POPULATION GENETIC STRUCTURE OF BLUEFIN TUNA IN THE NORTH ATLANTIC OCEAN. IDENTIFICATION OF VARIABLE GENETIC MARKERS

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SUMMARY

As part of the ICCAT Bluefin Year Program, the United States is sponsoring a preliminary investigation of genetic variation within north Atlantic bluefin tuna. The study employs a variety of molecular methodologies to survey the bluefin tuna nuclear and mitochondrial genomes for highly variable regions that will be informative for testing various hypotheses of population structure. To ensure that the genetic techniques will enable researchers to work with samples of small size (e.g., larvae and early juveniles), sub-optimal preservation, and to rapidly survey a large number of samples, all methodologies are investigating regions of DNA amplified by the polymerase chain reaction (PCR).

Complementary approaches are being employed to analyze amplified DNA in four different laboratories. The techniques include: (1) sequence analysis of the mitochondrial DNA control region; (2) restriction fragment length polymorphism (RFLP) analysis variable regions within or near nuclear genes of metabolic enzymes and other structural genes; (3) RFLP analysis of anonymous single copy nuclear genes; and (4) analysis of nuclear micro-satellite DNA. Upon the identification of polymorphic genetic regions, each laboratory will perform a blind genetic screening on the same 200 bluefin tuna collected from throughout the north Atlantic to provide an estimate of the genetic variation revealed by each technique.

After several polymorphic genetic characters have been identified it is anticipated that a team of international collaborators will use a subset of these methodologies to genetically characterize bluefin tuna larvae and early juveniles collected from putative spawning grounds in the north Atlantic. These data could then be used to evaluate hypotheses of spawning site fidelity and stock structure of bluefin tuna within the north Atlantic Ocean.

RESUMÉ

Dans le cadre du Programme ICCAT d'Année Thon rouge, les Etats-Unis ont financé une investigation préliminaire de la variation génétique chez le thon rouge de l'Atlantique Nord. L'étude utilise toute une variété de méthodologies moléculaires pour examiner les génomes nucléiques et mitochondriaux à la recherche de zones fortement variables qui fourniraient des informations pour tester diverses hypothèses quant à la structure de la population. Pour garantir que les techniques génétiques permettent aux chercheurs de travailler avec des échantillons de petite taille (par ex., larves et petits juvéniles), une conservation sous-optimale, et examiner un grand nombre d'échantillons, toutes les méthodologies étudient des régions de l'ADN amplifiées au moyen de la réaction en chaîne de la polymérase (PCR).

Des approches complémentaires sont utilisées dans quatre laboratoires différents pour analyser l'ADN amplifié. Ces techniques comprennent : 1) analyse séquentielle de la région de contrôle de l'ADN mitochondrial ; 2) analyse du polymorphisme restrictif de la longueur fragmentaire (RFLP) de zones variées à l'intérieur ou à proximité de gènes nucléiques d'enzymes métaboliques et autres gènes structuraux ; 3) analyse du RFLP de gènes nucléiques uniques anonymes,; et 4) analyse de l'ADN nucléique microsatellitaire. En identifiant les régions génétiques polymorphiques, chaque laboratoire effectuera un tri génétique aveugle sur les mêmes 200 thons rouges prélevés dans tout l'Atlantique Nord, pour obtenir une estimation de la variation génétique révélée par chaque technique.

Une fois identifiées plusieurs caractéristiques génétiques polymorphiques, on prévoit qu'une équipe de collaborateurs internationaux utilisera un sous-ensemble de ces méthodologies pour caractériser du point de vue génétique les larves et les petits juvéniles de thon rouge prélevés dans des zones supposées de frai dans l'Atlantique Nord. Ces données pourrait alors servir à évaluer les hypothèses concernant la fidélité au lieu de ponte et la structure de stock du thon rouge dans l'Atlantique Nord.

RESUMEN

Como parte del Programa ICCAT Año Internacional del Atún Rojo, Estados Unidos patrocina una investigación preliminar de la variación genética del atún rojo en el Atlántico norte. El estudio emplea una variedad de metodologías moleculares para hacer una prospección de los genomas mitocondriales y nucleares del atún rojo, para regiones muy variables, que será muy útil para comprobar varias hipótesis sobre estructura de población. Para asegurar que las técnicas genéticas permitirán a los investigadores trabajar con muestras de pequeño tamaño (por ejemplo, larvas y juveniles tempranos), una preservación no óptima y hacer rápidas prospecciones de un gran número de muestras, todas las metodologías investigan regiones de ADN amplificadas por medio de la reacción de la cadena de polimerasa (PCR).

