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STANDARD AND NOR-STAINED KARYOTYPES OF THREE SPECIES OF NORTH AMERICAN CYPRINID FISHES

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Recent studies in our laboratory have been focused on documenting the chromosomal sites of the nucleolar organizer regions (NORs) in North American cyprinid fishes (Amemiya and Gold, 1990a; Amemiya et al., 1991). Because NORs represent the chromosomal locations of the nuclear genes encoding the 18S, 5.8S, and 28S ribosomal RNAs (Ritossa and Spiegelman, 1965; Howell, 1982), they have been useful for identifying homologous chromosomes within and among cyprinid complements. Thus far, our studies have shown that chromosomal NORs vary considerably among North American cyprinids and often can be used to infer or test hypotheses of cyprinid relationships (Amemiya and Gold 1988, 1990a, 1990b; Amemiya et al., 1991).

In this note, we document the chromosomal NOR phenotypes (and standard karyotypes) of three North American cyprinid species—*Notropis greenei*, *Notropis rubellus*, and *Notropis tristis*. The last was formerly known as *Notropis topeka* (see Mayden and Gilbert, 1989). The systematic relationships of *N. greenei* and *N. tristis* are essentially unknown. Both Mayden (1989) and M. M. Coburn (personal communication) are of the opinion that *N. greenei*, a cyprinid confined primarily to the Ozark Uplands (Lee et al., 1980), belongs in the tidy genus *Notropis* (*sensu* Mayden, 1989). This genus includes, among others, the subgenera *Alburnops*, *Hydrophlox*, and *Notropis*, and the *N. texanus* and *N. volucellus* species-groups (Mayden, 1989). No systematic hypotheses regarding *N. tristis*, a cyprinid confined to parts of the Missouri, Mississippi, and Arkansas river drainages (Lee et al.,

TABLE 1. Summary of NOR-stained material examined.

	<i>Notropis greeni</i>	<i>Notropis rubellus</i>	<i>Notropis tristis</i>
Number of specimens examined	3	3	5
Number of metaphases examined	152	145	91
Number of (haploid) NOR chromosomes	1	1	1
NOR chromosome phenotype*	<i>D</i>	<i>D</i>	<i>D</i>

*NOR chromosome phenotype (after Gold and Amemiya, 1986; Amemiya and Gold, 1988): *D*, NOR terminal on the short arm of a medium-sized submetacentric chromosome.

1980), have been published, although Bailey (1959) suggested that *N. tristis* possibly might be related to *Notropis anogenus* and perhaps *Notropis ortenburgeri*. (The systematic relationships of these two species within North American Cyprinidae also is unknown, and neither has been karyotyped.)

Notropis rubellus, a cyprinid broadly distributed in the north-central and northeastern United States (Lee et al., 1980), was originally considered to belong to the subgenus *Notropis* (Hubbs and Ortenburger, 1929; Hubbs and Bonham, 1951). Snelson (1968), however, disagreed with this assessment, and suggested that *N. rubellus* might belong in the subgenus *Hydrophlox*. Swift (1970), in a comprehensive review of the cyprinid subgenera *Alburnops* and *Hydrophlox*, agreed with Snelson (1968) and placed *N. rubellus* in its own monotypic species group within *Hydrophlox*. Swift (1970) cautioned, however, as have others, that considerable morphological variation existed among specimens assigned to *N. rubellus*, and that this should be evaluated before definitive placement of the species in *Hydrophlox*.

Recently, R. L. Mayden (personal communication), using allozyme electrophoresis, and T. E. Dowling (personal communication), using analysis of restriction site variation in mitochondrial DNA (mtDNA), have hypothesized that *N. rubellus* may, in fact, belong to the subgenus *Notropis*. The allozyme data indicate that *N. rubellus* belongs in a clade with *N. atherinoides* and *N. stilbius*, two trenchant members of the subgenus *Notropis* (Coburn, 1982). The mtDNA data indicate that *N. rubellus* is closely allied with *N. atherinoides*.

