Japanese Journal of Ichthyology
Vol. 31, No. 4 1985

魚類学雑誌
31巻4号 1985年

# Karyologic and Electrophoretic Studies of the Genus *Cynoscion* (Sciaenidae, Perciformes) from the Northern Gulf of Mexico

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(Received June 16, 1984)

*Cynoscion arenarius* and *C. nebulosus*, the white and speckled seatrouts, are examples of the Sciaenidae or drums, an important family of inshore fishes in the commercial and sport fisheries of the Atlantic, Indian and Pacific oceans. The distribution of *C. arenarius* is apparently restricted to the Gulf of Mexico, but *C. nebulosus* occurs also in the western Atlantic from New York to Florida (Hoese and Moore, 1977). A third species in the Gulf, *C. nothus*, the silver seatrout, is known mainly from deeper, more offshore waters than the two sympatric congeners (Ginsburg, 1931). This paper provides the first formal karyotypes for the genus *Cynoscion* and is the fourth reported karyologic study for the family. Also, there have been very few electrophoretic studies of sciaenids. Shaw (1970) assayed nine enzyme systems coded by 16 loci in *Menticirrhus americanus, Bairdiella chrysura, Stellifer lanceolatus, Leiostomus xanthurus, Micropogon undulatus* and *Cynoscion arenarius* but did not report detailed data, only indices of genetic similarity. Weinstein and Yerger (1976a, b) studied *C. arenarius, C. nothus, C. regalis* and *C. nebulosus*, but assayed only general protein patterns from blood serum, eye lens and muscle, to which they would assign no genetic interpretations. Beckwitt (1983) assayed 19 enzymes coded by 36 loci for *Genyonemus lineatus* and 22 enzymes coded by 38 loci for *Seriphus politus*. Thus our study appears to be the first reported attempt to apply isozyme techniques to a group of congeretic species in the family Sciaenidae.

## Materials and methods

Techniques for preparing permanent chromosome microslides from liver, spleen, and gill tissue included those described by Patton (1967) as modified by LeGrande and Fitzsimons (1976). Chromosome types and numbers were determined primarily from camera lucida drawings with a Wild M20 KGS microscope and, less frequently, from tracings made from projections of chromosome spreads recorded on negative film (Polaroid 665). Chromosome types were designated by assigning the terms metacentric, submetacentric, subtelocentric, telocentric or their combinations on the basis of centromeric positions defined by Levan et al. (1964). The diploid count included the total number of chromosomes in a spread regardless of the type of chromosomes comprising the complement; the fundamental number was determined by assigning a value of one to subtelocentric and telocentric chromosomes and two to metacentric and submetacentric chromosomes. Arm ratios were calculated from idiograms drawn from projected images of chromosomes. Live seatrouts of the species *Cynoscion arenarius* and *C. nebulosus* were collected for karyology on 10 occasions from 1978 to 1983 from the northern Gulf of Mexico along coastal Louisiana.

*Cynoscion nebulosus, C. arenarius,* and *C. nothus* were assayed for eight enzyme systems (Table 2) by horizontal starch gel electrophoresis following the methods of Stein et al. (1984) with one exception; the only buffer used was the 0.1 M Tris-citrate at pH 7.5. Nomenclature for isozyme loci follows the recommendation of Buth (1938). Specimens for electrophoretic analysis were collected from the Gulf of Mexico, near Grand Isle, Louisiana.

Voucher specimens for karyology and electrophoresis were placed into the permanent collections of fishes at the Louisiana State University Museum of Zoology and the University of New Orleans (LSUMZ 2785–2787, 2789, 2790, 2792, and UNO 4008 for *Cynoscion arenarius*; LSUMZ 2784, 2788, 2791, and 2793–2795 for *C. nebulosus*; and UNO 4009 and 4011 for *C. nothus*).

