5 and 6, we could not find sources for specimens

Such low protein-level divergence (0.45%) is in keeping with the strong evolutionary constraints on the function of the CO-I protein as well as with the 0.98% mean amino acid divergence found in the sister suborder Oegopsida (Carlini and Graves 1999).

Assuming a constant rate of CO-I divergence, the common haplotypes of longfin squid appear to be older than those of arrow squid by nearly an order of magnitude. This could be an artifact of sampling which included the primary range of the temperate longfin squid, but only the peripheral northern range of the tropical arrow squid. In any event, all intraspecific haplotypes within the study area are recently derived in comparison to the species lineages. Such shallow population histories embedded in deep evolutionary lineages are common for marine taxa (Billington and Hebert 1991; Grant et al. 1998; Graves 1998). Explanations for this phenomenon range from demographic events (e.g. bottlenecks, regional extinctions, and secondary contact) to stochastic loss of female mtDNA lineages (accelerated by fluctuations in abundance and variance in reproductive success). For squid, O'Dor and Coelho (1993) argued that the biological instabilities of their life cycle inevitably lead to cyclic population collapses, which implies a role for all of the above mechanisms in reducing haplotype diversity within squid species.

For both longfin squid and arrow squid, the data contradict the null hypothesis of genetic homogeneity (i.e. F_{st} was not zero) within North American waters. Yet, although both species were composed of two populations, they had different phylogeographic patterns. Longfin squid displayed the classic pattern of Gulf and Atlantic populations (reviewed by Avise 1996). On the other hand, arrow squid populations diverged between longitudes 88°W and 89°W, with the eastern population encompassing nearly half the northern Gulf of Mexico as well as the Atlantic. Oceanographic features are often cited to explain phylogeographic patterns of marine fauna, but there are only three obvious possibilities relevant to the observed genetic breaks. First, the Florida peninsula and the strong easterly currents of the Florida Straits have been implicated in structuring populations of other marine species. Second, the generally westward flow of the Louisiana Coastal Current entrains freshwater (discharged by the Mississippi River) over the inner- and mid-continental shelf off Louisiana, reducing surface water salinities to <25–30‰ (Rabalais et al. 1999). Both longfin and arrow squid are typically found in higher salinities, and are seldom found at the discharge point of major rivers (Voss and Brakoniecki 1985), although laboratory studies show both species can survive for several days at salinities <30‰ (e.g. Hanlon et al. 1983). In addition, since at least 1985, excess nutrients carried westward from the Mississippi River have substantially increased phytoplankton blooms, creating widespread hypoxia in the lower 20–50% of the water column between the 5 and 30 m isobaths (60 m maximum). From about mid-May to mid-September of most years, severe hypoxia (dissolved O_2 at <2 mg l^{-1}) occurred over about 7,000–20,000 km^2

