Journal of Heredity 2005:96(4):417–423
doi:10.1093/jhered/esi036
Advance Access publication March 2, 2005

© The American Genetic Association. 2005. All rights reserved.
For Permissions, please email: journals.permissions@oupjournals.org.

Genetic Screening for Prey in the Gut Contents from a Giant Squid (*Architeuthis* sp.)

B. E. Deagle, S. N. Jarman, D. Pemberton, and N. J. Gales

From the Zoology Department, University of Tasmania, GPO Box 252-05, Hobart, 7001, Australia (Deagle); Australian Antarctic Division, Channel Highway, Kingston, 7050, Australia (Deagle, Jarman, and Gales); and Tasmanian Museum and Art Gallery, GPO Box 1164, Hobart, 7001, Australia (Pemberton).

Address correspondence to Bruce E. Deagle, Zoology Department, University of Tasmania, GPO Box 252-05, Hobart, 7001, Australia, or e-mail: bedeagle@utas.edu.au.

Giant squids (*Architeuthis* sp.) remain mysterious; they have evaded observation and are rarely taken from their deep sea habitat. Information on the diet of *Architeuthis* is scarce due to the limited number of specimens with morphologically recognizable remains in their digestive tracts. We explored the use of polymerase chain reaction (PCR)-based methods for detection of DNA in the prey remains and amorphous slurry from an *Architeuthis* gut sample. The DNA region amplified varied in size, allowing separation of fish and squid components. Sequence comparisons identified fish prey as *Macruronus novaezelandiae*. Isolation of *Architeuthis* DNA from an ingested tentacle and the presence of chitin fragments indicate cannibalism occurs in giant squid. Denaturing gradient gel electrophoresis was used to screen for less common DNA types, revealing a high frequency of PCR-generated false alleles, but no additional prey species.

Giant squids (*Architeuthis* sp.) have long captured the public's imagination because of the rarity of specimens, their enormous size, and their existence in an alien habitat. *Architeuthis* squids have been found over a huge geographic range in the Pacific, Atlantic, and Southern Oceans (Clarke 1966; Ellis 1995). They are common enough to form a significant part of sperm whales' caloric intake in many areas (Clarke and MacLeod 1982; Clarke and Young 1998), but ecological knowledge of these animals is minimal. One of the most fundamental pieces of information needed to understand a species' biology and role in an ecosystem is knowledge of its diet. Common methods for determining diet include direct observation, gut content analysis, and fecal analysis. For the giant squid, and many other marine species that are rarely observed or captured, morphological identification of semidigested prey remains in the digestive tract is the only method that has been widely applied. *Architeuthis* specimens collected through occasional landing or chance stranding are usually in poor condition and the gut is often "empty" with no morphologically recognizable content (Förch 1998). Even if material is present, classification of the remains based on morphological features is notoriously difficult in squid because of their tendency to tear apart and finely macerate prey items (Kear 1992). This has resulted in many scales, bones, and lumps of flesh found in giant squid digestive tracts being reported as unidentifiable (Bolstad and O'Shea 2004; Förch 1998; Lordan et al. 1998). The limited information published on the diet of *Architeuthis* indicates fish and cephalopods are their most important prey, with crustacean remains occasionally being observed (Förch 1998; Lordan et al. 1998). This is consistent with findings from studies of other large squids (Phillips et al. 2003).

The difficulties associated with diet determination through visual identification of squid gut contents has led to the use of other methods, such as fatty acid analysis (e.g., Phillips et al. 2001) and the identification of prey remains using immunological approaches (e.g., Kear 1992). Another approach that could help identify decomposed species in squid gut contents is the use of DNA identification techniques (Symondson 2002). Polymerase chain reaction (PCR)-based methods using species- or group-specific PCR primers have been used to detect specific species of larval fish from predatory fish stomachs (Rosel and Kocher 2002), and to identify species of krill flushed from the stomachs of Adelie penguins (*Pygoscelis adeliae*) (Jarman et al. 2002). Molecular analyses that look for a wide range of prey items in gut contents are considerably more challenging, but can provide useful data (e.g., Rollo et al. 2002).

In the present study we used molecular genetic methods to determine the prey species present in the gut of a giant squid collected in Tasmania, Australia. The DNA was extracted from both amorphous gut material and isolated prey remains. DNA sequences obtained were compared with those available in the public database and a sequence

Downloaded from https://academic.oup.com/jhered/article/96/4/417/2187607 by guest on 21 August 2022

similarity approach was applied to identify prey species. Denaturing gradient gel electrophoresis (DGGE) was used to screen amplified DNA fragments in order to check for DNA molecules present at low levels in the amplified mixture. The methodology presented will provide a framework for future studies, considerably increasing the potential for diet data collection from scarce specimens of giant squid and other rare marine animals such as beaked whales.

