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Short Communication

Molecular markers in Chinese carps and their interspecific hybrids

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Abstract

A sample consisting of 2 *Ctenopharyngodon idella* (grass carp) females, 2 *Aristichthys nobilis* (bighead carp) males, and 10 interspecific hybrids was analyzed using a multidisciplinary approach. The 10 hybrids were the only surviving specimens of a crossbreeding experiment. The cytological approach, involving the measurement of the largest axis of the erythrocytes, revealed that the parental species were diploid, whereas, among the hybrids, eight individuals were triploid, and two were diploid. Two of the triploid individuals were analyzed cytogenetically and showed 2n = 3X = 72 chromosomes. Using the biochemical and molecular approaches, it was possible to confirm that one of the *C. idella* specimens was the true mother of the hybrids. Those data also allowed to rule out both males from parenthood. The molecular analyses also indicated the absence of gynogenetic individuals among the hybrids. We discuss the use of a multidisciplinary approach as an efficient tool in the monitoring of aquaculture programs.

Key words: SPAR, carps, hybrids.

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Chinese carps are the main fish group used by Asian countries in aquaculture programs. The grass carp, *Ctenopharyngodon idella,* is currently used for the biological control of aquatic plants. Since the bighead carp, *Aristichthys nobilis*, and the grass carp display differential feeding patterns, these two species are commonly used in polyculture programs (Huet, 1978). In 1978, Márián and Krasznai reported the occurrence of a triploid sterile hybrid of grass and bighead carps that could be employed for the control of aquatic vegetation, with no risk of overpopulation.

The Chinese carps *Ctenopharyngodon idella* (grass carp), *Aristichthys nobilis* (bighead carp) and *Hypophthalmichthys molitrix* (silver carp) were introduced in Brazil in 1979 by the aquaculture program of the Centro de Pesquisas e Treinamento em Aqüicultura (Research and Training Center in Aquaculture) CEPTA/IBAMA, Pirassununga, State of São Paulo, Brazil (Almeida-Toledo *et al*., 1995).

Nowadays, in most Brazilian regions these Chinese carps are no longer cultivated for economic purposes, but the remaining hatchery stocks of this exotic fish represent a suitable sample for genetic analysis.

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A very good specific molecular marker, obtained by the SPAR (single primer amplification reaction) technique, was described by Gupta *et al.* (1994). This technique is based on PCR (polymerase chain reaction) amplification of DNA markers, using single primers of simple sequence repeats (SSR) or microsatellites, and has been used in studies of species diversity and geographic distribution of neotropical freshwater fish species (Fernandes-Matioli *et al*., 2000).

In the present study, we analyzed a parental stock of grass and bighead carps and their F1 hybrids, using SPAR nuclear genome markers, and we were able to characterize the parental species and their F1 hybrids, and to suggest parenthood, based on polymorphic patterns of the molecular markers.

Two *C. idella* females (C1 and C2), 2 *A. nobilis* males (A1 and A2), and 10 interspecific hybrids (H1 to H10) obtained from crosses performed in 1998 were analyzed. These individuals are maintained in culture conditions at the Estação de Aqüicultura do IBAMA (IBAMA Aquaculture Station)/Chapecó, State of Santa Catarina, Brazil, exclusively for research.

Blood samples were collected from the caudal artery and/or vein, using heparinized syringes, and conserved in 85-96% ethanol. DNA was isolated by the standard phenol:chloroform protocol (Sambrook *et al*., 1989). The nuclear genome of each individual was characterized using the SPAR-PCR technique (Gupta *et al*., 1994; Fernandes-

Matioli *et al*., 2000). Tetranucleotide repeat primer $(AAGC)_4$ was employed in the SPAR-PCR approach. The PCR reactions were as follows: 5-10 ng of DNA template were amplified in a final volume of 30 µL containing 10 mM Tris.HCl, pH 8.4, 0.5% nonidet P-40, 50 mM KCl, 4.0 mM $MgCl_2$, 100 µM each of dNTP, 5 pmol primers, and 1.25 units of *Taq*DNA polymerase (Life Technologies). Amplifications were performed in an Eppendorf Mastercycler Gradient thermocycler for 35 cycles, as follow: 15 cycles of 45 s at 94 °C, 60 s at 58 °C, and 60 s at 72 °C; and 20 cycles of 45 s at 94 °C, 60 s at 57 °C, and 60 s at 72 °C. All products were analyzed on 1.4% agarose gel stained by ethidium bromide. The patterns obtained by DNA amplification using $(AAGC)_4$ as a primer are referred to in this paper as *micro*13 patterns.

Two hybrids (triploids) were subjected to cytogenetic analysis. Chromosome preparation from kidney cells was performed following Foresti *et al*. (1981). Metaphases were stained with a 3% Giemsa staining solution.

For all the specimens, triploid and diploid hybrids were also identified by the measurement of the largest erythrocyte axis, according to Ueno (1984). In this approach, slides with blood smears were stained with a 2% Giemsa staining solution. The measurement of the largest erythrocyte axis was carried out using a MC80 Axioskop microscope.

The SPAR profiles are presented in Figure 1. The *micro*13 patterns obtained by amplification using $(AAGC)_4$ as a primer were species-specific for *C. idella* and *A. nobilis*, and the hybrids presented a combination of the parental patterns. We found a polymorphism within *C. idella*, characterized by an amplified fragment of about 900 bp (base pairs), present in individual C2, but not observed in the hybrids (Figure 1). According to previous information, individual C1 is the true mother. On the other hand, the two males presented at least three sharp amplified bands, with a relatively high molecular size, that are not observed in any of the hybrids. These differential patterns allowed us to conclude that none of the males studied was the true male parent of the hybrids analyzed.