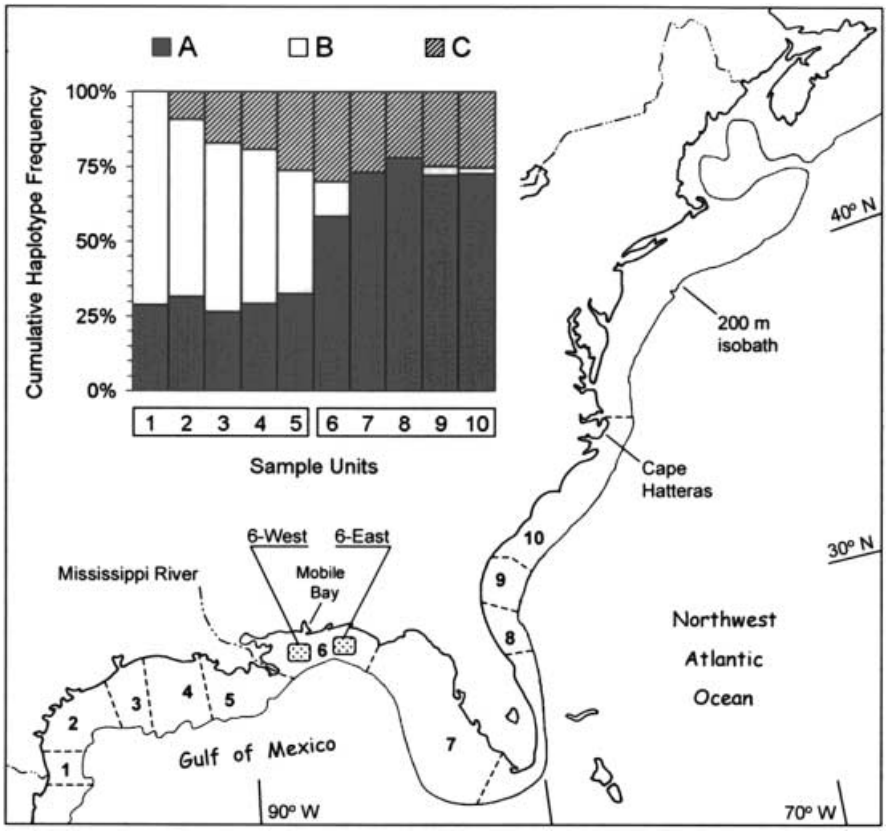

Fig. 4 *Loligo plei*. PCR-RFLP haplotype frequencies among sample units for the mtDNA CO-I gene. Sample units are separated by *dashed lines* on the map, with the number of specimens for units 1–10 (respectively) being 28, 32, 23, 31, 34, 86, 37, 45, 68, and 47. For each unit, its identification number marks the mean latitude and longitude of all trawl stations (weighted by the number of specimens per station). The bar graph collapses rare haplotypes (12 individuals) into their respective clusters (as shown in Fig. 2). *L. plei* is reported to have a continuous distribution around the Florida peninsula (Roper et al. 1984); however, between units 7 and 8, we could not find sources for specimens

of the Louisiana continental shelf, and trawlers were unable to capture shrimp or demersal fish within that zone (Rabalais and Turner 2001). Finally, longitude 88°W marks the boundary between the mud and carbonate sediments of the northern Gulf of Mexico, which could be relevant to these squid given their demersal daytime behavior. Longfin and arrow squid differ in their overall ranges as well as in their responses to temperature and salinity; thus, each factor may have acted differentially on the two species, leading to the observed differences in phylogeographic patterns.

The Florida peninsula provides a natural breakpoint in the population structure of longfin squid, which ranges over about 5,000 km of the continental shelf off eastern North America (as well as about 6,000 km off Central and South America). This species remains over the continental shelf, rarely being found even at oceanic islands close to the continents (Cohen 1976); hence, ever since the closure of the Suwannee Straits across northern Florida at least 1.75 million years ago (Bert 1986), the only migration route between the Gulf and Atlantic populations of longfin squid has been around southern Florida. Water flow in the Straits of Florida is primarily eastward along a narrow continental shelf, and temperatures typically exceed 24°C in the surface waters (being derived from the South Atlantic). The longfin squid normally inhabits waters with temperatures between 9°C and 22°C, and its response to temperature is strong enough to reverse its seasonal inshore–offshore migrations north of Cape Hatteras versus south of the Cape and off Venezuela (Whitaker 1978; Summers 1983; Arocha and Urosa 1991; Costa and da Costa Fernandez 1993). Although suitable temperatures for longfin squid exist along the narrow band of continental shelf in the Straits of Florida between 100 and 400 m deep (the maximum depth recorded for this species), currents there range from 20 to 50 cm s^{-1}, sometimes even exceeding 100 cm s^{-1} (Lee et al. 1994). Such currents would prevent passive dispersal of paralarvae from Atlantic longfin squid into the Gulf of Mexico, and possibly limit migration by adults. The present data are consistent with gene flow between the Gulf and Atlantic populations being primarily eastward through the Straits. For instance, haplotypes C and E were essentially restricted to the Atlantic and yet haplotype B, even though more common in the Gulf, occurred at appreciable frequency throughout the Atlantic.