En cuatro laboratorios diferentes, se aplican enfoques complementarios para analizar el ADN amplificado. Las técnicas incluyen: (1) análisis secuencial del ADN mitocondrial de la región de control; (2) polimorfismo de longitud de los fragmentos de restricción (RFPL) de regiones variables dentro o cerca de genes nucleares de enzimas metabólicos y otros genes estructurales, (3) análisis FRPL de copia anónima de genes nucleares individuales, y (4) análisis de microsatélite ADN nuclear. Tras identificar las regiones genéticas polimorfas, cada laboratorio efectuará un análisis genético ciego sobre los mismos 200 ejemplares de atún rojo recogidos en todo el Atlántico norte, con el fin de facilitar una estimación de la variación genética que revela cada una de las técnicas.

Una vez se hayan identificado varios caracteres genéticos polimorfos, un equipo de colaboradores internacionales aplicará un subconjunto de estas metodologías, con el fin de clasificar genéticamente las larvas de atún rojo y los juveniles tempranos recogidos en zonas putativas de desove del Atlántico norte. Estos datos se podrían emplear para evaluar la hipótesis de fidelidad a las zonas de desove y la estructura del stock en el Atlántico norte.

Introduction

The population genetic structure of the bluefin tuna (*Thunnus thynnus*) is poorly understood on global and local levels. Unlike many other species of the genus *Thunnus*, bluefin tuna spawning is restricted both in space and time. Within the North Atlantic Ocean bluefin tuna are known to spawn in two distinct areas: the Mediterranean Sea and the Gulf of Mexico (Richards 1976). Results of tag and release experiments demonstrated that fish move between the eastern and western North Atlantic, however, it is not known if members of either sex show fidelity to eastern or western North Atlantic spawning sites. If bluefin tuna exhibit spawning site fidelity, then one might expect in the absence of gene flow between areas (movement of individuals with successful reproduction) that genetic differences would accumulate. Because isolation of eastern and western North Atlantic bluefin tuna may be incomplete or relatively recent on an evolutionary time scale, genetic differences between spawning sites may be small. To effectively test for population structure in such a scenario, it is essential that one survey genetic characters that are rapidly evolving, allowing for the maximum resolution of genetic differences if they exist.

The rapid growth of molecular biology has provided a wealth of techniques to survey genetic variation within populations. As part of the ICCAT International Bluefin Year Program, the United States is sponsoring a preliminary investigation of genetic variation within North Atlantic bluefin tuna. The objective of this preliminary study is not to elucidate the population genetic structure of bluefin tuna in the North Atlantic, but to employ a variety of molecular genetic techniques to identify those regions of the bluefin tuna genome that reveal substantial genetic variation. Only after variable genetic characters have been identified will it be possible for a team of international fishery scientists to test hypotheses of genetically based stock structure.

In this paper we outline the variety of approaches being employed in our laboratories to reveal genetic variation within North Atlantic bluefin tuna. The purpose of the document is to inform the international community of our experimental objectives and to invite their input and collaboration into the design and execution of the second phase of the project, which will entail an analysis of bluefin tuna population genetic structure in the North Atlantic.

Experimental Approach

Sample collection. Because the intent of this project is to find variable genetic loci, no attempt was made to obtain "representative" samples of eastern and western bluefin tuna. Rather, a collection of large juvenile and adult bluefin tuna was obtained from a variety of locations throughout the North Atlantic and sent to a central laboratory where total genomic DNA was isolated from heart or other tissues by standard procedures (Sambrook et al. 1989). Samples were given random identification numbers and aliquots of each sample were sent to the four laboratories for analysis.

Because late juvenile and adult bluefin tuna are capable of crossing the North Atlantic, tests of various hypotheses of bluefin tuna stock structure will have to focus on larvae and early juveniles, life history stages that could not have moved between the two putative spawning areas. Thus the total DNA available for genetic analysis from the early life history stages may well be limiting. To maximize the number of genetic analyses that can be performed on any individual, all of the genetic methodologies described below rely on the polymerase chain reaction (PCR) to amplify specific regions of the nuclear and mitochondrial genomes.

