A Cytogenetic Analysis of the Karyotypes of the Golden Shiner, *Notemigonus crysoleucas*, the Rudd, *Scardinus erythrophthalmus*, and Their Reciprocal F_1 Hybrids

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ABSTRACT. Standard (non-differentially stained) karyotypes of the golden shiner, *Notemigonus crysoleucas*, rudd, *Scardinus erythrophthalmus*, and their reciprocal F_1 hybrids were documented. Both species possess $2N = 50$ chromosomes. The karyotype of the golden shiner is comprised of 7 pairs of metacentric chromosomes, 11 pairs of submetacentric chromosomes, and 7 pairs of subtelo-/telocentric chromosomes, giving a (diploid) chromosome arm number estimate of 86. The karyotype of the rudd is comprised of 6 pairs of metacentric chromosomes, 9 pairs of submetacentric chromosomes, and 10 pairs of subtelo-/telocentric chromosomes, giving a

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(diploid) chromosome arm number estimate of 80. Reciprocal F_1 hybrids possessed the expected number of chromosomes, i.e., 13 metacentric chromosomes, 20 submetacentric chromosomes, and 17 subtelo-/telocentric chromosomes. Specific marker chromosomes from both species were identified in the F_1 hybrids. These data demonstrate substantial chromosomal differences between the two species, which could lead to chromosomal segregation anomalies in the meiosis of F_1 hybrids; this could result in at least partial sterility.

INTRODUCTION

Baitfish farming is the third most valuable component of the aquaculture industry in the United States; in 1978, farm-raised baitfish were worth approximately $56 million (Anon. 1990). Baitfish are sold primarily to the recreational fishing industry, but a portion of the fish are sold to the ornamental fish industry to serve as "feeder" fish for piscivorous species. Baitfish are farmed in every state in the Southeast and as far north as New York (USDA 1988). The two principle species in the baitfish industry are the cyprinids *Notemigonus crysoleucas* (golden shiner) and *Pimephales promelas* (fathead minnow). In Arkansas, a total of 10,880 ha was used for baitfish production in 1989; about 75% of this total was devoted to the production of golden shiners (Collins 1990).

Recently, several Arkansas baitfish farmers imported the rudd, *Scardinus erythrophthalmus*, a cyprinid of European origin (see Figure 1). The rudd was acquired because it is similar to the golden shiner in appearance, and baitfish farmers hoped that it would be able to better withstand the rigors of handling. In addition, the rudd is a more attractive fish in that it has bright orange fins, which could make it a more attractive bait. Rudd also grow to a much larger size, and baitfish farmers anticipated that this might enable them to sell the fish to striped bass and/or ice fishermen, two consumer groups who desire large baitfish. However, many states have banned the importation of rudd because it is an exotic species. These rules have almost halted the development of the rudd as a farmed baitfish in the U.S.

One possible compromise might be to produce F_1 hybrids between the two species. Such F_1 individuals might be expected to exhibit hybrid vigor and to possess desirable commercial attributes of both species. In addition, given that the two species are quite distant from one another taxonomically, it also would not be unreasonable to expect that the F_1 hybrids might be sterile.

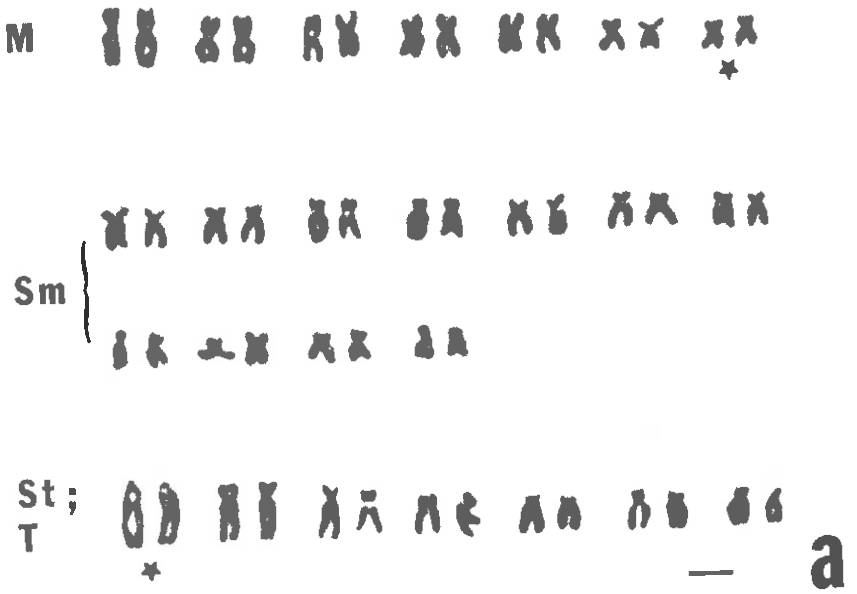
Chiarelli et al. (1969) reported that the rudd possessed $2N = 48$ chromosomes, although a subsequent study by Cataudella et al. (1977) reported that the species possessed $2N = 50$ chromosomes. The golden shiner has been documented to possess $2N = 50$ chromosomes (Leippman and Hubbs 1969; Gold and Avise 1977).