We found no evidence of population structure for longfin squid in the Atlantic, despite the presence of Cape Hatteras (a biogeographic boundary) within our sample range. This finding contrasted with that of Garthwaite et al. (1989), who postulated three populations between Cape Hatteras and Cape Cod (36°N–42°N). However, their conclusion was largely based on genetic observations at a single allozyme locus (*Pgm*), and they admitted that the postulated populations were not likely to be geographically stable over time. The lack of an effect on population structure by Cape Hatteras and the migratory abilities of longfin squid are inconsistent with this species having a phylogeographic

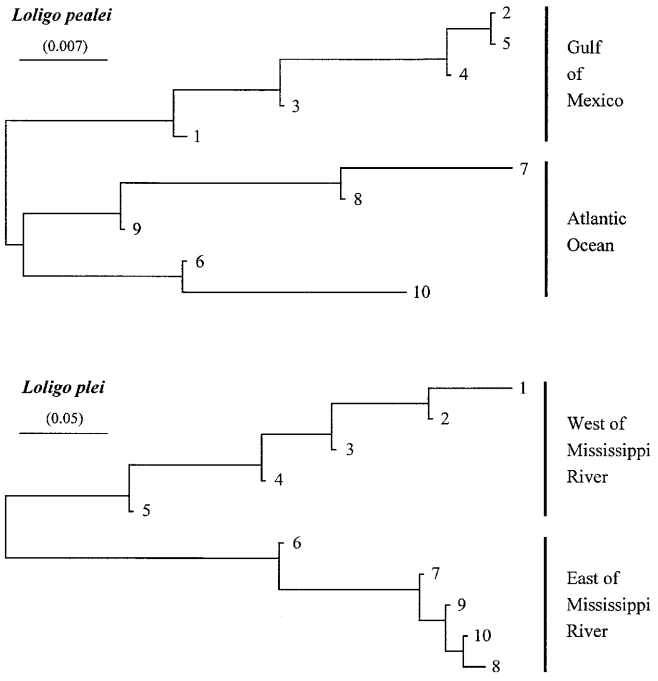

Fig. 5 *Loligo pealei*, *L. plei*. Neighbor-joining trees inferred from the matrices of coancestry coefficients for sample units. Coefficients for each species were calculated in AMOVA without collapsing the original haplotypes for the mtDNA CO-I gene into composite PCR-RFLP haplotypes. Scale bars represent genetic distances as defined by Reynolds et al. (1983): $D = -\ln(1-F_{st})$. Sample units are numbered as in Figs. 3 and 4

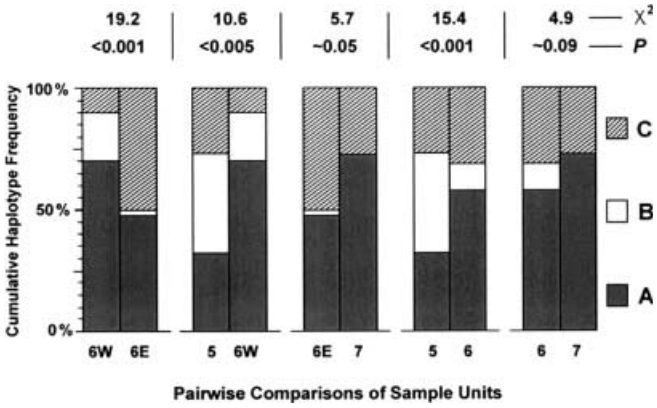

Fig. 6 *Loligo plei*. Chi-squared tests for homogeneity of PCR-RFLP haplotype frequencies across sample units in the northern Gulf of Mexico immediately east and west of longitude 88°W. Pairwise comparisons involved sample unit 5 ($n = 34$), unit 6 ($n = 86$), unit 7 ($n = 37$), subunit 6-West ($n = 40$), and subunit 6-East ($n = 46$). See Fig. 4 for locations of sample units and subunits

boundary at Cape Canaveral (immediately south of our Atlantic samples; Fig. 3). Thus, although samples from both coasts of peninsular Florida are required to confirm the boundary, present data implicate the southern tip of Florida as the phylogeographic break for longfin squid. This conclusion is consistent with Cohen's (1976) morphological analysis in which she split the species into Gulf versus Atlantic populations.

For arrow squid, explaining the phylogeographic pattern in the context of resident populations is more problematic. Between 88°W and 89°W in the northern Gulf of Mexico, there are only two obvious physical features with relevance to population structure in arrow squid. First, the mouth of the Mississippi River is located at 89°W, and the significant shifts in overall RFLP haplotype frequencies occurred there (Fig. 4). In particular, the dominant haplotype B of the western population was virtually absent in the eastern population. Second, there are carbonate sediments to the east and organic-laden mud sediments to the west of longitude 88°W (Wilhelm and Ewing 1972). The significant shift in haplotype frequencies within sample unit 6 occurred there (Fig. 6), and nearly all of the eastern haplotype B specimens were captured south of Mobile Bay (i.e. at 88°W). The coincidence of the Mississippi River and the sediment boundary with apparent genetic breaks is striking, but these features are unlikely to have caused the differences between western and eastern populations over evolutionary time scales.

