

## Cytogenetic studies in North American minnows (Cyprinidae)

### XVII. Chromosomal NOR phenotypes of 12 species, with comments on cytosystematic relationships among 50 species

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Chromosomal NOR phenotypes are documented for twelve species of North American cyprinid fishes whose NOR karyotypes have not been previously reported. Patterns of interspecific chromosomal NOR variation are then summarized for the fifty species of North American cyprinids surveyed to date. The degree of interspecific NOR variation among North American cyprinids suggests that the rate of chromosomal evolution within the group may have been higher than previously believed. The interspecific variants in chromosomal NORs are used as taxonomic and systematic characters in order to infer phylogenetic hypotheses of species relationships. The phylogenetic inferences drawn from the chromosomal NOR data provide a framework upon which future cytosystematic studies in North American cyprinids can be based.

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Cytogenetic studies in fishes have not been as successful as in other vertebrate groups. Standard karyotypes (chromosome and chromosome arm numbers) have been reported from less than 10 % of the more than 20,000 extant species of fishes (LEGRANDE 1990). The application of chromosome banding methodologies to fish chromosomes has been minimal (GOLD 1979; HARTLEY and HORNE 1985). The main difficulty in working with fish chromosomes has been obtaining high quality metaphase spreads. A few studies have used fish standard karyotypes to examine taxonomic or systematic problems (CHEN 1971; UYENO and MILLER 1973; THOMPSON 1979; LEGRANDE 1981; UYENO et al. 1983).

Recent improvements in fish cell culture (WIBERG 1984; AMEMIYA et al. 1984) and better methods for direct chromosome preparation (KLIGERMAN and BLOOM 1977) have advanced fish chromo-

some research by providing higher yields of metaphase spreads. Unfortunately, the only tractable banding methods in fish are limited to those which produce localized bands such as NOR- or C-bands (HARTLEY and HORNE 1985). Methods for revealing chromosomal NORs or nucleolus organizer regions on fish chromosomes have been used extensively (e.g., FORESTI et al. 1981; GALETTI et al. 1984; MOREIRA-FILHO et al. 1984; AMEMIYA and GOLD 1986; MAYR et al. 1987; THODE et al. 1988). The studies of fish chromosomal NORs have focused primarily on NOR variation within and among species (above references). Thus far, the published data on fish NORs indicate that intra- and interspecific NOR variants are common, and that the patterns of variation are similar to those observed in other lower vertebrates (SCHMID 1982; BICKHAM and ROGERS 1985).

Over the last decade, our laboratory has carried out cytogenetic investigations on North American cyprinid fishes. Cyprinids (minnows) represent the dominant freshwater ichthyofauna in North America, comprising over 250 extant species in some 35-

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40 genera (MILLER 1965; LEE et al. 1980). Much of the group appears to have evolved relatively recently and at heterogeneous rates (MILLER 1965; GOLD 1980; SMITH 1981). As might be expected for such a large, diverse group, taxonomic and systematic problems abound. These problems are typified by the extremely speciose genus *Notropis*, where several of the morphological characters generally used in cyprinid taxonomy and systematics apparently have been modified repeatedly during the evolution of the more than 100 extant species nominally placed in this genus (GILBERT and BAILEY 1962; SWIFT 1970; COBURN 1982; MAYDEN 1989). We have documented standard karyotypes of over 90 North American cyprinid species, including nearly 40 species of *Notropis* (GOLD et al. 1980, 1988; AMEMIYA and GOLD 1987a, 1990, unpublished). Gross karyotypic change in North American cyprinids appears to have been minimal: over 90% of all species assayed (including all *Notropis* species examined) possess diploid chromosome numbers of 50 (range = 48–52) and (estimated) diploid chromosome arm numbers between 92 and 100 (range = 80–100) (GOLD et al. 1980; AMEMIYA and GOLD 1987a). Heteromorphic sex chromosomes have yet to be unequivocally identified. The typical North American cyprinid karyotype comprises a continuous series of primarily submetacentric to acrocentric chromosomes ranging in length from ca. 1.5 to 5.5  $\mu\text{m}$  in a mid-metaphase plate. The overall uniformity of cyprinid chromosomes has severely limited the utility of standard or gross karyotypes for resolving taxonomic or systematic problems.

We have recently focused our cytogenetic studies of North American cyprinids to assays of intra- and interspecific variation in chromosomal NORs (GOLD 1984; GOLD and AMEMIYA 1986; AMEMIYA and GOLD 1988, 1990; GOLD et al. 1988). These studies have documented substantial NOR variation both within and among species, and also demonstrated that chromosomal NORs can serve as characters for inferring hypotheses of phylogenetic relationships. This paper is a continuation of our survey of chromosomal NOR differentiation in North American cyprinids. The purposes of the paper are threefold: first, to document the chromosomal NOR phenotypes of twelve species of North American cyprinids whose NOR karyotypes have not been previously reported; secondly, to summarize the patterns of interspecific chromosomal NOR variation observed among the fifty North American cyprinid species examined to date; and finally, to discuss the systematic/phylogenetic implications of the chromosomal NOR data.