Methods

Architeuthis Sample

The *Architeuthis* specimen was caught on June 14, 1999, by a trawler fishing for blue grenadier at a depth of 500–700 m off the west coast of Tasmania. The squid was a male weighing 190 kg. It was frozen on board the boat and kept frozen in storage until dissection in September 2002. It was opened along the ventral surface by cutting the mantle cavity via a longitudinal incision from the funnel to the rear. The posterior viscera were exposed and the esophagus traced through to the cecum, stomach, intestine, and rectum region. The gut contents were removed from the base of the esophagus, stomach, and cecum and stored in 95% ethanol. Only the knowledge that the sample was from the gut of a local *Architeuthis* specimen was provided to the researchers carrying out the genetic work (no information on location/method of capture or potential prey species was given).

DNA Extraction

DNA was extracted from amorphous particles and recognizable prey remains that were isolated under a dissecting microscope. The settled volume of the amorphous particles was approximately 1 L. The isolated prey remains included more than 50 scales, all of similar size and shape; three small (5–10 mm) tentacle fragments; one bone fragment; and 12 chitinous squid beak fragments (<5 mm). For DNA extraction, the amorphous particles were resuspended and samples of 2 ml were centrifuged for 5 min, yielding approximately 200 mg of pelleted material. We extracted DNA from two pellets in independent procedures. In a third set of extractions, DNA was extracted from individual scales ($n = 10$), tentacle fragments ($n = 2$), and the bone fragment.

All extractions were done using the hexadecyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987). In this procedure, all steps were carried out in 1.5 ml tubes and centrifugations were at 14,000 rpm in a microfuge. The samples were homogenized in 175 μl CTAB buffer (2% CTAB; 100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA; 0.2% 2-mercaptoethanol), 2 μl proteinase K (20 mg/ml) was added, and samples were incubated at 65°C for 1 h. Sequential extractions were done with 150 μl of chloroform, isoamyl alcohol (24:1), 150 μl of phenol:chloroform: isoamyl alcohol (25:24:1) and 150 μl of chloroform:isoamyl alcohol (24:1), with 5 min centrifugations between steps. The DNA was precipitated by addition of 150 μl isopropanol (−20°C) and pelleted by a 20 min centrifugation. The DNA was washed with 400 μl of 70% ethanol, centrifuged for 5 min, air dried, and resuspended in 30 μl of distilled water. The concentration of purified DNA was determined using a PicoFluor fluorometer (Turner Designs, Sunnyvale, CA). Near the end of the study, DNA was also extracted from fish tissue obtained from a local fish market using the method outlined above.

Primer Design

Conserved PCR primers have previously been developed that amplify segments of mitochondrial DNA (mtDNA) from a broad range of animal taxa (Palumbi 1996). These primers are often used in phylogenetic studies, and in order to provide a suitable amount of sequence data, the size of the products is generally greater than 500 bp. Since DNA from the squid gut was likely to be degraded, we wanted to amplify a shorter fragment (approximately 200 bp) and therefore designed a new primer pair. The 3′ end of the mitochondrial ribosomal 16S gene (flanked by conserved primers 16Sar-5′ and 16Sa-3′; Palumbi 1996) was chosen as a potential target. This region has been widely characterized, providing a large dataset to help identify unknown sequences. Sequences were obtained from GenBank for a taxonomically diverse group of 30 fish (osteichthyes and chondrichthyes), 30 cephalopods, and several crustaceans. These were aligned and suitable primers selected (see Table 1 for primer sequences and representative alignments). The primer binding region is highly conserved in cephalopods and fish, with slightly more variation found in crustaceans.

It is possible to design degenerate primers that would incorporate the small amounts of variation that were observed. However, potential incorporation of mismatches in PCR products may have confused interpretation of our DGGE analysis, and the mismatches seemed unlikely to significantly affect primer binding, so we chose nondegenerate primers based on the squid sequence. The amplified region is short and variable, maximizing the likelihood of amplification and of obtaining informative sequence data. An additional useful feature of the amplified region is that it varies in length within and between the major taxa targeted. Based on the complete set of species used in primer design, the average size of the fragment is 258 bp ± 8.4 bp in fish and 180 bp ± 25.9 bp in cephalopods, with no overlap identified between these groups. The size range in crustaceans overlaps that of the cephalopods.