Because the arrow squid inhabits shallow, warm waters with salinities >30‰ (Hixon et al. 1980), the Mississippi River affects its distribution by generating hypoxia in bottom waters and by reducing the salinity of surface waters. But, widespread hypoxia in the northern Gulf of Mexico has occurred for less than a century (Rabalais et al. 1999; Rabalais and Turner 2001), making it an unlikely candidate for having established separate populations. Even in a contemporary sense, hypoxia does not present an insurmountable barrier between the western and eastern arrow squid populations. For instance, in July 1995, although hypoxia extended across sample units 4 and 5 (covering ~17,000 km^2 off the coast of Louisiana), we captured specimens seaward of the hypoxic zone from Texas to the Mississippi River. By contrast, salinity reductions near the Mississippi River are a more long-term feature of the Gulf of Mexico, yet currents should eventually have swept paralarvae both west and east of the river. Also, river flows fall dramatically during dry years and periods of dry climate, so the Mississippi River discharge can only be an intermittent barrier to adult arrow squid. Finally, the sediment boundary has been present long enough to affect the evolution of arrow squid, and Bert (1986) deemed it relevant to a speciation event in the stone crab *Menippe mercenaria*, which is a permanent bottom-dweller after a short period as a pelagic larva. The arrow squid is also demersal during the day, and it can hide from predators by taking on the color and texture of the sediments. Nonetheless, given the large repertoire of colors and patterning behavior exhibited by this species (Hanlon and Messenger 1996), it is unclear how a sediment change would create population structure in arrow squid.

An alternate interpretation of the observed haplotype frequencies is that, rather than a true phylogeographic break between resident arrow squid populations, we detected genetic structure emanating from

more southern populations of this tropical squid. In this context, the sharp genetic break may simply represent the farthest eastward migration by squid from the southwestern Gulf of Mexico. North American waters constitute only about 15% (~3,500 km) of the range of arrow squid, whereas the primary range includes the southern Gulf of Mexico, the Caribbean Sea (Moynihan and Rodaniche 1982), and the Atlantic coast of South America. Also, during the winter, only small arrow squid are found in the northwestern Atlantic (Whitaker 1978) and in the northern Gulf of Mexico (Herke 1999), except off southern Florida (Voss and Brakoniecki 1985). Given the normal temperature range (12–30°C) for this tropical species, the seasonal shift in age structure implies an autumnal migration by larger arrow squid to warmer waters off southern Florida, in the Caribbean Sea, or in the southern Gulf of Mexico. Such overwintering areas would be reservoirs for repopulating the northern Gulf of Mexico and the northwestern Atlantic Ocean when the waters reach 12°C during the following year. The inverse relationship between the frequencies of haplotype B and haplotype C is consistent with eastward migration from a southwestern Gulf population of haplotypes A and B, along with westward migration from a southern Florida–Caribbean Islands population of haplotypes A and C (Fig. 4). The more gradual reduction in the frequency of haplotype C (versus B) may be due to the nearshore currents, which are predominantly westward along the Louisiana coast (Rabalais et al. 1999).

The genetic breakpoints found in the two species occurred at different geographic locations. This lack of congruence may be related to differences between longfin and arrow squid in depth distribution and temperature tolerance. For instance, even though both species can be captured at the same site within the Gulf, there is a tendency for longfin squid to dominate the outer continental shelf, between the 40 and 200 m isobaths, whereas arrow squid dominate the midshelf, between the 20 and 40 m isobaths (Cohen 1976; Hixon et al. 1980). If the genetic break observed for arrow squid truly represents separate resident populations, we postulate that this more offshore position of longfin squid isolates it from the genetic barrier experienced by arrow squid. Alternatively, under the hypothesis of annual recolonization by arrow squid, there is little reason to expect longfin squid to show a phylogeographic break at the Mississippi River. Similarly, with respect to the classic Atlantic–Gulf split seen for longfin squid, the Florida peninsula is unlikely to present a barrier to arrow squid. First, paralarvae from the eastern Gulf should enter the Atlantic via the Loop Current (part of the Gulf Stream; Vukovich et al. 1979). Second, arrow squid tolerate higher temperatures than do longfin squid; thus, they can avoid the strong, eastward currents of the Florida Straits by swimming westward around the Florida Keys. Finally, if Atlantic arrow squid are an annual extension of a more southern population, the Florida Straits should not bar squid from migrating north. For instance, a gyre 200 km in diameter forms several times a year off the Dry Tortugas (north of western Cuba), with each episode lasting up to 3 months. This Tortugas Gyre apparently helps retain fish larvae on the southwest continental shelf of Florida (Lee et al. 1994), so it could be responsible for arrow squid crossing the Florida Straits.

