

# Cytogenetic studies in North American minnows (Cyprinidae). XIV Chromosomal NOR phenotypes of eight species from the genus *Notropis*

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## Abstract

The karyotypes and chromosomal nucleolus organizer region or NOR phenotypes of eight species from the diverse North American cyprinid genus *Notropis* are documented. Five of the species are representatives of the *Notropis* subgenus *Cyprinella*, and three are representatives of the previously unexamined *Notropis* subgenera *Hydrophlox* and *Luxilus*. All eight species possessed  $2n = 50$  chromosomes and a single pair of chromosomal NORs. In four of the five *Cyprinella* species, the NOR was located terminally on one arm of a medium-sized metacentric chromosome; in the fifth *Cyprinella* species, the NOR was located terminally on the short arm of a large submetacentric chromosome which also was the largest chromosome in the complement. In the remaining three species (from *Hydrophlox* and *Luxilus*), the NOR was located terminally on the short arm of a medium-sized submetacentric chromosome. The results of this and earlier studies indicate that the patterns of variation in chromosomal NORs within the genus *Notropis* are more complex than previously believed and that the plesiomorphic NOR condition in *Notropis* has yet to be unequivocally identified.

## Introduction

Differential banding of metaphase chromosomes has provided a powerful tool for the study of systematic and phylogenetic relationships among a wide variety of vertebrates. Although much of the work in this area has focused on mammals (Dutrillaux *et al.*, 1982a,b; Baker *et al.*, 1982,1983; Rogers *et al.*, 1984), considerable chromosome banding data also have been published on birds, turtles, snakes and amphibians (Stock and Mengden, 1975; Stock and Bunch, 1982; Bickham and Baker, 1976; Mengden and Stock, 1980; Schmid, 1980). Conversely, comparatively few studies of chromosome banding used in a systematics context have been published on fishes. The reasons for this are in large part due to the difficulties in obtaining sufficient quantities of well-spread metaphases from fish tissues (Gold, 1979).

Recently, we have developed reliable methods for obtaining suitable harvests of fish chromosomes and for differentiating chromosomal nucleolus organizer regions or NORs on fish chromosomes (Gold and Ellison, 1983; Amemiya *et al.*, 1984; Amemiya and Gold, 1986). These methods have been used to study the inter- and intraspecific patterns of chromosomal NOR variation among North American cyprinid fishes (Gold, 1984; Gold and Amemiya, 1986; Amemiya and Gold, 1988). Although problems of homologization of NOR-bearing chromosomes

across species still remain (Amemiya and Gold, 1988), these studies have clearly shown that (1) species-specific differences in chromosomal NOR phenotypes exist among North American cyprinids and are taxonomically informative, and (2) systematic or phylogenetic hypotheses inferred from the interspecific NOR chromosome differences are not substantially discordant with hypotheses of relationships inferred from other (primarily morphological) data bases.

In this paper, we document the chromosomal NOR phenotypes of eight species from the diverse North American cyprinid genus *Notropis*. Briefly, the genus *Notropis* is the most speciose of all North American cyprinid genera and contains well over 100 living representatives (Miller, 1965; Coburn, 1982; Mayden, 1985). About half of the nominal *Notropis* species have been placed on morphological bases into six fairly well-defined, putatively monophyletic subgenera, although other putatively monophyletic assemblages or species-groups within the genus also have been proposed (Coburn, 1982; Mayden, 1985). Mayden (1985), in fact, has suggested that many of the putatively monophyletic assemblages within *Notropis* should be elevated to generic rank.

In our previous studies (Gold, 1984; Gold and Amemiya, 1986; Amemiya and Gold, 1988), we examined chromosomal NOR phenotypes of representatives from the *Notropis* subgenera *Cyprinella*, *Lythrurus* and *Notropis*, as well as several species whose relationships within *Notropis* were either unknown or incompletely established. Of the eight species studied here, five are representatives of the subgenus *Cyprinella*, and three are representatives of the previously unexamined subgenera *Hydrophlox* and *Luxilus*. The purposes of the study were to continue our sampling of chromosomal NOR phenotypes within *Notropis* and to begin construction of a chromosomal hypothesis of species relationships within the subgenus *Cyprinella*.