PCR Amplification and Cloning

Standard PCRs were performed on 2 μl of template in a 25 μl volume containing 0.4 μM of each primer, 0.125 mM dNTPs, 2.0 mM $MgCl_2$, 1× AmpliTaq Gold buffer, and 0.625 units AmpliTaq Gold (Applied Biosystems, Foster City, CA). Thermal cycling conditions were as follows: 94°C for 10 min, then 35 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 45 s) followed by 72°C for 2 min. Samples were separated on a 2.0% agarose gel. PCR products were cloned into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA). Positive

Downloaded from https://academic.oup.com/jhered/article/96/4/417/2187607 by guest on 21 August 2022

Table 1. Primers used in the current study aligned with homologous sequences from representative target taxa

Phylum	Class	Family	Genus + species	Accession no.	16S1F (5′ → 3′) GGACGAGAAGACCCT	16S2R (5′ → 3′) CGCTGTTATCCCTATGGTAACT	Product size (bp)[a]
Chordata	Mammalia	Physeteridae (sperm whales)	*Physeter macrocephalus*	AJ277029	A——————————————	——————————————G———————	228
	Elasmobranchii	Squalidae (dogfish sharks)	*Squalus acanthia*	Y18134	A——————————————	——————————————G———————	261
		Rajidae (skates)	*Raja radiata*	AF106038	A——————————————	——————————————G———————	261
	Actinopterygii	Amiidae (bowfins)	*Amia calva*	AY442347	A——————————————	——————————————G———————	280
		Congridae (conger eels)	*Conger myriaster*	AB038381	A——————————————	——————————————G———————	271
		Clupeidae (herrings, sardines)	*Sardinops melanostictus*	AB032554	A——————————————	——————————————G———————	249
		Cyprinidae (minnows, carps)	*Cyprinus carpio*	X61010	A——————————————	——————————————G———————	255
		Salmonidae (salmonids)	*Oncorhynchus mykiss*	L29771	A——————————————	——————————————G———————	259
		Neoscopelidae (lanternfishes)	*Neoscopelus microchir*	AP002921	A——————————————	——————————————G———————	254
		Gadidae (cods, haddocks)	*Gadus morhua*	X99772	A——————————————	——————————————G———————	254
		Berycidae (alfonsinos)	*Beryx splendens*	AP002939	A——————————————	——————————————G———————	255
		Zeidae (dories)	*Zenopsis nebulosus*	AP002942	A——————————————	——————————————G———————	251
		Gasterosteidae (sticklebacks)	*Gasterosteus aculeatus*	AP002944	A——————————————	——————————————G———————	251
		Sparidae (porgies)	*Pagrus major*	AP002949	A——————————————	——————————————G———————	254
Mollusca	Cephalopoda	Architeuthidae (giant squids)	*Architeuthis dux*	AY377629	———————————————	——————————————————————	190
		Onychoteuthidae (hook squids)	*Moroteuthis ingens*	X79580	———————————————	——————————————————————	190
		Loliginidae (squids)	*Loligo bleekeri*	AB009838	———————————————	——————————————————————	183
		Loliginidae (squids)	*Sepioteuthis lessoniana*	AY131035	———————————————	——————————————————————	186
		Ommastrephidae (squids)	*Nototodarus gouldi*	AY380810	———————————————	——————————————————————	191
		Ommastrephidae (squids)	*Todarodes pacificus*	AB158364	———————————————	——————————————————————	191
		Octopodidae (octopus)	*Octopus vulgaris*	AJ390312	——————A————————	——————————————————————	203
		Octopodidae (octopus)	*Hapalochlaena maculosa*	AY545107	———————————————	——————————————————————	189
		Sepiidae (cuttlefishes)	*Sepia pharaonis*	AF369117	———————————————	——————————————————————	184
		Sepiidae (cuttlefishes)	*Sepiella maindroni*	AF369959	———————————————	——————————————————————	191
Arthropoda	Malacostraca	Euphausiidae (krills)	*Euphausia superba*	AB084378	T—————T————————	——————————————AA——————	200
		Euphausiidae (krills)	*Nyctiphanes australis*	AF177181	T—————T————————	——————————————AA——————	202
		Penaeidae (penaeid shrimps)	*Penaeus monodon*	AF217843	——————T————————	——————————————AA——————	207
		Penaeidae (penaeid shrimps)	*Xiphopenaeus kroyeri*	AF192093	——————T————————	——————————————AA——————	207
		Palinuridae (spiny lobsters)	*Jasus edwardsii*	AF337979	——————T————————	——————————————AA——————	210

[a] The expected PCR product size is consistently larger in fish than in squid.

Downloaded from https://academic.oup.com/jhered/article/96/4/417/2187607 by guest on 21 August 2022

transformants were identified using blue/white color selection, insert size was checked by digestion with *Eco*RI, and separation of bands was done on a 2.0% agarose gel.