## Results and discussion

**Karyology.** Twenty-two *Cynoscion arenarius* were processed; 19 yielded analyzable spreads. Data were obtained from 18 of 20 *C. nebulosus*. No discernable differences in chromosome number or configuration were detected between *Cynoscion arenarius* and *C. nebulosus*, and no

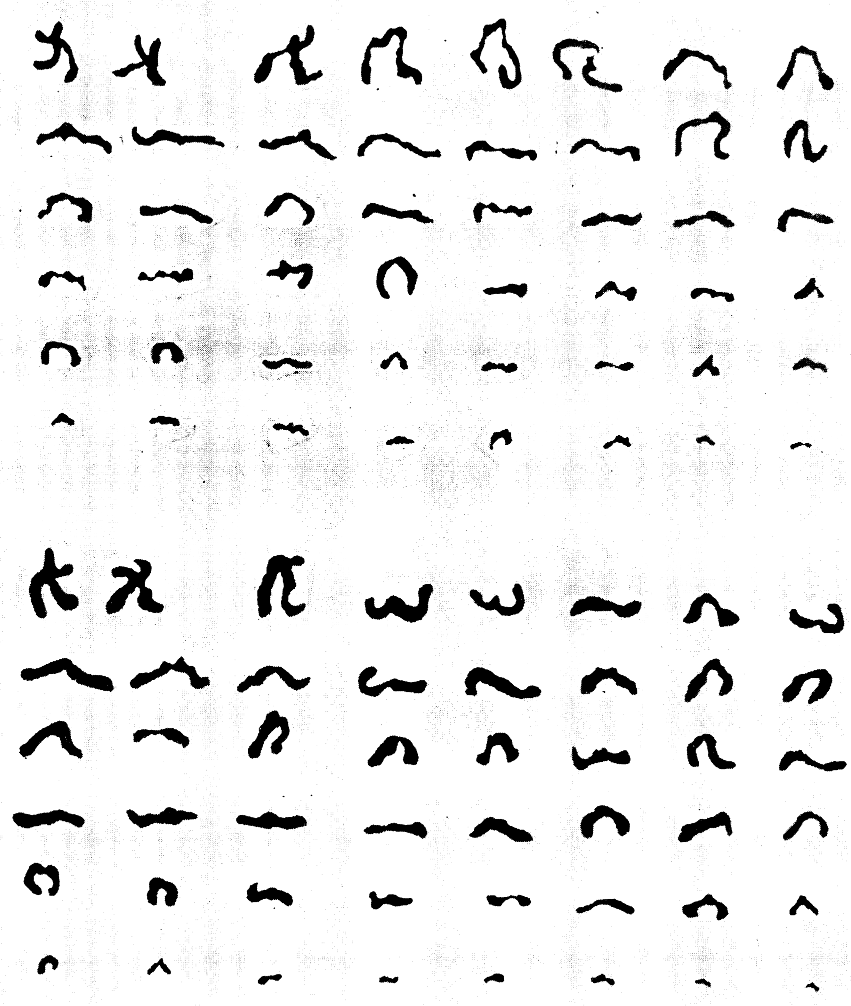

Fig. 1. Chromosome complements of *Cynoscion arenarius* (LSUMZ 2789, top) and *C. nebulosus* (LSUMZ 2791, bottom).

karyotypic differences were noted between sexes of either species (Fig. 1, Table 1). In both species the modal diploid number of 48 occurred in 92 and 94 percent, respectively, of the total cells counted. Counts below 48 probably represented chromosome loss during microslide preparations, undetected overlap among incompletely spread chromosomes, the natural occurrence of fewer chromosomes in some nuclei, or some combination of these. Fundamental numbers below 50 may have been produced in chromosomes with median-zone centromeres by the arms being folded closely together, which in lightly stained material, make them difficult to distinguish from chromosomes with terminal or near-terminal centromeres. In both species, the predominant configuration of chromosomes included one pair of biarmed elements and 23 pairs of acrocentrics with little or no arm development. In well over half the analyzable chromosome spreads, the two biarmed chromosomes were clearly visible, but, in cells with contracted chromosomes, subtelocentrics were often indisguishable from telocentrics. Except for the two metacentric-submetacentric chromosomes, size differences between chromosomes were sufficiently gradual to prevent accurate sorting into homologous pairs. The counts exceeding the modal number of subtelocentric-telocentric chromosomes probably are the result of an inability to recognize lightly stained biarmed elements with folded arms, but the occurrence of an extra metacentric-submetacentric chromosome in two spreads from one specimen of *C. arenarius* is not an artifact. In both spreads with the third biarmed chromosome, the diploid count was 46; in the same fish, spreads with the usual two biarmed elements had a total count of 48.