### Materials and methods

The fish examined in this study were collected by seine from natural populations. The species (collection localities) were as follows: *Notropis baileyi* (Okatoma Creek, Covington Co., Mississippi); *Notropis camurus* (Thompson's Creek, West Feliciana Par., Louisiana); *Notropis galacturus* (Spring River, Sharp Co., Arkansas); *Notropis nubilus* (Lee Creek, Crawford Co., Arkansas); *Notropis pilsbryi* (Jenkyns Creek, Jasper Co., Missouri, and the West Fork of the White River, Washington Co., Arkansas); *Notropis proserpinus* (Pecos River, Val Verde Co., Texas); *Notropis spilopterus* (Chagrin River, Lake Co., Ohio); and *Notropis whipplei* (Ouachita River, Montgomery Co., Arkansas). All individuals collected were returned live to our laboratory in College Station, Texas, and maintained in aerated aquaria. Voucher specimens, including the individuals karyotyped, will ultimately be deposited in the Texas Cooperative Wildlife Collection at Texas A and M University.

Metaphase chromosome preparations were made either directly from kidney tissue of colchicine- or colcemid-injected specimens (after Gold, 1984) or from cultured fibroblasts (after Amemiya *et al.*, 1984). NOR-banding was accomplished

by the controlled silver technique of Howell and Black (1980) as modified by Gold and Ellison (1983). Chromosomes stained with silver (and counterstained with Giemsa) were photographed in bright-field using Kodak Technical Pan 2415 film (ASA 40) developed in Diafine (Acufine) or HC-110 dilution D (Kodak). Determinations of NOR-band position(s) and size and of relative size and centromere position of NOR-bearing and other chromosomes were made from positive prints using a digitizer, a small laboratory computer, and the BANDSCAN program described in Gold and Amemiya (1986).

