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Geographic variability in the grey mullet *Mugil cephalus*: preliminary results of mtDNA and chromosome analyses

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ABSTRACT

Crosetti, D., Avise, J.C., Placidi, F., Rossi, A.R. and Sola, L., 1993. Geographic variability in the grey mullet *Mugil cephalus*: preliminary results of mtDNA and chromosome analyses. *Aquaculture*, 111: 95–101.

The grey mullet, *Mugil cephalus*, plays an important role in the fisheries and aquaculture of tropical and subtropical regions of the world. This species is considered cosmopolitan, but its distribution appears peculiar with regard to the coastal ecology of the species. A multidisciplinary study of the geographic variability of this species, through a cytogenetic, molecular and morphometric characterization, was undertaken to detect whether genetically distinct populations occur. The preliminary results from analyses of mitochondrial DNA and of chromosomes of seven different populations around the world are reported. The different populations analysed are well discriminated by mtDNA analyses: samples are clustered in four groups, Mediterranean, East Atlantic, Central Pacific and East Pacific, with a maximum sequence divergence of 3.3%. The karyotype of all the populations studied is uniformly composed of 48 acrocentric chromosomes, and neither heterochromatin distribution nor nucleolus organizer regions allow the identification of chromosomal markers useful in distinguishing these genetically differentiated groups of populations.

INTRODUCTION

The Mugilidae family includes a hundred species, grouped in ten genera. Mullets have a conservative external morphology (Thomson, 1981), and the question is raised whether taxonomic characters traditionally observed on preserved material from museum collections are sufficient to identify genetically distinct populations.

Within the genus *Mugil*, found in temperate and tropical waters worldwide,

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the grey mullet, *M. cephalus*, is considered cosmopolitan (Thomson, 1963; Fig. 1). This distribution appears peculiar with regard to the coastal ecology of the species, with limited dispersal into pelagic waters and consequent limited gene flow among populations.

The grey mullet plays an important role in the fisheries and aquaculture of tropical and subtropical regions of the world (Nash and Shehadeh, 1980), especially in cultural practices based on natural food webs. The development of its culture requires a basic knowledge on the species biology and genetics, in order to avoid an erroneous flow of information about rearing practices used on fish from different geographical areas.

A multidisciplinary approach to the study of the geographic variability of the species *M. cephalus* was undertaken, through a cytogenetic, molecular and morphometric characterization. This research aims at detecting genetically isolated populations within this cosmopolitan species, and at describing the level of intraspecific diversification, examining samples from different regions of the world.

Besides possible practical applications to mullet culture, information on the geographic variability of the grey mullet is of interest to the problem of speciation in the marine environment, where reproductive isolation presents peculiar modalities. The conjoint approach of morphometry and genetics allows the re-examination of the data from traditional approaches with those from more modern techniques recently introduced in population biology.

Gene-enzyme analyses and multivariate morphometrics will be carried out when sampling is completed. Only the preliminary results from mtDNA and chromosome analyses are reported in this paper.

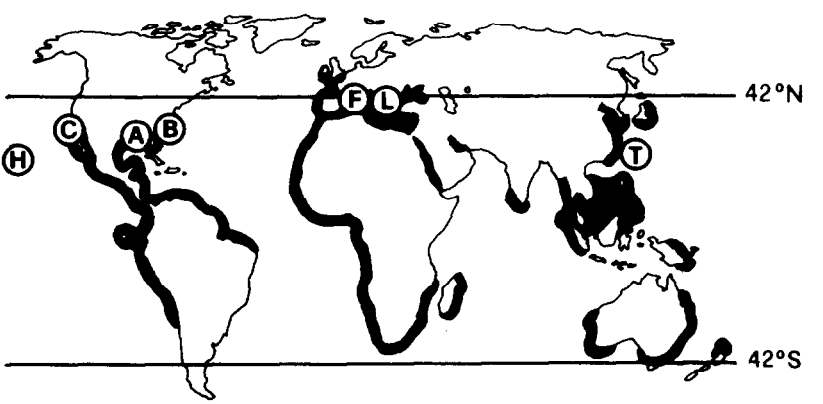

Fig. 1. Geographic distribution of *Mugil cephalus* (from Thomson, 1963) and collection sites. A, Florida, USA; B, North Carolina, USA, east coast; C, California, USA; F, Fiumicino, Italy, west coast; H, Hawai, USA; L, Lesina, Italy, east coast; T, Taiwan.

MATERIAL AND METHODS

Wild mullet populations were collected from different regions of the world. Seven populations have been analysed until now: two Mediterranean populations from Italy (L and F), Hawaii (H), Taiwan (T), West Florida, USA (A), North Carolina, USA (B), and California, USA (C) (Fig. 1). Each population is represented at least by nine specimens, usually by 20.