DGGE Analysis

In order to identify additional sequences in our PCR products we used DGGE, a technique that can separate variable DNA sequences (Myers et al. 1987). Separation is accomplished by electrophoresis of the DNA fragments in a polyacrylamide gel containing a gradient with an increasing concentration of denaturants. The mobility of the fragments is determined by their melting behavior as they denature, and this is highly sequence dependent. DGGE was performed using the DCode system (Bio-Rad, Hercules, CA). Acrylamide gels (7.5%) were poured using a model 475 gradient delivery system (Bio-Rad) and run at 56°C. For samples separated by DGGE, the 16S1R primer was redesigned to incorporate a G-C clamp (gggcgggggcggcgggacgggcgcggggc-gcggcgggcg-CGCTGTTATCCCTATGGTAACT; Sheffield et al. 1989), the annealing temperature was lowered to 50°C, and other conditions were the same as in the standard PCR. Template was 25 ng genomic DNA or 1 μl of a 1:100 dilution of unclamped PCR product or plasmid DNA. Electrophoretic conditions (gradient range, voltage, and length of run) that resulted in clear band separation were determined by experimenting with several different species of fish and squid.

Sequence Analysis

Sequencing reactions were carried out with the Big Dye prism dideoxy sequencing dye terminator kit (Applied Biosystems). Electrophoresis was performed on a PRISM 377 automated DNA sequencer (Applied Biosystems). DNA sequences where compared with publicly available sequences in GenBank using a BLAST search (Altschul et al. 1990). Sequence data were aligned using CLUSTAL_X (Thompson et al. 1997). To determine sequence relatedness, we used the neighbor-joining algorithm (Saitou and Nei 1987) in MEGA version 2.01 (Kumar et al. 2001) based on distances calculated using Kimura's two-parameter model (Kimura 1980). All nucleotides transitions and transversions were included in the analysis; nucleotide positions containing insertions/deletions in the alignment were excluded.

Results

Amorphous Component

The concentration of DNA purified from the two amorphous squid gut samples were 12 ng/μl and 8 ng/μl. PCR amplification of DNA extracted from both gave a strong "squid-size" band (approximately 190 bp) and a much weaker "fish-size" band (approximately 250 bp). The clones obtained from these amplifications contained two insert sizes corresponding to the two PCR bands observed (78 small and 2 large). In order to increase the number of clones with the larger insert, a secondary band-stab PCR (Bjourson and Cooper 1992) was carried out, using the faint large band from the first PCR as template. Clones produced from the band-stab PCR gave almost equal proportions of the two inserts (26 small and 24 large).

Sequencing was initially carried out on seven clones containing short inserts and six clones with the longer insert. The short-insert clones gave sequences 189 bp in length; six were identical and the seventh differed by a single nucleotide substitution. The longer-insert clones gave sequences 252 bp in length; again they were identical except for a single nucleotide substitution in one sequence. The consensus sequences were compared with entries in GenBank. In the case of the small fragment (GenBank accession AY392149), the sequence exactly matched mitochondrial 16S sequences from *Architeuthis dux*. The next closest matches were from a variety of squid species, all with more than 25 nucleotide differences over the region. The longer DNA sequence (GenBank accession AY392146) matched most closely with mitochondrial 16S sequences from fish species in the order Gadiformes, with four of the top five matches being within the family Gadidae. An identity matrix (giving the proportion of identical residues between sequences) shows that the longer DNA sequence and each of the top five BLAST matches are about 80% identical, with none being a likely species match. Based on this information, we obtained tissue samples from two local Gadiformes (*Mora moro* and *Macruronus novaezelandiae*) and one species belonging to the sister order Zeiformes (*Cyttus traversi*). DNA was extracted from these species and the 16S mtDNA fragment amplified and sequenced (GenBank accession AY392146–48). Analysis of these sequences showed a perfect match between the unknown sequence from the squid gut and *M. novaezelandiae* (Figure 1).

Individually Isolated Prey Remains

The amplification of DNA from the 10 scales resulted in six samples producing fish-size bands and weak squid-size bands. The remaining four scale samples gave weak or no obvious fish-size bands. The bone fragment and tentacle fragments produced only fish- and squid-size bands, respectively. Cloning and sequencing of these PCR products revealed all sequences matched either *M. novaezelandiae* (blue grenadier) or *Architeuthis* sequences previously obtained. While the bone and tentacles gave only blue grenadier or *Architeuthis* sequences, a mixture of sequences was obtained from the scales, indicating *Architeuthis* DNA present in the gut was associated with the fish scales. The intensity of fish-size bands from the scales and the bone fragment were much stronger than the fish bands observed in the amorphous PCR products, indicating that the vast majority of blue grenadier sequences obtained from these samples originated from the scales or bone.