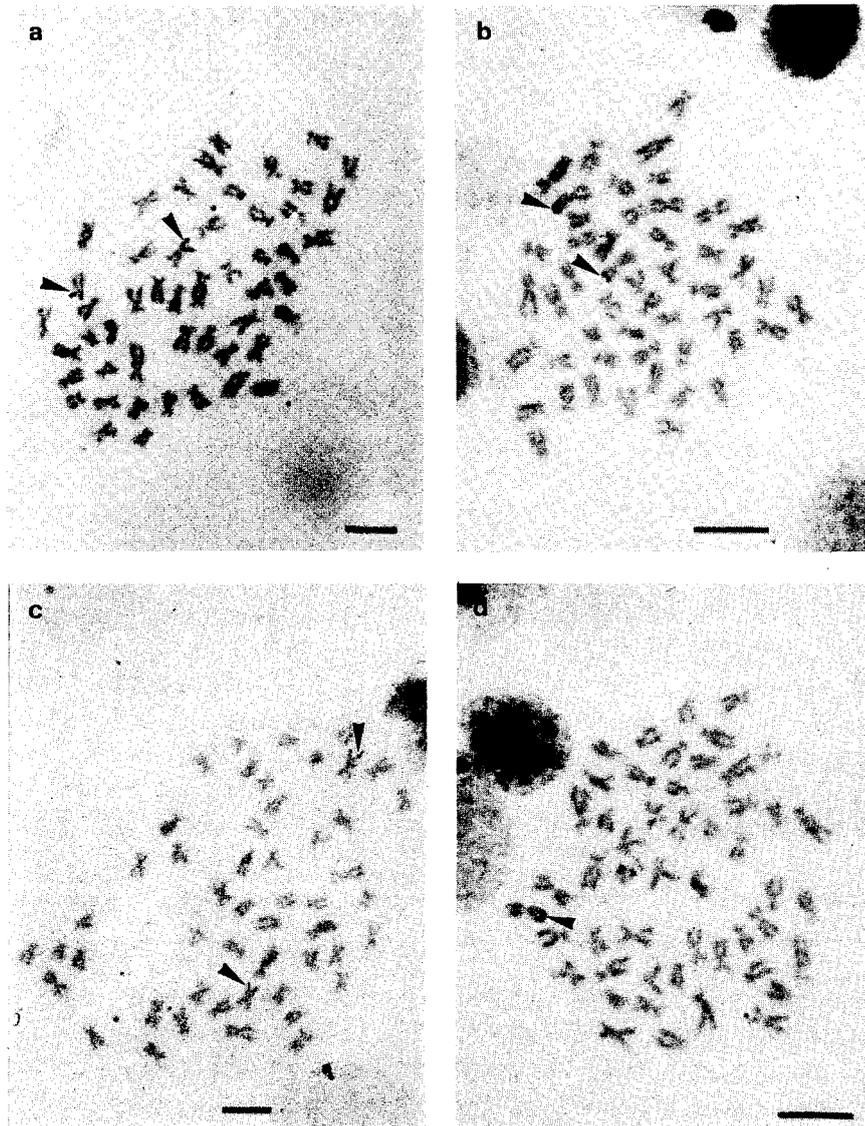
## Results

Summary data of the silver-stained material from the eight species are shown in Table 1, and representative silver-stained metaphases are shown in Figures 1 and 2. The species are arranged in Table 1 according to subgenus following Gilbert (1964), Swift (1970), and Mayden (1985). All individuals from all species possessed  $2n = 50$  chromosomes, as has been the case for all *Notropis* species examined to date (Gold *et al.*, 1980; Amemiya and Gold, 1987; Amemiya, unpublished). The chromosome numbers of all but *N. camurus* and *N. proserpinus* are reported here for the first time (*op. cit.*).

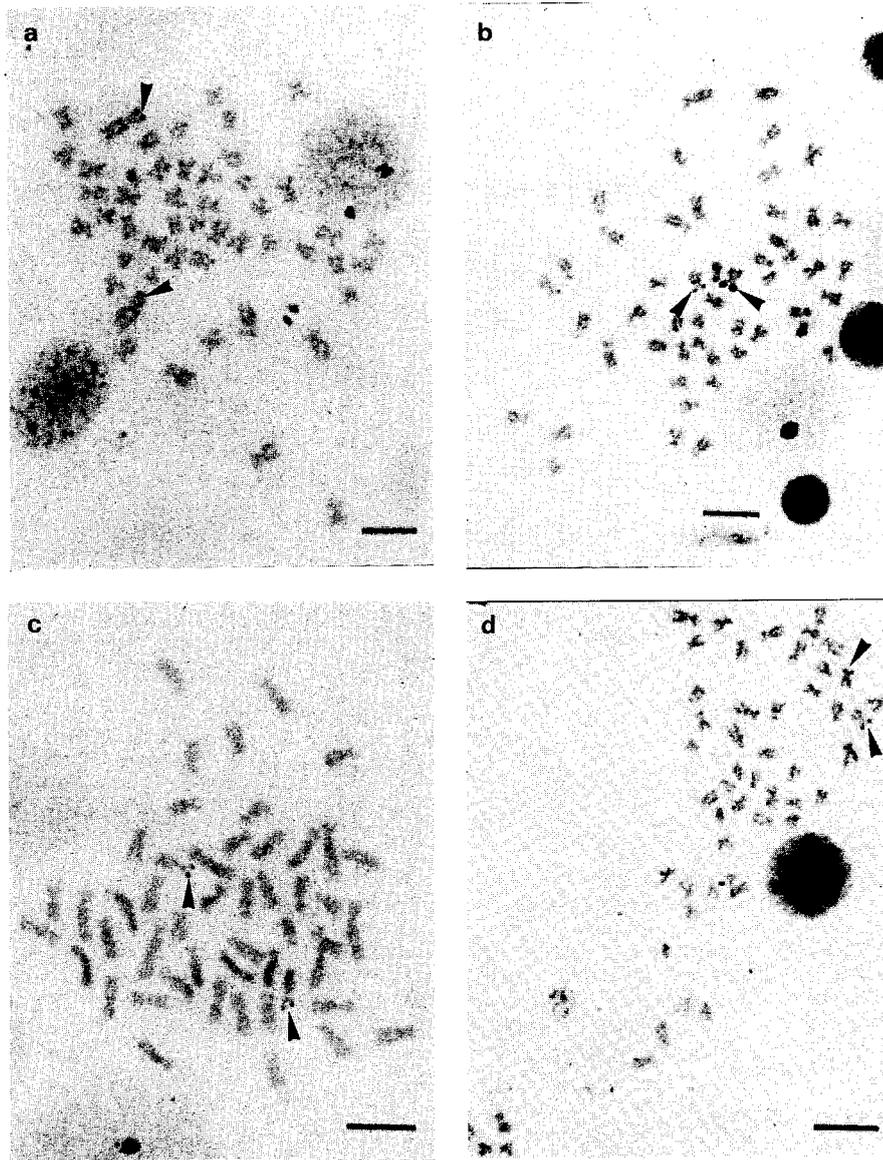
**Table 1** Summary of NOR-stained material examined