The tissues sampled for mitochondrial DNA analyses (ovary, liver, heart) were collected and preserved fresh in a buffer solution (MSB–EDTA) at 4°C. MtDNA was isolated following the cesium chloride protocol (Lansman et al., 1981). Small fishes were processed by alkaline lysis (Tamura and Aotsuka, 1988), where muscle and brain were also used. A complete survey was possible only on five populations (L, F, B, T, H), because of the small size of the fish collected or the poor quality of the tissues in (A) and (C) respectively.

Each sample was digested with 13 restriction enzymes (*Ava*I, *Ava*II, *Bam*HI, *Bcl*I, *Bgl*II, *Hin*dII, *Hin*dIII, *Kpn*I, *Nde*I, *Pvu*II, *Spe*I, *Sst*II, *Stu*I). Restriction fragments were end-labeled using Klenow and ^{35}S-dNTP$_{(s)}$, and, following electrophoresis through 0.9–1.4% agarose gels, revealed by autoradiography. Fragment size was compared against the 1-kilobase ladder standard. No attempt was made to score fragments smaller than 0.4 kb. Estimates of nucleotide sequence divergence (p) between mtDNA clones were generated by the fragment approach of Nei and Li (1979) and the resulting distance matrix was clustered using the UPGMA algorithm (Sneath and Sokal, 1973).

Karyological preparations were made in the field, or on fish transported live to the laboratory. Although specimens from all the populations sampled were examined, better results were obtained when live fish were available in the laboratory, i.e. eight specimens from Fiumicino (F), Hawaii (H), North Carolina (B), Taiwan (T). Somatic metaphases, obtained from pooled spleen, gill and cephalic kidney cells were stained with different techniques, to identify the nucleolus organizer regions, NORs, and the pattern of heterochromatin distribution. NORs were silver-stained by the method of Howell and Black (1980) and C-banding was performed according to Sumner (1972). NORs and the heterochromatin were further investigated after staining with chromomycin A_3, CMA_3, and DAPI, as described by Sola et al. (1992).

RESULTS

MtDNA

For the 13 enzymes, a total of 103 fragments were scored, and 14 different haplotypes (a–m) were found (Fig. 2). *Ava*I, *Pvu*II and *Sst*II yielded the same fragments in all individuals, and were therefore not discriminant.

Grey mullet shows extensive mtDNA diversity, with a maximum sequence divergence of 3.3% (Fig. 2). The two populations F and L, captured respec-

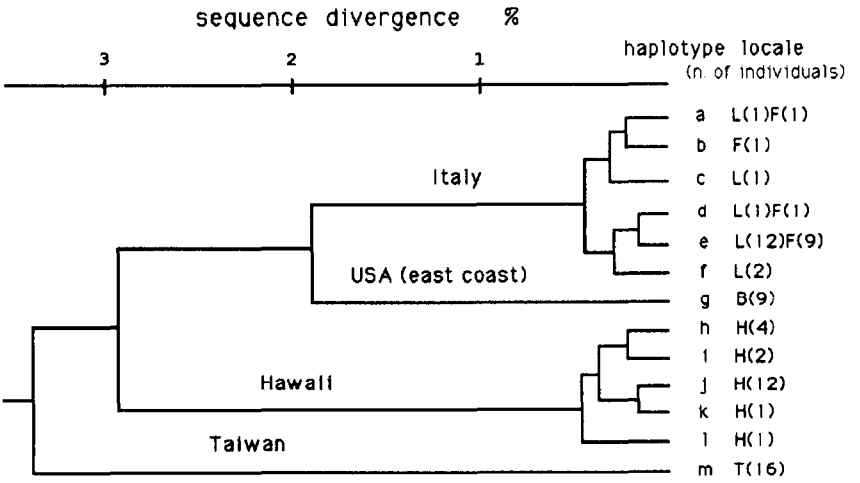

Fig. 2. UPGMA phenogram summarizing relationships among the mtDNA haplotypes in grey mullet.

tively on the west and east coast of Italy, although polymorphic, do not show genetic differentiation. The nearest population to the Mediterranean is North Carolina (USA), a population where all individuals show the same pattern. Estimates of sequence divergence indicate that the North Carolina population mtDNA differs from the Mediterranean populations mtDNA by 1.9%. No major differences are found between North Carolina (B) and Florida (A) for the seven enzymes run on both populations, the fragments of which were comparable. However, Florida (A) shows a small polymorphism. Hawaii (H) and Taiwan (T) are genetically well apart, and do not cluster in a single group representing a Pacific gene pool. Hawaii is closer to the cluster representing the Atlantic and Mediterranean populations, diverging by approximately 2.8%. Whereas Hawaii shows some polymorphism, Taiwan shows none.