To investigate the possibility of chromosomal differences between the two species, which might lead to meiotic segregation problems in (and concomitant sterility of) F_1 hybrids, the standard or non-differentially stained karyotypes of both species and of their reciprocal F_1 hybrids were determined. The purposes of the study were to document the chromosome number of rudd now maintained in North America, to determine if differences exist between the karyotypes of the two species, and to examine the karyotypes of the reciprocal F_1 hybrids.

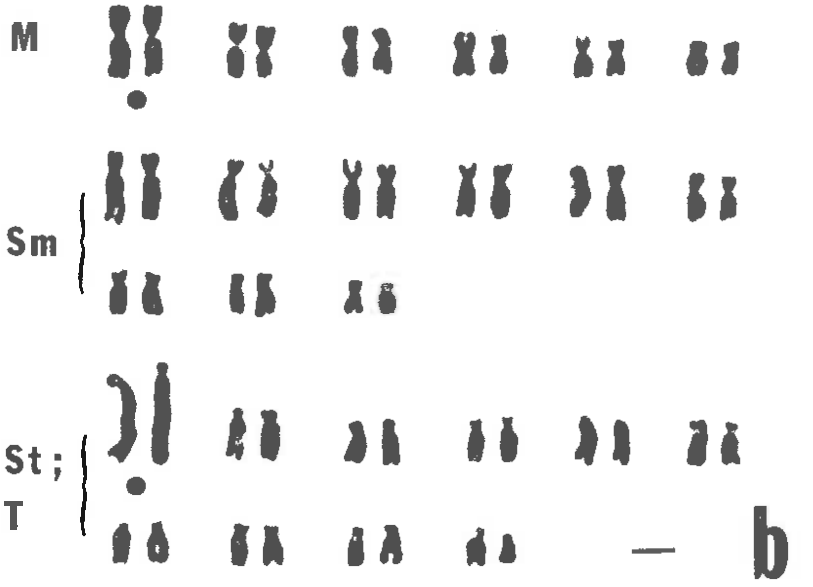
MATERIALS AND METHODS

Individuals of both species were obtained from Anderson Minnow Farms, Lonoke, Arkansas; transported to the Joe Hogan State Fish Hatchery, Arkansas Game and Fish Commission, Lonoke; and maintained in separate ponds. In the spring of 1989, reciprocal crosses between the two species were carried out at the hatchery by stocking separate ponds with either golden shiner females and rudd males or rudd females and golden shiner males. Spawning mats were placed in the ponds, and the eggs were allowed to hatch. The F_1 hybrids were then grown in the ponds, using standard commercial baitfish culture practices (Giudice et al. 1981). Individuals of the parental species and the reciprocal F_1 hybrids were obtained by seining the ponds in the spring of 1990. Fish were then shipped live to Texas A&M University. The maternal parent of all F_1 hybrids was confirmed by comparison of restriction fragments of the mitochondrial DNA (mtDNA) of each individual, as described in Gold et al. (1991).

FIGURE 1. (a) Karyogram of the golden shiner, *Notemigonus crysoleucas*. (b) Karyogram of the rudd, *Scardinus erythrophthalmus*. Chromosomes are arranged into rows as follows: M = metacentric chromosomes; Sm = submetacentric chromosomes; St and T = subtelo-/teleocentric chromosomes. Stars and circles indicate "marker" chromosomes from the golden shiner and rudd, respectively. Bars are the equivalent of 5 μm.