The purpose of the present study was to determine if the chromosomal NOR phenotypes of the three species could be used to evaluate their relationships within North American Cyprinidae. Specimens of all three species were obtained by seine from natural populations. The taxa (collection localities) were as follows: *N. greeni* (Lee Creek, Arkansas River drainage, Crawford County, Arkansas); and *N. rubellus* and *N. tristis* (Mill Creek, Kansas River drainage, Wabaunsee County, Kansas). The specimens were returned live to the laboratory and maintained in aerated aquaria until sacrificed. Metaphase chromosomes were prepared from cultured fibroblasts following methods outlined in Gold et al. (1990a). Silver-staining for chromosomal NORs was carried out using the method of Howell and Black (1980). Bright field photomicroscopy followed procedures in Gold and Amemiya (1986).

Summary data of the silver-stained material examined from the three species are given in Table 1; silver-stained metaphases from each species are shown in Figure 1. All individuals examined from each species possessed $2N = 50$ chromosomes, as do most North American cyprinids (Gold et al., 1980; Amemiya and Gold, 1990a). The chromosome numbers of all three species are reported here for the first time. All three possessed a single pair of NOR-bearing chromosomes, with the NOR situated on the short arm of a medium-sized submetacentric chromosome. Hereafter, this will be referred to as NOR phenotype *D*,

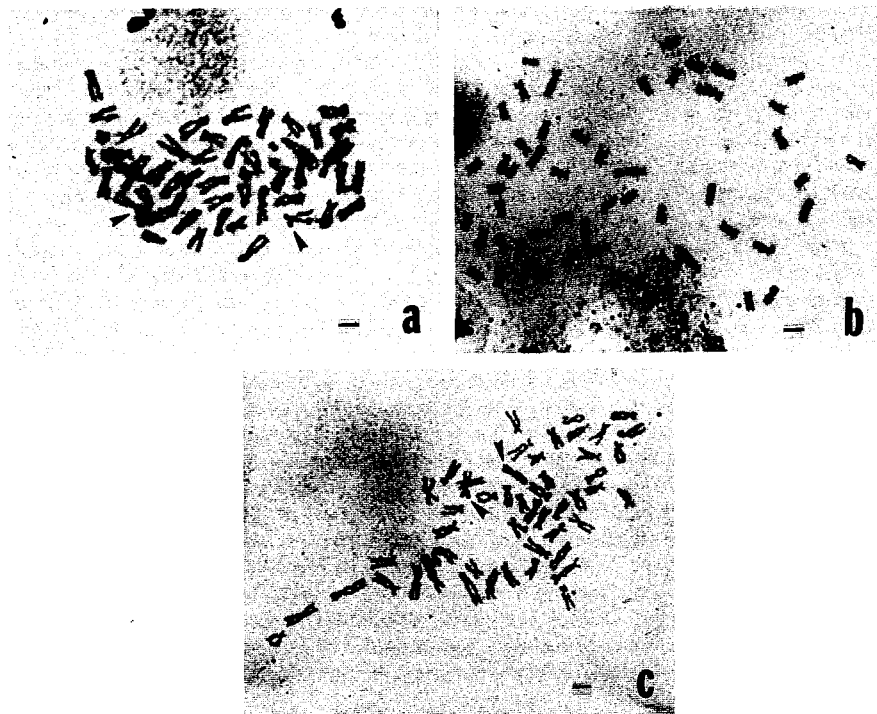


FIGURE 1. Silver-stained metaphases of a) *Notropis greenei*, b) *Notropis rubellus*, and c) *Notropis tristis*. Chromosomal NORs are indicated by arrowheads. Bars are the equivalent of 5 μ m.

following the nomenclature for cyprinid NOR chromosomes developed by Gold and Amemiya (1986) and Amemiya and Gold (1988).