Sampling beyond North American waters is needed to fully describe the phylogeographic patterns of these two species, but we can address the null hypotheses of this study. First, within each species, nucleotide divergences among PCR-RFLP haplotypes (of the mitochondrial gene CO-I) were much lower than typically seen between species of *Loligo*. Therefore, neither longfin nor arrow squid is composed of cryptic species within the northern Gulf of Mexico and the northwestern Atlantic Ocean. Second, across this entire region, haplotype frequencies within each species are not consistent with a model of panmixia (i.e. F_{st} is not equal to zero); further, unlike some pelagic fishes, both species consist of two populations exhibiting strong genetic differentiation. Finally, population structure for longfin squid is concordant with the classic Gulf of Mexico–Atlantic Ocean phylogeographic pattern seen for other marine taxa in the region; however, arrow squid populations are separated near 88°W–89°W into a northwestern Gulf population and a northeastern Gulf–Atlantic population. Whether that separation is a true phylogeographic divide or simply an endpoint for annual recolonization by arrow squid from the southern Gulf of Mexico remains to be demonstrated.

Acknowledgements Primary funding came from the Louisiana State Board of Regents and from a National Science Foundation grant (no. OPP-9707806) to D.W. Foltz. In addition, the Louisiana Universities Marine Consortium provided a generous grant of $2,000. The initial identified specimens of *Loligo pealei* and *L. plei* were graciously provided by H. Perry and G. Sánchez (Gulf Coast Research Laboratory, Ocean Springs, Miss.). Most specimens were collected by the National Marine Fisheries Service (NMFS) and the South Carolina Department of Natural Resources (SCDNR). V. Nordahl (NMFS, Northeast Fisheries Science Center, Woods Hole, Mass.) as well as C. Barans and P. Webster (SCDNR) were especially helpful in providing specimens from the Atlantic Ocean. For the northern Gulf of Mexico, N. Sanders (NMFS, Southeastern Fisheries Science Center, Pascagoula, Miss.) allowed S.W. Herke to participate in the summer 1995 Shrimp–Groundfish Survey by the R.V. "Oregon" II; furthermore, the crew collected samples for S.W. Herke when he could not attend. M. Grace (NMFS, Southeastern Fisheries Science Center) extended the same courtesy onboard the R.V. "Chapman" for the fall 1995 Small Pelagics Survey. Commercial fishermen supplied a crucial sample of *L. plei* from the Dry Tortugas–Key West area at the behest of D. Gregory (National Sea Grant marine extension agent, Key West, Florida). Finally, Dr. M. Noor, Dr. A. Rocha-Olivares, and two anonymous reviewers provided helpful comments on a previous draft of this manuscript.

References

Anderson FE (2000a) Phylogenetic relationships among loliginid squids (Cephalopoda: Myopsida) based on analyses of multiple data sets. Zool J Linn Soc 130:603–633

Anderson FE (2000b) Phylogeny and historical biogeography of the loliginid squids (Mollusca: Cephalopoda) based on mitochondrial DNA sequence data. Mol Phylogenet Evol 15:191–214

Arocha F, Urosa LJ (1991) Some biological studies of *Loligo plei* and occurrence of *Loligo pealii* (Cephalopoda, Myopsida) in northeastern Venezuela. Biol Mar 42:145–152

Augustyn CJ, Grant WS (1988) Biochemical and morphological systematics of *Loligo vulgaris vulgaris* Lamarck and *Loligo vulgaris reynaudii* D'Orbigny nov. comb. (Cephalopoda: Myopsida). Malacologia 29:215–233

Avise JC (1992) Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63:62–76

Avise JC (1996) Toward a regional conservation genetics perspective: phylogeography of faunas in the southeastern United States. In: Avise JC, Hamrick JL (eds) Conservation genetics: case histories from nature. Chapman and Hall, New York