Metaphase chromosomes from individuals of the parental species and their reciprocal F_1 hybrids were prepared using both short- and long-term cell cultures, as described in Gold et al. (1990). Microscope slides were prepared using the methods of Kligerman and Bloom (1977) as modified by Rayburn and Gold (1982). Slides were stained for 3-5 minutes in 2-4% Giemsa in 10^{-2} phosphate buffer, air dried, cleared in xylene for 10 minutes, and mounted in Permount. Bright field photomicroscopy followed procedures given in Gold and Amemiya (1986). Metaphases were examined from seven individuals of each of the parental species and from 15 individuals from each of the reciprocal hybrids.



RESULTS AND DISCUSSION

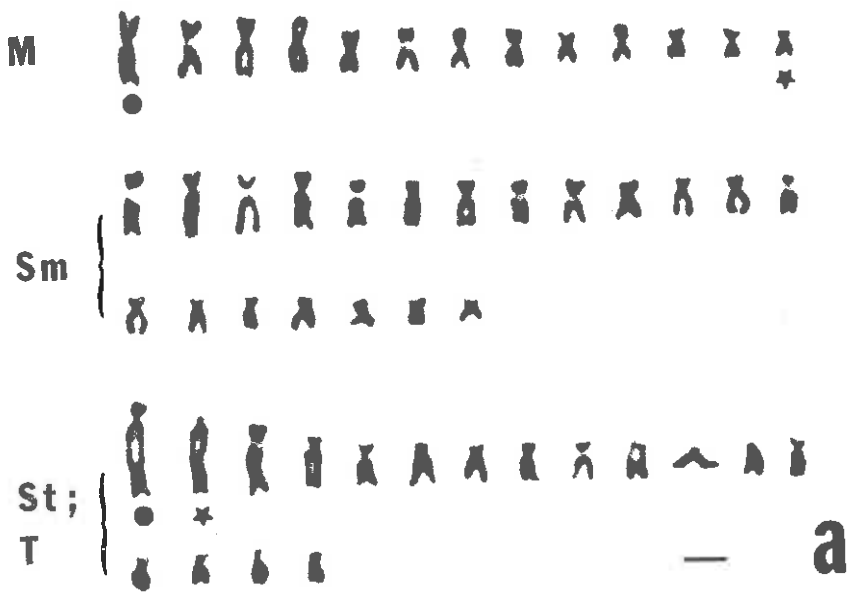
Karyograms of the two parental species are shown in Figure 1. The golden shiners possessed $2N = 50$ chromosomes; within the complement, there were 7 pairs of metacentric chromosomes, 11 pairs of submetacentric chromosomes, and 7 pairs of subtelo-/telocentric chromosomes. By counting the meta- and submetacentric chromosomes as biarmed and the subtelo-/telocentric chromosomes as uniarmed, the estimated (diploid) chromosome arm number of the golden shiner was 86. The rudd also possessed $2N = 50$ chromosomes, with 6 pairs of metacentric chromosomes, 9 pairs of submetacentric chromosomes, and 10 pairs of subtelo-/telocentric chromosomes. The estimated (diploid) chromosome arm number of the rudd was 80. Specific "marker" chromosomes observed in both species include a small pair of metacentric chromosomes and a large pair of subtelo-/telocentric chromosomes in the golden shiner complement, and a large pair of metacentric chromosomes and a

very large pair of subtelo-/telocentric chromosomes in the rudd complement.

Karyograms of reciprocal F_1 hybrids are shown in Figure 2. All of the F_1 hybrids had the expected karyotype of 13 metacentric chromosomes, 20 submetacentric chromosomes, and 17 subtelo-/telocentric chromosomes. The estimated (diploid) chromosome arm number of the hybrids was 83. All four "marker" chromosomes were also evident, particularly the very large subtelo-/telocentric chromosome derived from the rudd parent.