DGGE Analysis

Denaturing gradient gel electrophoresis conditions that resulted in good separation of fragments from the fish

Downloaded from https://academic.oup.com/jhered/article/96/4/417/2187607 by guest on 21 August 2022

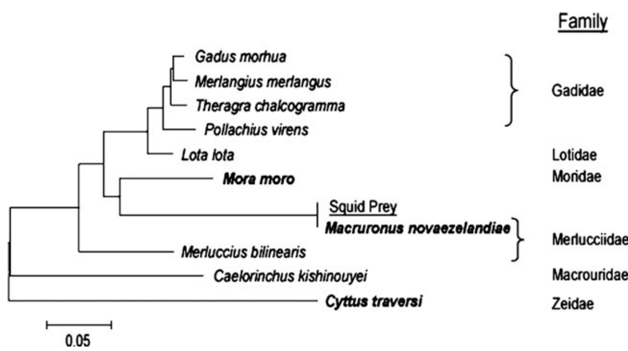

Figure 1. Neighbor-joining tree based on the 252 bp mtDNA sequence obtained from the *Architeuthis* gut sample aligned with sequences of the five closest Blast matches (*Gadus morhua*, *Merlangius merlangus*, *Theragra chalcogramma*, *Pollachius virens*, and *Lota lota*), GenBank sequence of a related fish belonging to a genus previously identified as *Architeuthis* prey (*Caelorinchus kishinouyei*), sequences of fish species obtained during the present study (**bold**), and an additional GenBank sequence from the family Merlucciidae (*Merluccius bilinearis*). All of the species belong to the order Gadiformes, with the exception of *C. traversi*, which belongs to the sister order Zeiformes.

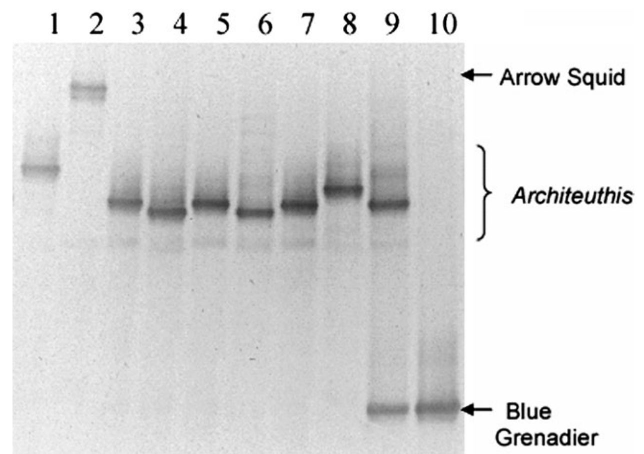

Figure 2. Denaturing gradient gel electrophoresis separation of mtDNA 16S PCR products. Lanes 2 and 10 are amplified from genomic DNA of arrow squid (*Nototodarus* sp.) and blue grenadier (*M. novaezelandiae*), respectively. Lane 9 is an amplification of DNA extracted from the amorphous slurry component of the *Architeuthis* gut contents. Remaining lanes are amplified from clones derived from the same source. Sequences of the clones shown either match the *Architeuthis* consensus (lanes 3, 5, and 7) or are closely related (lane 1, variant H; lane 4, variant B; lane 6, variant D; lane 8, variant F). See Table 2 for sequences.

surveyed were 30–70% gradient at 50 V for 8 h on a 16 cm gel. For squid fragments, the best separation was achieved with a 0–50% gradient at 60 V for 8 h on a 16 cm gel (Figure 2). The analysis of the PCR amplifications from the amorphous component and the band-stab gave two bands corresponding to *Architeuthis* and blue grenadier bands (Figure 2, lane 9). While these results indicate that the majority of DNA present in the sample comes from these two species, there is the potential for less abundant DNA sequences to be present.

To check this possibility, 26 fish-size and 80 squid-size clones were amplified and screened for sequence variation. The analysis of the fish-size clones showed 23 samples matched the electrophoretic mobility of the common blue grenadier sequence (GenBank accession AY392146); the three remaining clones had unique DGGE bands. Sequencing of these clones revealed one was the single base pair variant of the blue grenadier sequence that had been previously identified and the two other sequences were unique—but differed by only one or two base substitutions from the common blue grenadier sequence (Table 2). Screening of 80 squid-size clones revealed 70 clones running parallel to the common *Architeuthis* sequence (GenBank accession AY392149) and 10 not matching the reference sequence (Figure 2). The 10 variant clones were sequenced, revealing eight different sequences, all of which were closely related to the previously obtained *Architeuthis* sequences (Table 2).