Bert TM (1986) Speciation in western Atlantic stone crabs (genus *Menippe*): the role of geological processes and climatic events in the formation and distribution of species. Mar Biol 93:157–170

Billington N, Hebert PDN (1991) Mitochondrial DNA diversity in fishes and its implications for introductions. Can J Fish Aquat Sci 48[Suppl 1]:80–94

Brierley AS, Thorpe JP, Pierce GJ, Clarke MR, Boyle PR (1995) Genetic variation in the neritic squid *Loligo forbesi* (Myopsida: Loliginidae) in the Northeast Atlantic Ocean. Mar Biol 122:79–86

Briggs JC (1995) Global biogeography. Elsevier Amsterdam

Brodziak J (1998) Revised biology and management of long-finned squid (*Loligo pealei*) in the Northwest Atlantic. Calif Coop Ocean Fish Investig Rep 39:61–70

Brodziak JKT, Macy III WK (1996) Growth of long-finned squid, *Loligo pealei*, in the Northwest Atlantic. Fish Bull (Wash DC) 94:212–236

Carlini DB, Graves JE (1999) Phylogenetic analysis of cytochrome *c* oxidase I sequences to determine higher-level relationships within the coleoid cephalopods. Bull Mar Sci 64:57–76

Charlesworth B (1998) Measures of divergence between populations and the effect of forces that reduce variability. Mol Biol Evol 15:538–543

Cohen AC (1976) The systematics and distribution of *Loligo* (Cephalopoda, Myopsida) in the western North Atlantic, with descriptions of two new species. Malacologia 15:299–367

Costa PAS, da Costa Fernandez F (1993) Seasonal and spatial changes of cephalopods caught in the Cabo Frio (Brazil) upwelling ecosystem. Bull Mar Sci 52:751–759

Crandall KA, Templeton AR (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. Genetics 134:959–969

Excoffier L (1993) MINSPNET. Available at http://anthro.unige.ch/~excoffie/

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Garthwaite RL, Berg Jr CJ, Harrigan J (1989) Population genetics of the common squid *Loligo pealei* LeSueur, 1821, from Cape Cod to Cape Hatteras. Biol Bull (Woods Hole)177:287–294

Gold JR, Richardson LR (1998a) Mitochondrial DNA diversification and population structure in fishes from the Gulf of Mexico and western Atlantic. J Hered 89:404–414

Gold JR, Richardson LR (1998b) Population structure in greater amberjack, *Seriola dumerili*, from the Gulf of Mexico and the western Atlantic Ocean. Fish Bull (Wash DC) 96:767–778

Grant WS, Clark A-M, Bowen BW (1998) Why restriction fragment length polymorphism analysis of mitochondrial DNA failed to resolve sardine (*Sardinops*) biogeography: insights from mitochondrial DNA cytochrome *b* sequences. Can J Fish Aquat Sci 55:2539–2547

Graves JE (1998) Molecular insights into the population structures of cosmopolitan marine fishes. J Hered 89:427–437

Hanlon RT (1998) Mating systems and sexual selection in the squid *Loligo*: how might commercial fishing on spawning squids affect them? Calif Coop Ocean Fish Investig Rep 39:92–100

Hanlon RT, Messenger JB (1996) Cephalopod behaviour. Cambridge University Press, New York

Hanlon RT, Hixon RF, Hulet WH (1983) Survival, growth, and behavior of the loliginid squids *Loligo plei*, *Loligo pealei*, and *Lolliguncula brevis* (Mollusca: Cephalopoda) in closed sea water systems. Biol Bull (Woods Hole) 165:637–685

Herke SW (1999) Phylogeography of two *Loligo* squid (Cephalopoda: Myopsida) in the Gulf of Mexico and the northwestern Atlantic Ocean. PhD dissertation, Louisiana State University, Baton Rouge

Hixon RF, Hanlon RT, Gillespie SM, Griffin WL (1980) Squid fishery in Texas: biological, economic and market considerations. Mar Fish Rev 42:44–50

Kumar S, Tamura K, Nei M (1993) MEGA: molecular evolutionary genetics analysis, ver. 1.01. The Pennsylvania State University, University Park

Lee TN, Clarke ME, Williams E, Szmant AF, Berger T (1994) Evolution of the Tortugas Gyre and its influence on recruitment in the Florida Keys. Bull Mar Sci 54:621–646