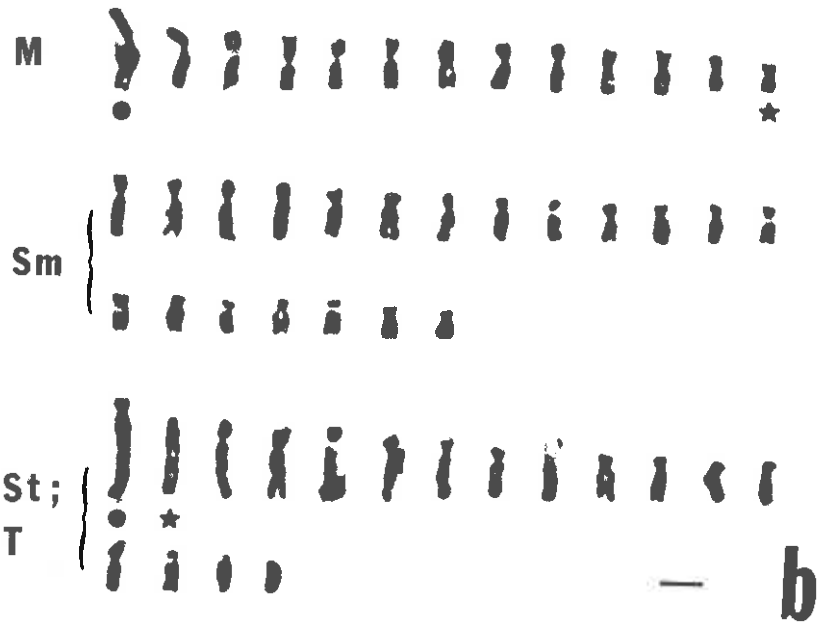
The foregoing demonstrates that the two species differ quite substantially in karyotype, despite the fact that both species have the same chromosome number. Although standard (non-differentially

FIGURE 2. (a) Karyogram of F_1 hybrid from the cross golden shiner ♀ x rudd ♂. (b) Karyogram of F_1 hybrid from the cross rudd ♀ x golden shiner ♂. Chromosomes are arranged in rows as follows: M = metacentric chromosomes; Sm = submetacentric chromosomes; St and T = subtelo-/telocentric chromosomes. Stars and circles indicate "marker" chromosomes from the golden shiner and rudd, respectively. Bars are the equivalent of 5 μm.



stained) karyotypes do not permit assessment of chromosomal homologies between species, there are apparent differences between the two species in the number of chromosomal arms. The estimated chromosome arm numbers, i.e., 86 in the golden shiner versus 80 in the rudd, suggest that a minimum of three different chromosomal rearrangements have occurred since the two species diverged from a common ancestor.

The occurrence of additional chromosomal rearrangements separating the two species may be inferred from the presence of the "marker" chromosomes. Both the large metacentric chromosome and the very large subtelo-/telocentric chromosome in the rudd karyotype have no apparent counterpart in the golden shiner karyotype; similarly, the small metacentric chromosome and the large subtelo-/telocentric chromosome in the golden shiner karyotype have no apparent counterpart in the rudd karyotype. Finally, Gold et al. (1991) demonstrated that the single pair of nucleolar organizer region, or NOR-bearing, chromosomes in the two species were non-homolo-



gous, suggesting that at least one chromosomal rearrangement involving the NOR separated the two species.

The existence of substantial chromosomal differences between the two species suggests the possibility that chromosome pairing during meiosis in F_1 hybrids could be affected, with the net result being the production of genetically aneuploid gametes. This, in turn, could result in at least partial sterility of F_1 individuals. This prediction of at least partial sterility would depend, in large part, on the specific nature of the chromosomal rearrangements differentiating the two species. For example, differences in the number of chromosomal arms or in chromosomal size could stem from inversion or translocation rearrangements, in which case the production of genetically aneuploid gametes would be expected in chromosomal heterozygotes (Swanson et al. 1981). Alternatively, such differences could also stem from additions or deletions of chromosomal material. Were the latter to primarily involve chromosomal heterochromatin, the effects on chromosomal pairing at meiosis might be minimal (John and Miklos 1979).

At this point, the question of whether F_1 hybrids between the two species might exhibit at least partial sterility (and hence be useful in a commercial context) remains open. There is sufficient chromosomal evidence to suggest that chromosomal pairing difficulties might occur in the meiosis of F_1 hybrids. Research on gonadal development of the F_1 hybrids and on their fertility/sterility is being conducted.

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