Discussion

This study reports on the development and application of genetic tools for the identification of prey remains recovered from *Architeuthis* gut contents. Primers were designed that amplify a region of 16S mtDNA that differs in size between fish and squid, allowing separation of DNA recovered from these potential prey groups. Our analysis of an *Architeuthis* gut sample revealed both fish- and squid-size PCR products. These PCR products were screened for sequence variants (i.e., different species of fish or squid) using DGGE.

Fish DNA was amplified from scales, bones, and the slurry component of the *Architeuthis* gut sample. These sequences were initially characterized (based on publicly available sequence data) as belonging to a single Gadiforme species; sequencing of local Gadiformes allowed us to identify the prey species as blue grenadier (called hoki in New Zealand). This fish species occurs in the waters of southern Australia and around New Zealand, and they are a dominant component of the upper continental slope fish fauna around Tasmania (May and Blaber 1989). The capture of *Architeuthis* by commercial trawlers targeting blue grenadier in Australia (Pemberton D, unpublished data) and New Zealand (Bolstad and O'Shea 2004) has suggested these fish form a component of the diet of *Architeuthis*; however, blue grenadier had not previously been recorded in the gut contents of *Architeuthis*. The absence of direct evidence for this link had led previous researchers to conclude that *Architeuthis* probably preys on the same food items as blue grenadier rather than the blue grenadier itself (Bolstad and O'Shea 2004). It is interesting to note that the three *Architeuthis* specimens found stranded in southern Tasmania (first in 1986, then again in 1992 and 2002) were all found between June and early September, which is the same time of year that blue grenadier from Australian waters gather to spawn in dense aggregations off western Tasmania (Gunn et al. 1989).

Downloaded from https://academic.oup.com/jhered/article/96/4/417/2187607 by guest on 21 August 2022

Table 2. Variable sites identified in nucleotide sequences obtained from the amorphous slurry component of the *Architeuthis* gut contents

	Position of variable sites	No. of sequences	Frequency from DGGE
Architeuthis			
	1 1 1 1 1 1 2 4 1 1 1 4 5 7 9 8 4 5 6 6 9		
Consensus	T T T C C T C C	6	70/80
Variant A	C - - - - - - -	1	1/80*
B	- - C - - - - -	1	1/80*
C	- C - - - - - -	1	1/80*
D	- - - - - C - -	1	1/80*
E	- - - - - - - T	2	2/80
F	- - - - T - - T	2	2/80
G	- - - T T - - T	1	1/80
H	- - - T T - T T	1	1/80
Blue grenadier			
	1 5 8 6 4 1		
Consensus	G C T	5	23/26
Variant A	- T -	1	1/26
B	- - G	1	1/26
C	A - G	1	1/26

* These variants were not separable from each other under the DGGE conditions used.

The squid DNA sequences that we isolated from the giant squid gut closely matched *Architeuthis* and the majority of these sequences are likely to have originated from the gut lining of the predator. Detection of cannibalism using DNA-based methods is possible, but only through the development and use of individual-specific DNA markers. Using only information from more conserved markers, such as the ones used in the current study, it is not possible to differentiate between predator and prey of the same species. However, the ability to identify morphologically ambiguous tissue fragments is a strong point of the genetic identification approach, and amplification of DNA extracted from the small tentacle fragments found in the squid produced only *Architeuthis* sequences. This finding suggests that cannibalism has occurred, a conclusion further supported by the presence of crushed squid beak in the gut and the lack of any DNA from different squid species in the 80 clones that were screened using DGGE. Cannibalism has been widely reported in other squids (Phillips et al. 2003; Quetglas et al. 1999; Santos and Haimovici 1997) and has recently been described in *Architeuthis* (Bolstad and O'Shea 2004). It should be noted that autophagy or accidental self-ingestion cannot be ruled out as a potential source of the tentacle fragments (see discussion in Bolstad and O'Shea 2004).