Taxon Genus <i>Notropis</i>	Number of specimens examined	Number of metaphases examined	Number of (haploid) NOR chromosomes	NOR chromosome phenotypes*
Subgenus <i>Cyprinella</i>				
(1) <i>N. camurus</i>	8	111	1	H
(2) <i>N. galacturus</i>	2	22	1	H
(3) <i>N. proserpinus</i>	6	130	1	H
(4) <i>N. spilopterus</i>	4	500	1	C'
(5) <i>N. whipplei</i>	9	89	1	H
Subgenus <i>Hydrophlox</i>				
(6) <i>N. baileyi</i>	3	35	1	D
(7) <i>N. nubilus</i>	4	106	1	D
Subgenus <i>Luxilus</i>				
(8) <i>N. pilsbryi</i>	10	50	1	D

\*NOR chromosome phenotypes: C, terminal on the short-arm of a large-sized submetacentric; D, terminal on the short arm of a medium-sized submetacentric; H, terminal on one arm of a medium-sized metacentric. A prime symbol (') indicates the chromosome is the largest in the complement.



**Figure 1** Silver-stained metaphases of (a) *Notropis camurus*, (b) *Notropis galacturus*, (c) *Notropis whipplei*, and (d) *Notropis proserpinus*. Chromosomal NORs are indicated by arrowheads. Bar scale equals 5  $\mu\text{m}$ .



**Figure 2** Silver-stained metaphases of (a) *Notropis spilopterus*, (b) *Notropis pilsbryi*, (c) *Notropis baileyi*, and (d) *Notropis nubilus*. Chromosomal NORs are indicated by arrowheads. Bar scale equals 5  $\mu\text{m}$ .