In a paper describing a karyologic technique using fish scale epithelium, Ramirez (1980) provided a photomicrograph of a chromosome spread from *Cynoscion arenarius*. He indicated a dipliod count of 48 chromosomes but did not

Table 1. Chromosome numbers and types in 8♂♂, 6♀♀ and 5 immatures of *Cynoscion arenarius* and 4♂♂, 7♀♀ and 7 immatures of *C. nebulosus*. For each heading, data for *C. arenarius* (A) are on the left, and, for *C. nebulosus* (B), on the right. Parentheses indicate number of cells. Modal counts are underscored.

| Chromosome numbers | | | | Chromosome types | | | |
|---|---|---|---|---|---|---|---|
| Diploid | | Fundamental | | Metacentric-sumbetacentric | | Subtelocentric-telocentric | |
| A | B | A | B | A | B | A | B |
| 39 (1) | | | | 1 (1?) | 1 (2) | 37 (1) | |
| | | 41 (1) | | | | | 42 (1) |
| | | 43 (1) | | <u>2 (57)</u> | <u>2 (82)</u> | 43 (4) | |
| | 44 (1) | | | | | 44 (2) | 44 (2) |
| | 45 (1) | | | 3 (2) | | 45 (2) | 45 (3) |
| 46 (5) | 46 (1) | | 46 (1) | | | <u>46 (48)</u> | <u>46 (73)</u> |
| 47 (2) | 47 (4) | 47 (1) | | | | 47 (1?) | 47 (2) |
| <u>48 (85)</u> | <u>48 (101)</u> | 48 (1) | 48 (1) | | | | |
| | | 49 (5) | 49 (5) | | | | |
| | | <u>50 (53)</u> | <u>50 (75)</u> | | | | |

Table 2. Enzyme systems examined, loci identified and the primary tissue in which each locus was scored.

| Enzyme | E.C. Number | Locus | No. of alleles | Tissue |
|---|---|---|---|---|
| Phosphoglucomutase | 2.7.5.1 | Pgm-A | 3 | Muscle |
| Glucosephosphate isomerase | 5.3.1.9 | Gpi-A | 1 | Muscle |
| | | Gpi-B | 4 | Muscle |
| Lactate dehydrogenase | 1.1.1.27 | Ldh-A | 1 | Muscle |
| | | Ldh-B | 2 | Muscle |
| | | Ldh-C | 1 | Eye |
| Aspartate aminotransferase | 2.6.1.1 | M-Aat-A | 2 | Liver |
| | | S-Aat-A | 2 | Muscle |
| | | S-Aat-B | 2 | Liver |
| Glycerol-3-phosphate dehydrogenase | 1.1.1.8 | G-3-pdh-A | 1 | Muscle |
| Malate dehydrogenase | 1.1.1.37 | M-Mdh-A | 1 | Muscle |
| | | S-Mdh-A | 1 | Muscle |
| | | S-Mdh-B | 1 | Muscle |
| Phosphogluconate dehydrogenase | 1.1.1.44 | Pgdh-A | 4 | Liver |
| Isocitrate dehydrogenase | 1.1.1.42 | Idh-A | 1 | Eye |
| | | Idh-B | 2 | Eye |

identify chromosome types. Our examination of the figure confirmed the count of 48 and indicated the presence of at least one metacentric-submetacentric element. A second biarmed chromosome was tentatively identified although it was overlapped by a telocentric chromosome. In another paper describing chromosome preparation techniques, Gregory et al. (1980) presented a karyotype with chromosomes stained for C-bands from the silver perch *Bairdiella chrysura*. The complement included 52 telocentrics. However, Black and Howell (in Gold et al., 1980) reported 48 as both the diploid and fundamental chromosome number for *B. chrysura*. LeGrande (pers. comm.) suggested that the discrepancy could be resolved with additional preparations from fresh specimens but noted that changes in chromosome number in established cell lines, such as those used by Gregory et al., are not uncommon. Patro and Prasad (1979) described the karyotypes of the Indian Ocean sciaenids *Johnius carutta* and *J. volgeri* as having a diploid complement of 48 subtelocentric-telocentric chromosomes.