## Materials and methods

The cyprinids examined in this study were collected by seine from natural populations in the southern United States. The taxa examined and their respective collection sites are given in Appendix I. Most specimens were returned live to College Station, Texas, and maintained in well-aerated aquaria until processing. A few specimens from Alabama were processed in Dr. Mike Howell's laboratory at Samford University, Birmingham, Alabama. Chromosome preparations from seven specimens of *Notropis bellus* were kindly provided by Dr. Howell. Voucher specimens, including the individuals karyotyped, were deposited in our laboratory collection and will ultimately be catalogued in the Texas Cooperative Wildlife Collection (TCWC) at Texas A & M University.

Metaphase chromosomes were prepared either directly from solid tissues (GOLD 1984) or from cultured fibroblasts (AMEMIYA et al. 1984). Ag-NOR-banding was accomplished via the controlled silver technique of HOWELL and BLACK (1980). Chromomycin A<sub>3</sub> NOR-banding followed AMEMIYA and GOLD (1987b). Bright field and fluorescence photomicroscopy followed procedures outlined in AMEMIYA and GOLD (1986). Determination of NOR band position(s) and size, and of relative size and centromere position of NOR-bearing and other chromosomes were made off positive prints using a digitizer, a small laboratory computer, and the BANDSCAN program described in GOLD and AMEMIYA (1986).

## Results and discussion

### I. Description of NOR phenotypes from twelve species

The NOR chromosome data from the twelve cyprinid species examined are summarized in Table 1. Representative NOR-stained metaphases for some of these taxa are shown in Fig. 1 and 2. All individuals from all twelve species possessed  $2n=50$  chromosomes, as has been the case for most North American cyprinids (including all *Notropis* species) surveyed to date (GOLD et al. 1980; AMEMIYA and GOLD 1987a, 1990; GOLD et al. 1988). The chromosome numbers of *Hybopsis storeriana*, *Nocomis asper*, *Notropis bellus*, *N. chalybaeus*, *N. chromosomus*, and *N. girardi* are reported for the first time.

The letter designations for the NOR chromosome phenotypes given in Table 1 were developed by