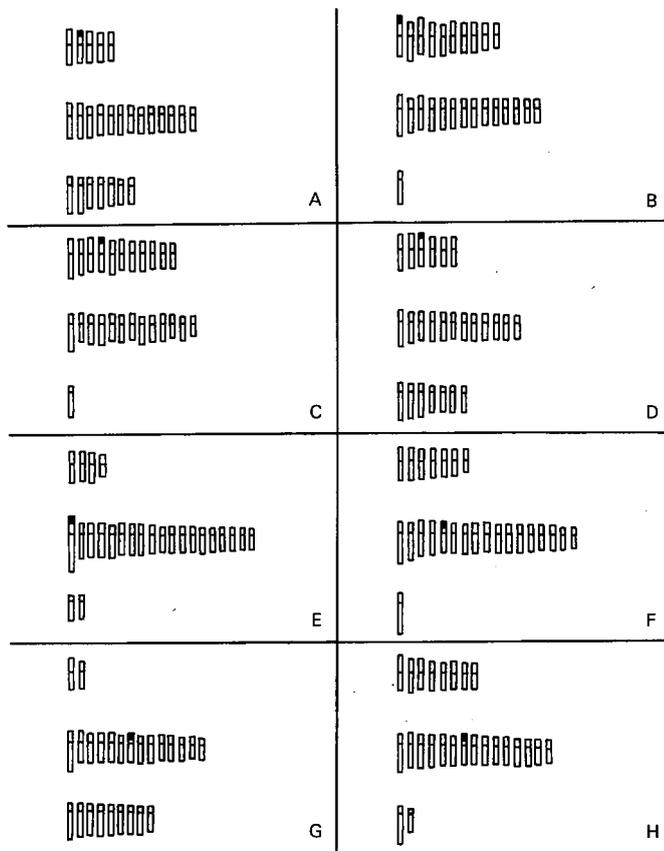
**Table 2** Summary of chromosomal NOR phenotypes in *Notropis*

Taxon Genus <i>Notropis</i>	Number of (haploid) NOR chromosomes	NOR chromosome phenotypes*
Subgenus <i>Alburnops</i>		
(1) <i>N. potteri</i>	1	F'
Subgenus <i>Cyprinella</i>		
(2) <i>N. camurus</i>	1	H
(3) <i>N. galacturus</i>	1	H
(4) <i>N. lepidus</i>	1	C'
(5) <i>N. lutrensis</i>	1	C'
(6) <i>N. proserpinus</i>	1	H
(7) <i>N. spilopterus</i>	1	C'
(8) <i>N. venustus</i>	1	C'
(9) <i>N. whipplei</i>	1	H
Subgenus <i>Hydrophlox</i>		
(10) <i>N. baileyi</i>	1	D
(11) <i>N. nubilus</i>	1	D
Subgenus <i>Luxilus</i>		
(12) <i>N. pilsbryi</i>	1	D
Subgenus <i>Lythrurus</i>		
(13) <i>N. ardens</i>	2	F'C
(14) <i>N. umbratilis</i>	2	F'H
Subgenus <i>Notropis</i>		
(15) <i>N. amabilis</i>	1	F'
(16) <i>N. jemezianus</i>	1	I'
(17) <i>N. oxyrhynchus</i>	1	J
(18) <i>N. shumardi</i>	1	F'
<i>N. dorsalis</i> species-group		
(19) <i>N. longirostris</i>	1	D
(20) <i>N. sp. cf longirostris</i> **	1	D
<i>N. texanus</i> species-group		
(21) <i>N. texanus</i>	1	D
Unknown affinities		
(22) <i>N. braytoni</i>	1	C'
(23) <i>N. emiliae</i>	1	E'

\* NOR chromosome phenotypes: C, terminal on the short arm of a large-sized submetacentric; D, terminal on the short arm of a medium-sized submetacentric; E, interstitial (subterminal) on the short arm of a large-sized submetacentric; F, terminal on the short arm of a large-sized acro-/subtelocentric; H, terminal on one arm of a medium-sized metacentric; I, terminal on the long arm of a large-sized acro-/subtelocentric; and J, terminal on the short arm of a small-sized submetacentric. A prime symbol (') indicates the chromosome is the largest in the complement.

\*\* An undescribed species from the Mobile Bay drainage (Heins *et al.*, 1980).

Chromosomal NOR data are from the present paper, Gold (1984), Gold and Amemiya (1986), and Amemiya and Gold (1988). Placement of species within putatively monophyletic assemblages (subgenera or species-groups) essentially follows Coburn (1982) and Mayden (1985).