Moynihan M, Rodaniche AF (1982) The behavior and natural history of the Caribbean reef squid *Sepioteuthis sepioidea*: with a consideration of social, signal and defensive patterns for difficult and dangerous environments. Adv Ethol 25:1–150

O'Dor RK, Coelho ML (1993) Big squid, big currents and big fisheries. In: Okutani T, O'Dor RK, Kubodera T (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo

O'Dor RK, Webber DM (1986) The constraints on cephalopods: why squid aren't fish. Can J Zool 64:1591–1605

Pierce GJ, Hastie LC, Guerra A, Thorpe RS, Howard FG, Boyle PR (1994) Morphometric variation in *Loligo forbesi* and *Loligo vulgaris*: regional, seasonal, sex, maturity and worker differences. Fish Res (Amst) 21:127–148

Rabalais N, Turner RE (eds) (2001) Coastal hypoxia: consequences for living resources and ecosystems. In: Coastal and estuarine studies series 58. American Geophysical Union, Washington, DC

Rabalais N, Turner RE, Justić D, Dortch Q, Wiseman Jr J (1999) Characterization of hypoxia: topic 1 report for the integrated assessment on hypoxia in the Gulf of Mexico. In: NOAA coastal ocean program decision analysis series no. 15. NOAA Coastal Ocean Program, Silver Spring, Md.

Reynolds J, Weir BS, Cockerham CC (1983) Estimation of the coancestry coefficient: a basis for short-term genetic distance. Genetics 104:767–779

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Roper CFE, Sweeney MJ, Nauen CE (1984) FAO species catalogue, vol 3. Cephalopods of the world: an annotated and illustrated catalogue of species of interest to fisheries. FAO Fish Synop 3:1–277

Sánchez G, Perry HM, Trigg CB (1996) Morphometry of juvenile and subadult *Loligo pealei* and *L. plei* from the northern Gulf of Mexico. Fish Bull (Wash DC) 94:535–550

Schneider S, Kueffer J-M, Roessli D, Excoffier L (1997) Arlequin ver. 1.1: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva

Shaw PW, Pierce GJ, Boyle PR (1999) Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi*, demonstrated with microsatellite DNA markers. Mol Ecol 8:407–417

Söller R, Warnke K, Saint-Paul U, Blohm D (2000) Sequence divergence of mitochondrial DNA indicates cryptic biodiversity in *Octopus vulgaris* and supports the taxonomic distinctiveness of *Octopus mimus* (Cephalopoda: Octopodidae). Mar Biol 136:29–35

Summers WC (1983) *Loligo pealei*. In: Boyle PR (ed) Cephalopod life cycles, vol I. Academic Press, New York

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Vecchione M (1988) In-situ observations on a large squid-spawning bed in the eastern Gulf of Mexico. Malacologia 29:135–141

Vecchione M, Brakoniecki TF, Natsukari Y, Hanlon RT (1998) A provisional generic classification of the family Loliginidae. In: Voss NA, Vecchione M, Toll RB, Sweeney MJ (eds) Systematics and biogeography of cephalopods. Smithson Contrib Zool 586:215–222

Voss GL, Brakoniecki TF (1985) Squid resources of the Gulf of Mexico and Southeast Atlantic coasts of the United States. N Atlant Fish Org (NAFO) Sci Coun Stud 9:27–37

Voss G, Opresko L, Thomas R, Hanlon R (1973) The potentially commercial species of octopus and squid of Florida, the Gulf of Mexico and the Caribbean Sea. University of Miami Sea Grant Program (NOAA Sea Grant no. 04-3-158-27), Miami, Fla

Vukovich FM, Crissman BW, Bushnell M, King WJ (1979) Some aspects of the oceanography of the Gulf of Mexico using satellite and in situ data. J Geophys Res 84:7749–7768

Whitaker JD (1978) A contribution to the biology of *Loligo pealei* and *Loligo plei* (Cephalopoda: Myopsida) off the southeastern coast of the United States. MS thesis, College of Charleston, Charleston, S.C.

Wilhelm O, Ewing M (1972) Geology and history of the Gulf of Mexico. Geol Soc Am Bull 83:575–599

Yeatman J, Benzie JAH (1994) Genetic structure and distribution of *Photololigo* spp. in Australia. Mar Biol 118:79–87