Thus far, we have documented chromosomal NOR phenotypes of nearly 75 species of North American Cyprinidae, excluding the three species examined in this study (Amemiya and Gold, 1990a; Gold et al., 1990b, unpublished data; Powers and Gold, 1992). Of these, 19 species possess a single pair of NOR chromosomes of the *D* phenotype. Twelve additional species possess a pair of *D* NOR chromosomes as well as a second or third pair of a different NOR phenotype. Twenty-eight of these 31 species currently are placed in the "*Notropis*"-like shiner (*sensu* Mayden, 1989) clade. On the surface, this suggests that the three species examined in this study probably belong in this clade, an hypothesis not inconsistent with the current placement of two of the species (*N. greenei* and *N. rubellus*) in the "*Notropis*"-like shiner assemblage (Mayden, 1989). It is interesting to note that nearly all the cyprinids with a single pair of *D* NOR chromosomes currently are placed in the genus *Notropis* (*sensu* Mayden, 1989). The exceptions are *Notropis ludibundus*—formerly *N. stramineus* (see Mayden and Gilbert, 1989)—and three species (*N. ammophilus*, *N. dorsalis*, and *N. longirostris*) placed by Mayden (1989) in the genus *Hybopsis*, but which Coburn and Cavender (1991) placed in the lineage Mayden (1989) referred to as the genus *Notropis*. One hypothesis is that all of the species (including the three examined in this study) belong in a clade defined by the presence of a single pair of *D* NOR chromosomes. However, relationships of *N. greenei*, *N. rubellus*, and *N. tristis* within this putative clade cannot be determined solely on the basis of chromosomal NOR phenotype because

homologies of the various *D* NOR chromosomes have yet to be tested using other types of chromosomal banding (for example, serial or G-bands).

The chromosomal NOR phenotype of *N. rubellus* is of interest relative to the previously noted disagreements over the taxonomy of this species. We have examined NOR phenotypes of six of the 14 extant species in the subgenus *Notropis* and of three of the nine extant species in the subgenus *Hydrophlox* (Amemiya and Gold, 1990a). All three species from *Hydrophlox* (*N. baileyi*, *N. chrosomus*, and *N. nubilus*) possess a single pair of *D* NOR chromosomes. Among the six species examined from the subgenus *Notropis*, only *N. stilbius* possesses a *D* NOR chromosome. The remainder, including *N. atherinoides*, possess a NOR on a large-sized acro-/subtelocentric chromosome which also is the largest chromosome in the complement (three species), on a small submetacentric chromosome (one species), or both (one species). Gold and Li (1991) hypothesized that a NOR located on a large acro-/subtelocentric chromosome may be plesiomorphic or primitive for the "*Notropis*"-like shiners, and hence for the subgenus *Notropis* as well. Taken together, these data suggest either that *N. rubellus* does, in fact, belong in the subgenus *Hydrophlox* (or at least in a clade defined by a single pair of *D* NOR chromosomes), or that *N. rubellus* and *N. stilbius* represent sister species within the subgenus *Notropis*. The latter assignment assumes that *N. stilbius* is a valid member of the subgenus *Notropis*. Further studies designed to test homologies of the *D* NOR chromosomes are now in progress.

We thank T. Buchanan, J. Jenkin, P. Powers, C. Ragland, K. Shaw, and T. Schmidt for assistance in procuring the specimens examined in this study, J. Jenkin for some of the data regarding *N. rubellus*, and L. Richardson and T. Schmidt for critical comments on an early draft of the manuscript. The work was supported in part by the National Science Foundation under grants BSR-8415428 and INT-8815517, and in part by the Texas Agricultural Experiment Station under Project H-6703. The paper represents number XXIII in the series "Cytogenetic studies in North American minnows (Cyprinidae)."

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FIRE ANT MANAGEMENT ON A VIRGIN BLACKLAND PRAIRIE

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Since its introduction near Mobile, Alabama, in the 1930s, the red imported fire ant, *Solenopsis invicta* Buren, has expanded its range to include the entire southeastern United States from North Carolina to Texas. The polygynous or multiple-queen form of the fire ant is thought prevalent over more than 50 percent of the range of the species in Texas, including Collin County in the northern part of the state (Porter et al., 1991). An invasion by polygynous fire ants, which have a much greater mound density than the monogynous or