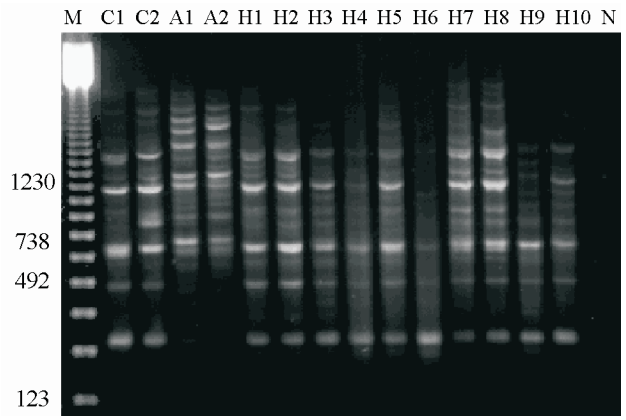

Figure 1 - Products of amplification by the SPAR technique, representing *micro*13 patterns. M = standard molecular marker (123 pb DNA ladder, GibcoBRL); C1 and C2 = *C. idella*, female; A1 and A2 = *A. nobilis*, male; H1 to H10 = interspecific hybrids; N = negative control.

The parental species *C. idella* and *A. nobilis* used in the present study are morphologically distinct. Interspecific hybrids usually exhibit a complex mixture of morphological characteristics inherited from dam and sire, presenting a wide polymorphism involving morphological and meristic traits within F1 (see, for example, Makayeva and Sukhanova, 1966; Aliev, 1967; Andriyasheva, 1973). As shown by Beck and Biggers (1982), offspring produced by hybridizing *C. idella* with *A. nobilis* can yield diploid, triploid and gynogenetic individuals. The cytogenetic analysis of two hybrids revealed a triploid number of 2n = 3X = 72 chromosomes. Ploidy was also verified by the measurement of the largest erythrocyte axis. The latter technique is better, since it avoids the animal sacrifice. The genome composition of the hybrids could not be inferred from their morphological features; thus, the molecular marker approach may be a useful tool for the analysis of the genetic characteristics of hybrids.

It is also known from the literature that the hybridization between *C. idella* and *A. nobilis* produces offspring in which a significant percentage is of triploid hybrids (see, for example, Magge and Philip, 1982; Allen and Wattendorf, 1987). Beck and Biggers (1982) reported that diploid *C. idella* and *A. nobilis* hybrids are more likely to be deformed and stunted in growth than triploids. This fact might cause a higher mortality of diploid individuals during their development, which could add to a bias in the ratio between triploid and diploid individuals in the offspring. According to information obtained from the breeders of the present sample, a high mortality was observed in the offspring obtained in 1998 and, among the remaining fish, 20% of diploid hybrids were observed, which corroborates the hypothesis discussed above.

Our results using SPAR-PCR, although not useful for evidencing the diploid or triploid character of the hybrids, revealed that the parental species presented specific amplification patterns, which we designated here as *micro*13 patterns. The comparison between the *micro*13 patterns found in the parental species and those found in the hybrids allowed us to confirm that the parental female C1 was the true mother, and to exclude both *A. nobilis* individuals analyzed as true fathers of the hybrids (Figure 1).

Although a sample used in the present study was small, the results obtained highlight the suitability of molecular markers for interspecific hybrid studies and characterization. The molecular approaches helped to clarify distinct genetic aspects of the individuals, such as their kinship relationships.

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References

Aliev DS (1967) Morphology of young-of-the-year hybrids of phytophagous fish. Vopr Ikhtiol 7:191-194.

Allen SKJr and Wattendorf RJ (1987) Triploid grass carp: Status and management implications. Fisheries 12:20-24.

Almeida-Toledo LF, Bigoni APV, Bernadino G and Toledo-Filho SA (1995) Chromosomal location of NORs and Bands in F1 hybrids of bighead carp and silver carp reared in Brasil. Aquaculture 135:227-284.

Andriyasheva MA (1973) Viability of phytophagous fish hybrids. Izv. Gos. Nauchno-Issled. Inst Ozern Rech Rybn Khoz 85:73-86.

Beck ML and Biggers CJ (1982) Chromosomal investigation of *Ctenopharyngodon idella* X *Aristichthys nobilis* hybrids. Experientia 38:319.

Fernandes-Matioli FMC, Matioli SR and Almeida-Toledo LF (2000) Species diversity and geographic distribution of *Gymnotus* (Pisces: Gymnotiformes) through analysis of nuclear $(GGAC)_n$ microsatellites. Genet Mol Biol 23:1-5.

Foresti F, Almeida-Toledo LF and Toledo-Filho SA (1981) Polymorphic nature of nucleolus organizer regions in fishes. Cytogen Cell Genet 31:137-144.

Gupta M, Chyi YS, Romero-Severson J and Owen, JL (1994) Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theor Appl Genet 89:998-1006.

Huet M (1978) Tratado de Piscicultura. 2nd ed. Edições Mundi-Prensa, pp 189-190.

Magge SM and Philipp DP (1982) Biochemical genetic analyses of the grass carp X bighead carp F1 hybrid and the parental species. Trans Amer Fish Soc 111:593-602.

Makayeva AP and Sukhanova AI (1966) Development of hybrids of phytophagous fishes. Vopr Ikhtiol 6:477-497.

Márián T and Krasznai Z (1978) Karyological investigations on *Ctenopharyngodon idella* and *Hypophthalmichthys nobilis* and their cross-breeding. Aquacul Hungarica 1:44-50.

Sambrook J, Fritsch EF and Maniatis T (1989) Molecular cloning. A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, New York.

Ueno K (1984) Induction of triploid carp and their hematological characteristics. Jpn J Genetics 59:585-591.

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