Genetic identification from amorphous gut material is appealing, since data collection is not limited to undigested tissue and hard part remains. Since DNA from several species of prey may be present in this mixture, heterogeneous amplification products must be separated for identification. To identify different PCR products, we took advantage of the size differences in the amplification products and also applied DGGE, a technique that has been widely applied in microbial ecology to characterize PCR products from mixed-species templates (Muyzer 1999). Direct DGGE analysis of PCR products from the squid gut identified both the blue grenadier and *Architeuthis* amplification products. Since rare amplification products are likely to be hard to detect using direct PCR, we also screened individual clones derived from these PCR products. In the 80 squid-size and 26 fish-size clones analyzed, no new prey species were identified. However, this analysis did detect multiple sequences closely matching *Architeuthis* and blue grenadier. Possible origins of these sequences include heteroplasmy, amplification from multiple genetically different individuals, or PCR-induced mutations resulting from the amplification of degraded DNA. These possibilities are not mutually exclusive, so it is difficult to discount any completely; however, several facts indicate the majority of these sequences are PCR artifacts. First, most of the changes are C-T transitions, which is consistent with *Taq* polymerase errors generated from damaged template through jumping PCR and cytosine deamination (Hofreiter et al. 2001). Second, all alleles are separated from the next closest allele by a single nucleotide substitution, suggesting in situ generation. Finally, the nucleotide substitutions are inconsistent with patterns of conserved versus variable sites observed in closely related species (five of the seven substitutions in *Architeuthis* occur in sites that are conserved among the 30 other species of cephalopod surveyed for primer design).

Although the majority of DNA molecules obtained from the squid gut amorphous slurry were undamaged, the high frequency of false alleles obviously can be problematic when screening for prey species represented by a low frequency of DNA. It may be possible to lower the background level of false alleles through the use of a polymerase possessing 3′-5′ exonuclease activity (proofreading) or by treatment of the DNA extraction with uracil N-glycolase (Hofreiter et al. 2001). The development of group-specific primers that exclude DNA from the predator and/or amplify only a portion of potential prey would also allow detection of prey items present in small amounts (Jarman et al. 2004).

The scarcity of *Architeuthis* specimens necessitates a detailed analysis of each one if our knowledge of this species is to increase substantially. The use of DNA-based methods to study diet allows identification of prey recovered from gut contents, including prey remains that could not be identified using morphological methods. The universality of genetic methods could also allow a standard protocol of gut content analysis to be developed, maximizing information gain from sporadically collected samples. One of the factors currently limiting the use of this approach is that prey identification relies on DNA sequence data being available for a wide range of potential prey species. With the rapid increase in available DNA sequence data (e.g., Miya et al. 2003) and development of taxonomic systems based on DNA sequences (Hebert et al. 2003; Ross et al. 2003; Tautz et al. 2003), genus or species identification of DNA sequences should become increasingly possible.

Downloaded from https://academic.oup.com/jhered/article/96/4/417/2187607 by guest on 21 August 2022

Acknowledgments

Comments from two anonymous reviewers helped improve the manuscript. The collection of the *Architeuthis* specimen was a result of the interest and tenacity of Mick Barron, who was an Australian Fisheries Management Authority observer. We also thank Peter Rockliff, of the fishing company Petuna, and Tasmanian Museum staff, Kathryn Medlok, Debbie Robertson, Brian Looker, and Elizabeth Turner. This work was funded by the Australian Antarctic Division and an Australian Postgraduate Award (to B.D.).

References

Altschul SF, Gish W, Miller W, Myers EW, and Lipman DJ, 1990. Basic local alignment search tool. J Mol Biol 215:403–410.

Bjourson AJ and Cooper JE, 1992. Band-stab PCR: a simple technique for the purification of individual PCR products. Nucleic Acids Res 20:4675.

Bolstad KS and O'Shea S, 2004. Gut contents of a giant squid *Architeuthis dux* (Cephalopoda: Oegopsida) from New Zealand waters. N Z J Zool 31:15–21.

Clarke MR, 1966. A review of the systematics and ecology of the oceanic squids. Adv Mar Biol 4:91–300.

Clarke MR and MacLeod N, 1982. Cephalopod remains from the stomachs of sperm whales caught in the Tasman Sea. Mem Natl Mus Victoria 43:25–42.

Clarke M and Young R, 1998. Description and analysis of cephalopod beaks from stomachs of six species of odontocete cetaceans stranded on Hawaiian shores. J Mar Biol Assoc UK 78:623–641.

Doyle JJ and Doyle JL, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15.

Ellis R, 1995. Monsters of the sea. London: Robert Hale.

Förch EC, 1998. The marine fauna of New Zealand: Cephalopoda: Oegopsida: Architeuthidae (giant squid). Memoir 110. Wellington, New Zealand: National Institute of Water and Atmospheric Research Biodiversity.

Gunn JS, Bruce BD, Furlani DM, Thresher RE, and Blaber SJM, 1989. Timing and location of spawning blue grenadier, *Macruronus novaezelandiae* (Teleostei, Merlucciidae), in Australian coastal waters. Aust J Mar Freshwater Res 40:97–112.