**Figure 3** Haploid idiograms of NOR karyotypes of the eight species. Chromosomes are arranged into rows in each karyotype on the basis of centromere position (after Levan *et al.*, 1964): metacentric chromosomes are in the top row, submetacentric chromosomes are in the middle row, and acrocentric (subtelocentric) chromosomes are in the bottom row. Within rows, chromosomes are arranged according to relative size. NORs are indicated by darkened areas. (a) *Notropis camurus*; (b) *Notropis galacturus*; (c) *Notropis whipplei*; (d) *Notropis proserpinus*; (e) *Notropis spilopterus*; (f) *Notropis pilsbryi*; (g) *Notropis baileyi*; and (h) *Notropis nubilus*.

All eight species were found to possess only a single pair of NOR-bearing chromosomes (Table 1). In four of the five *Cyprinella* species (*N. camurus*, *N. galacturus*, *N. proserpinus* and *N. whipplei*), the single NOR was located terminally on one arm of a medium-sized metacentric chromosome [NOR phenotype H]. However, in the fifth *Cyprinella* species (*N. spilopterus*), the single NOR was located terminally on the short arm of a large submetacentric chromosome which also was the largest chromosome in the complement [NOR phenotype C']. In the remaining three species (*N. baileyi* and *N. nubilus* of *Hydrophlox* and *N. pilsbryi* of *Luxilus*), the single NOR was located terminally on the short arm of a medium-sized submetacentric chromosome [NOR phenotype D].

The letter designations for the NOR chromosome phenotypes were developed by Gold and Amemiya (1986) and Amemiya and Gold (1988), and are based on the position of the NOR on the chromosome (terminal, subterminal, *etc.*), the centromere position of that chromosome (metacentric, submetacentric, *etc.*), and the relative size of the chromosome within the complement. Only the last criterion was somewhat difficult (or subjective) to assess given the similarity in size of most North American cyprinid chromosomes (*e.g.* Figures 1 and 2).

In order to assist in differentiating relative chromosome sizes, computer-assisted measurements of NOR karyotypes of the eight species were used, as shown in Figure 3. It is important to note, however, that the haploid idiograms shown in Figure 3 are intended only to demonstrate the NOR position and relative size and centromere position of NOR-bearing chromosomes within the complements of each species, and not to unambiguously identify the NOR-bearing chromosomes from all other chromosomes of similar size and centromere position. As an example, the H NOR chromosome in *N. galacturus* (Figure 3b) appears to be larger than the H NOR chromosome in *N. whipplei* (Figure 3c). This may be artifactual given the expected differential contraction and condensation of chromosomes during mitosis.

Intraspecific heteromorphisms were observed in roughly 10–12% of all specimens examined regardless of species. These heteromorphisms included either differences between chromosomally homologous NOR sites in the size of silver-stained NORs or the absence of silver stainability at one of two chromosomally homologous NOR sites. An example of the former is shown in Figure 1c, and an example of the latter is shown in Figure 1d. These types of intraspecific NOR heteromorphisms appear to be relatively common in fish, including cyprinids (Foresti *et al.*, 1981; Gold, 1984; Moreira-Filho *et al.*, 1984; Gold and Amemiya, 1986), and are qualitatively different from the NOR variations observed between species (Gold and Amemiya, 1986; Amemiya and Gold, 1988).

## Discussion

A summary of the chromosomal NOR phenotypes observed among the twenty-three *Notropis* species studied to date is shown in Table 2. Based on a smaller data set of nine *Notropis* species, Gold and Amemiya (1986) hypothesized that a single NOR chromosome pair of the C' phenotype was the primitive or plesiomorphic condition within *Notropis*. This hypothesis was based on the observations that (1) eight of the nine species possessed only a single pair of NOR-bearing chromosomes, (2) seven of the ten NOR chromosomes identified had NORs terminally located on the short arm of a large bi-armed chromosome, and (3) the C' NOR chromosome was the most frequent NOR chromosome phenotype observed and was found in three of the four *Notropis* subgroups surveyed.