Cytogenetics

A total of 244 metaphases from eight specimens were examined. The karyotype was found to be uniformly composed of 48 acrocentric chromosomes for all the sites studied (Fig. 3a) and corresponds to the previous descriptions of *Mugil cephalus* karyotypes from Italy (Cataudella et al., 1974) and Louisiana, USA (Le Grande and Fitzsimons, 1976). The heterochromatin distribution (Fig. 3b,c) does not provide chromosomal markers that distinguish populations differentiated by mtDNA, nor was sex-dependent heterochromatin observed. Beside the usual presence of heterochromatin on the centromeres, C-bands are evident on the telomeres of chromosomes 1 in specimens from all populations. After Ag-staining, NORs are evident on the same telomeric location (Fig. 3d), indicating that the C-positiveness of the telomeric bands is related to the presence of NORs. The telomeres of chromo-

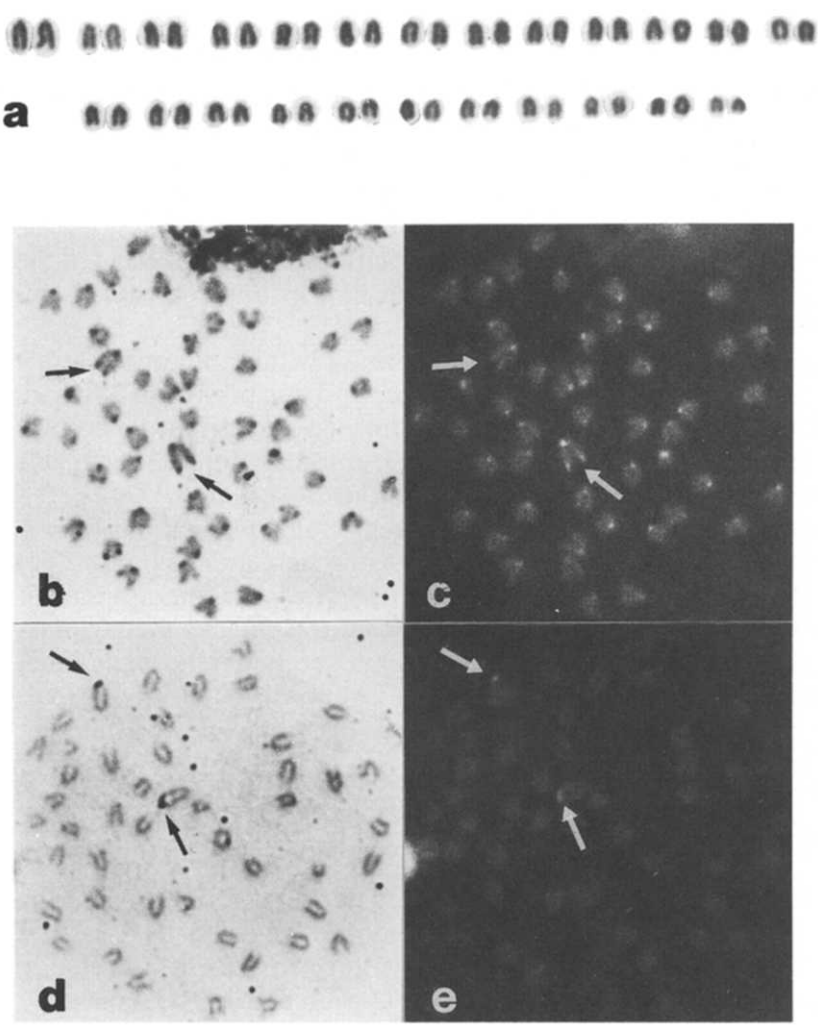

Fig. 3. (a) Giemsa-stained karyotype; (b) C-banded metaphase plate, subsequently DAPI-stained (c); (d) Ag-stained metaphase plate, subsequently CMA_3-stained (e). Arrows indicate telomeres of chromosomes 1, where NORs are located. Male and female specimens from all populations show the same chromosomal pattern after the different staining techniques.

somes 1 show a bright fluorescence when chromomycin A_3-stained (Fig. 3e). All specimens share this unique NOR-chromosomes pair, and no other NOR-bearing chromosomes have been observed. A similar NOR pattern is also shown through Ag- and CMA_3-staining in grey mullets from Texas, USA (Amemiya and Gold, 1986).

DISCUSSION

The different populations analysed are well discriminated by mtDNA analyses: samples are clustered in four groups, Mediterranean, East Atlantic, Central Pacific and East Pacific. Despite their belonging to the same ocean, the two Pacific populations are well distinct, Taiwan being the most different from all the populations sampled. It is, however, difficult to determine to what extent the genetic differences found among populations may be reflected in the phenotype of these fish, and therefore in their biology and consequent cultural practices.

The homogeneity in karyotypes is not surprising within the conservativeness of the "all acrocentric" 48 chromosomes karyotype in percoid fish (Ohno, 1974). However, the homogeneity in NOR number and distribution in specimens from locales so far apart is worth noting, especially considering that it is quite common to find variable NORs in fish (see Sola et al., 1992).

MtDNA analysis and cytogenetics on different mullet populations show a differing resolution in applications to problems in systematics, as pointed out by Hillis and Moritz (1990). The absence of differences in chromosome analyses, even with different banding techniques, confirms the fact that cytotaxonomy is usually best applied to higher taxonomic levels. On the other hand, restriction analyses of mtDNA are best suited for intraspecific comparisons, as estimates of nucleotide sequence divergence discriminated the different mullet populations examined.

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