Hebert PDN, Cywinska A, Ball SL, and deWaard JR, 2003. Biological identifications through DNA barcodes. Proc R Soc Lond B Biol 270:313–321.

Hofreiter M, Jaenicke V, Serre D, von Haeseler A, and Pääbo S, 2001. DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. Nucleic Acids Res 29:4793–4799.

Jarman SN, Gales NJ, Tierney M, Gill PC, and Elliott NG, 2002. A DNA-based method for identification of krill species and its application to analysing the diet of marine vertebrate predators. Mol Ecol 11:2679–2690.

Jarman SN, Deagle BE, and Gales NJ, 2004. Group-specific polymerase chain reaction for DNA-based analysis of species diversity and identity in dietary samples. Mol Ecol 13:1313–1322.

Kear AJ, 1992. The diet of Antarctic squid: comparison of conventional and serological gut content analyses. J Exp Mar Biol Ecol 156:161–178.

Kimura M, 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J Mol Evol 16:111–120.

Kumar S, Tamura K, Jakobsen IB, and Nei M, 2001. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 17:1244–1245.

Lordan C, Collins MA, and Perales-Raya C, 1998. Observations on morphology, age and diet of three *Architeuthis* caught off the west coast of Ireland in 1995. J Mar Biol Assoc UK 78:903–917.

May JL and Blaber SJM, 1989. Benthic and pelagic fish biomass of the upper continental slope off eastern Tasmania. Mar Biol 101:11–25.

Miya M, Takeshima H, Endo H, Ishiguro NB, Inoue JG, Mukai T, Satoh TP, Yamaguchi M, Kawaguchi A, Mabuchi K, et al. 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. Mol Phylogenet Evol 26:121–138.

Muyzer G, 1999. DGGE/TGGE a method for identifying genes from natural ecosystems. Curr Opin Microbiol 2:317–322.

Myers RM, Maniatis T, and Lerman LS, 1987. Detection and localization of single base changes by denaturing gradient gel electrophoresis. In: Methods in enzymology (Colowick SP and Kaplan NO, eds). New York: Academic Press; 501–527.

Palumbi SR, 1996. Nucleic acids II: the polymerase chain reaction. In: Molecular systematics (Hillis DM, Moritz C, and Mable BK, eds). Sunderland: Sinauer Associates; 205–247.

Phillips KL, Jackson GD, and Nichols PD, 2001. Predation on myctophids by the squid *Moroteuthis ingens* around Macquarie and Heard Islands: stomach contents and fatty acid analyses. Mar Ecol Prog Ser 215:179–189.

Phillips KL, Nichols PD, and Jackson GD, 2003. Size-related dietary changes observed in the squid *Moroteuthis ingens* at the Falkland Islands: stomach contents and fatty-acid analyses. Polar Biol 26:474–485.

Quetglas A, Alemany F, Carbonell A, Merella P, and Sanchez P, 1999. Diet of the European flying squid *Todarodes sagittatus* (Cephalopoda: Ommastrephidae) in the Balearic Sea (western Mediterranean). J Mar Biol Assoc UK 79:479–486.

Rollo F, Ubaldi M, Ermini L, and Marota I, 2002. Ötzi's last meals: DNA analysis of the intestinal content of the Neolithic glacier mummy from the Alps. Proc Natl Acad Sci USA 99:12594–12599.

Rosel PE and Kocher TD, 2002. DNA-based identification of larval cod in stomach contents of predatory fishes. J Exp Mar Biol Ecol 267:75–88.

Ross HA, Lento GM, Dalebout ML, Goode M, Ewing G, McLaren P, Rodrigo AG, Lavery S, and Baker CS, 2003. DNA surveillance: Web-based molecular identification of whales, dolphins, and porpoises. J Hered 94:111–114.

Saitou N and Nei M, 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Santos RA and Haimovici M, 1997. Food and feeding of the short-finned squid *Illex argentinus* (Cephalopoda: Ommastrephidae) off southern Brazil. Fish Res 33:139–147.

Sheffield VC, Cox DR, Lerman LS, and Myers RM, 1989. Attachment of a 40-base-pair G+C-rich sequence (G-C clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. Proc Natl Acad Sci USA 86:232–236.

Symondson WOC, 2002. Molecular identification of prey in predator diets. Mol Ecol 11:627–641.

Tautz D, Arctander P, Minelli A, Thomas RH, and Vogler AP, 2003. A plea for DNA taxonomy. Trends Ecol Evol 18:70–74.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, and Higgins DG, 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882.

Received May 5, 2004
Accepted November 4, 2004

Corresponding Editor: C. Scott Baker

Downloaded from https://academic.oup.com/jhered/article/96/4/417/2187607 by guest on 21 August 2022