Subsequent study and examination of additional specimens (Amemiya and Gold, 1988; Amemiya, unpublished observations), have shown that the NOR chromosomes in *Notropis shumardi* and *Notropis potteri* are acro-/subtelocentric (the

F' NOR phenotype) and not submetacentric (the C' NOR phenotype) as reported by Gold and Amemiya (1986). On that basis, Amemiya and Gold (1988) suggested that a single NOR located terminally on the short arm of the largest chromosome in the complement (the C' or F' NOR phenotypes) might represent the plesiomorphic condition in *Notropis*.

The finding in this study of D NOR chromosomes in species of the *Notropis* subgenera *Hydrophlox* and *Luxilus* necessitates a re-evaluation of the possible plesiomorphic NOR condition in *Notropis*. As shown in Table 2, four of the eight putatively monophyletic assemblages within *Notropis* now sampled contain species with NOR sites located on the short arm of a medium-sized submetacentric chromosome (the D NOR phenotype), and four assemblages contain species with NORs on the short arm of the largest chromosome in the complements (the C' or F' NOR phenotypes). On the basis of commonality (Watrous and Wheeler, 1981), either [*i.e.* the D or the C' (or F') NOR phenotypes] could be plesiomorphic within *Notropis*, and until further species are assayed the problem will remain unresolved. Outgroup comparisons (*sensu* Watrous and Wheeler, 1981) provide little information in this case since none of the non-*Notropis* North American cyprinid species thus far examined possess a D, C', or F' NOR chromosome (Gold and Amemiya, 1986; Amemiya and Gold, 1988).

The situation in the *Notropis* subgenus *Cyprinella* is somewhat better resolved. Two phenetic groups within *Cyprinella*, one containing species with C' NOR chromosomes (*N. lepidus*, *N. lutrensis*, *N. spilopterus*, and *N. venustus*) and one containing species with H NOR chromosomes (*N. camurus*, *N. galacturus*, *N. proserpinus*, and *N. whipplei*) are evident (Table 2). Phylogenetically, a strong argument can be made that the C' NOR chromosome is the plesiomorphic NOR condition for the subgenus and that the H NOR chromosomes are (by definition) derived or apomorphic. Briefly, the C' NOR chromosome and its presumed derivative, the E' NOR chromosome (Amemiya and Gold, 1988), have been found in two *Notropis* species outside of *Cyprinella* (Table 2), and in both cases the NOR chromosomes appear homologous to the C' NOR chromosomes in *N. lepidus*, *N. lutrensis*, and *N. venustus* based on similarities in C-banding patterns (Amemiya and Gold, 1988).

Assuming that the C' NOR chromosome is plesiomorphic in *Cyprinella*, the systematic inference is that the H NOR chromosomes represent a chromosomal synapomorphy which unites *N. camurus*, *N. galacturus*, *N. proserpinus*, and *N. whipplei* into a monophyletic clade within *Cyprinella*. Monophyly of these four species within *Cyprinella*, however, is not supported by morphological data (Mayden, 1985), and it remains to be seen whether the H NOR chromosomes in the four species are in fact homologous. Experiments to test homologies of the H NOR chromosomes using C- and other banding procedures are currently in progress.

In summary, the results of this study indicate that the patterns of variation in chromosomal NORs within the cyprinid genus *Notropis* are more complex than previously believed. Species-specific differences in chromosomal NOR phenotypes do exist among *Notropis* species, yet at present there is no definitive

indication as to which NOR condition is plesiomorphic for the genus. Sampling additional species may help resolve this issue, but it will be crucial to employ alternative banding methodologies to critically assess NOR chromosome homologies. Given the general difficulty in working with fish chromosomes (Gold, 1979), this will not be an easy task.

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