Table 3. Allele frequencies at eight loci in three species on *Cynoscion*. Numbers in parentheses are sample sizes.

| Locus | *arenarius* | *nebulosus* | *nothus* |
|---|---|---|---|
| S-Aat-A | (15) | (6) | (9) |
| a | 1.00 | 1.00 | — |
| b | — | — | 1.00 |
| S-Aat-B | (15) | (3) | (6) |
| a | 0.83 | 1.00 | 0.17 |
| b | 0.17 | — | 0.83 |
| Pgdh-A | (15) | (3) | (6) |
| a | 0.97 | — | — |
| b | — | 1.00 | — |
| c | — | — | 1.00 |
| d | 0.03 | — | — |
| Idh-B | (15) | (6) | (9) |
| a | 0.97 | 1.00 | 1.00 |
| b | 0.03 | — | — |
| Pgm-A | (15) | (6) | (9) |
| a | 0.93 | — | 0.94 |
| b | 0.03 | — | 0.06 |
| c | 0.03 | 1.00 | — |
| Gpi-B | (15) | (6) | (9) |
| a | 0.53 | 1.00 | — |
| b | 0.47 | — | — |
| c | — | — | 0.94 |
| d | — | — | 0.06 |
| Ldh-B | (15) | (6) | (9) |
| a | 1.00 | — | — |
| b | — | 1.00 | 1.00 |
| M-Aat-A | (15) | (3) | (9) |
| a | 1.00 | — | 1.00 |
| b | — | 1.00 | — |

Table 4. Nei's (1972) genetic distances (above diagonal) and Rogers' (1972) genetic distances (below diagonal) between three species of *Cynoscion*.

| | | | | |
|---|---|---|---|---|
| 1 | *C. nebulosus* | — | 0.309 | 0.438 |
| 2 | *C. arenarius* | 0.288 | — | 0.314 |
| 3 | *C. nothus* | 0.361 | 0.284 | — |

Thus, reported diploid chromosome numbers for the Sciaenidae include 48 for four species and 48 or 52 for a fifth one.

**Electrophoresis.** The assay of eight enzymes revealed 16 loci (Table 2). Eight (Gpi-A, Ldh-A, Ldh-C, G-3-pdh-A, M-Mdh-A, S-Mdh-A, S-Mdh-B, and Idh-A) exhibited no variation and one other (Idh-B) was invariant with the exception of one heterozygous individual. The remaining seven loci varied significantly among the three species (Table 3).

In contrast to their karyotypic similarity, *Cynoscion arenarius* and *C. nebulosus* appear to be quite distinct at the gene level; they differ appreciably at five of the 16 loci assayed in this study (Table 3). They are also distinct from a third congener, *C. nothus*. But genetic distances (Nei, 1972; Rogers, 1972; Swofford and Selander, 1981) among the three species, based on the 16 loci reported in this study (Table 4), indicate that they are closer to each other than the average genetic distance for other congeneric species of fishes (Avise, 1976; Ayala, 1975).

**Acknowledgments**

We thank J. W. Korth, A. J. Doucette (Louisiana State University), W. F. Font (University of Wisconsin), and R. J. F. Smith (University of Saskatchewan) for help in collecting, D. S. Stein for technical assistance, and D. L. Boesch and W. Delaune for the use of the Port Fourchon facility of the Louisiana Universities Marine Consortium. The comments on the manuscript by W. H. LeGrande (University of Wisconsin) and J. W. Korth are appreciated.

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### メキシコ湾北部の *Cynoscion* 属魚類（ニベ科）の核学的，電気泳動学的研究

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メキシコ湾北部に分布する *Cynoscion* 属の 2 種，*C. arenarius* と *C. nebulosus* の核型が分析された．またこれら 2 種に *C. nothus* を加えて，電気泳動によるアイソザイム分析が行われた．*C. arenarius* と *C. nebulosus* は同一核型を有し，それは 2n=48，中部・次中部着糸型 1 対と次端部・端部着糸型 23 対で表された．*Cynoscion* 属 3 種の 8 つの酵素 (Pgm, Gpi, Ldh, Aat, G-3-pdh, Mdh, Pgdh, Idh) から，合計 16遺伝子座がみいだされた．これらの電気泳動的資料の解析と既報の知見から，3 種の遺伝的隔たりが，他の属の種間におけるよりもより近いことが推察された．