Molecular Genetic Analyses

Sequence analysis of the mitochondrial genome. Mitochondrial DNA (mtDNA) is maternally inherited in vertebrates, providing an opportunity to trace maternal lineages, and in conjunction with analyses of nuclear genes, discriminate between female- and male-mediated gene flow (Avise 1994). Specific regions within the mitochondrial genome evolve at different rates, with the control region (or D-loop) exhibiting some of the highest rates (Avise 1994). To survey

variation in the mtDNA control region, specific primers have been constructed that amplify a variable section of the bluefin mtDNA control region. The nucleotide sequence of these amplified regions is being determined by standard di-deoxy nucleotide sequencing (Sanger et al. 1977).

RFLP analysis of specific (known) nuclear genes. Regions of several nuclear genes are strongly conserved among organisms and exhibit little sequence variation. However, regions within these genes that are transcribed but not translated (introns and flanking regions), are often free of selective constraints and evolve at relatively rapid rates (Avise 1994). The strategy of these studies is to look for variation in variable regions within or adjacent to strongly conserved genes.

To survey variation in these structural genes, two alternate strategies are being employed. In one, a genomic DNA library is constructed, consisting of large fragments of bluefin tuna DNA inserted into phages (viruses). These are then screened with probes consisting of highly conserved sequences of a structural gene to find those phage that contain part or all of the gene. Alternately, bluefin tuna complementary DNA (cDNA) for metabolic genes is being isolated and the 3' untranslated regions identified for PCR amplification. For both approaches, the fragments of the desired are then sequenced to determine if they include a highly variable region. If so, PCR primers are made to amplify the highly variable region from standard DNA isolations and the amplified products are screened with a suite of restriction enzymes to survey for genetic variation. In these analyses some of the specific nuclear genes being screened include lactate dehydrogenase, triose phosphate isomerase, actin, and the internal transcribed spacer of ribosomal RNA.

RFLP analysis of anonymous nuclear genes. In this procedure developed by Karl and Avise (1994), fragments of genomic DNA averaging 1,000 to 1,500 base pairs are incorporated into a DNA library. The library is screened to find those genes which exist in low copy number, and PCR primers are developed to amplify that region from standard DNA isolations. The fragments are then amplified from several individuals and screened with a battery of restriction enzymes to assess genetic variation.

RFLP analysis of microsatellite DNA. Microsatellite DNA consists of regions of repeating di-, tri-, and tetra-nucleotide sequences that occur throughout the nuclear genome. The number of repeating units within a microsatellite DNA locus evolves very rapidly, and provides an excellent character for surveying population structure (Wright and Bentzen 1994). To assay microsatellite DNA, a genomic library is constructed as detailed above, with inserts averaging 200 - 400 bp in size. The library is then screened with probes of repeating nucleotides to locate phages containing microsatellite loci. Regions of DNA flanking the repeating units are sequenced to develop primers to amplify the microsatellite locus from standard DNA isolations. The amplified regions are then screened for variation.

Each laboratory will employ a subset of the methodologies described above to survey genetic variation in the same 200 bluefin tuna. The results from each variable marker will then be compared to determine which characters reveal levels of genetic variation appropriate for analysis of the population genetic structure of North Atlantic bluefin tuna. Levels of variation will be used to determine the sample sizes required for subsequent population genetic studies.

With a suite of molecular genetic characters that reveal variation within North Atlantic bluefin tuna it will possible to evaluate different hypotheses of population genetic structuring. In collaboration with international fisheries scientists, we propose to use these tools to effect a genetic analysis of early life history stages of bluefin tuna from the Mediterranean Sea and Gulf of Mexico to determine the degree of genetic connectivity between the two spawning sites.

References

Avise, J.C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Karl, S.A., and J.C. Avise. 1993. PCR-based assays of Mendelian polymorphisms from anonymous single-copy nuclear DNA: techniques and applications for population genetics. Mol. Biol. Evol. 10:342-361.

Richards, W.J. 1976. Spawning of bluefin tuna (*Thunnus thynnus*) in the Atlantic Ocean and adjacent seas. Int. Com. Conserv. Atl. Tunas, Coll. Vol. Sci. Paps. 5:267-278.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, New York.

Sanger, F. S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463-5467.

Wright, J.M., and P. Bentzen. 1994. Microsatellites: genetic markers for the future. Rev. Fish. Biol. 4:384-388.