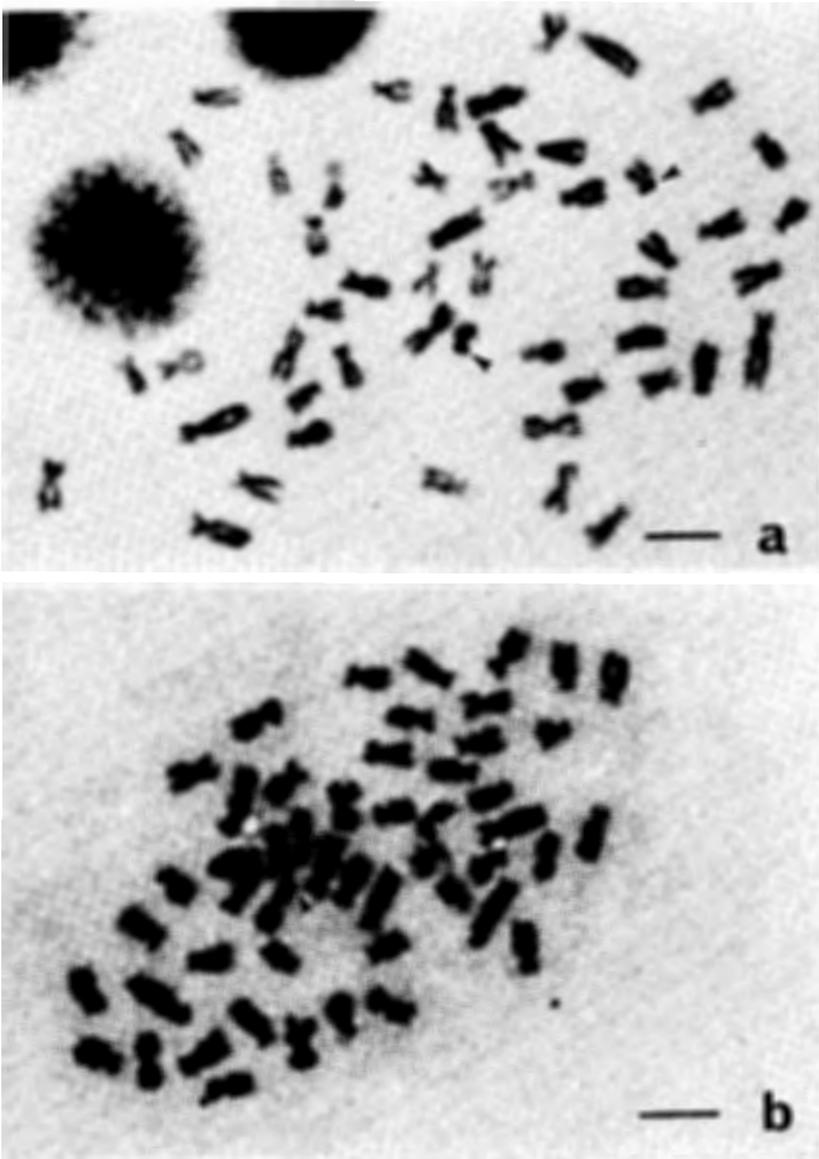
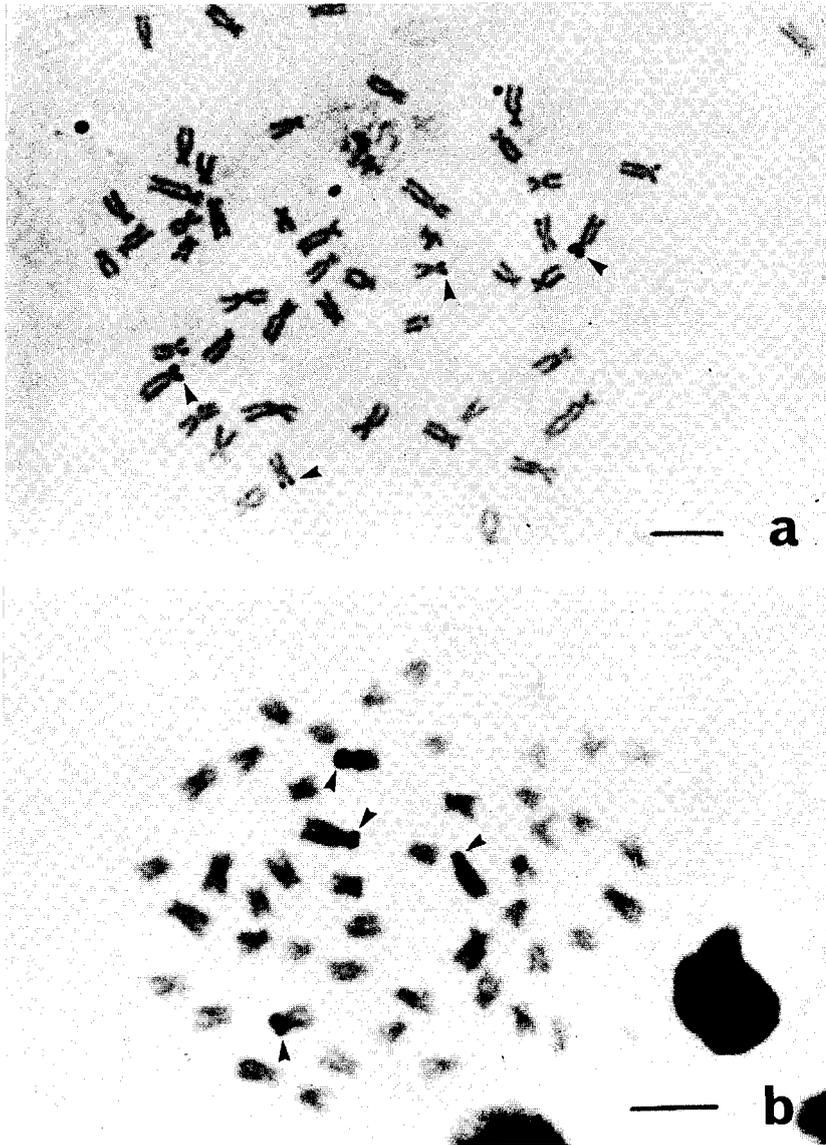


Fig. 1a and b. NOR karyotypes of two species with single NORs. a AgNOR-stained metaphase of *Erycymba buccata* (NOR phenotype B) and b *Notropis chalybaeus* (NOR phenotype D). Chromosomal NORs are indicated by arrows. Bars = 5  $\mu$ m.

GOLD and AMEMIYA (1986) and AMEMIYA and GOLD (1988). Characterization of NOR phenotypes was based on the position of the NOR on the chromosome (terminal, interstitial), the centromere position of that chromosome (submedian, median, etc.), and the relative size of the chromosome within the complement. Given the similarity in size of most North

American cyprinid chromosomes (Fig. 1 and 2), the last criterion was difficult to assess objectively. To aid in the differentiation of relative chromosome sizes, we employed computer-generated haploid idiograms of digitized NOR karyotypes. The idiograms for the twelve species reported in this paper are shown in Fig. 3 and are intended only to demon-



**Fig. 2a and b.** NOR karyotypes of two species with multiple NORs. **a** AgNOR-stained metaphase of *Hybopsis storeriana* (NOR phenotypes D, F); and **b** CMA-stained metaphase of *Notropis fumeus* (NOR phenotypes F', H). Chromosomal NORs are indicated by arrows. Note the photograph in **b** is an image reversal of the original fluorescence photomicrograph; the reversed image provides better demonstration of the chromosomal NORs. Bars = 5  $\mu$ m.

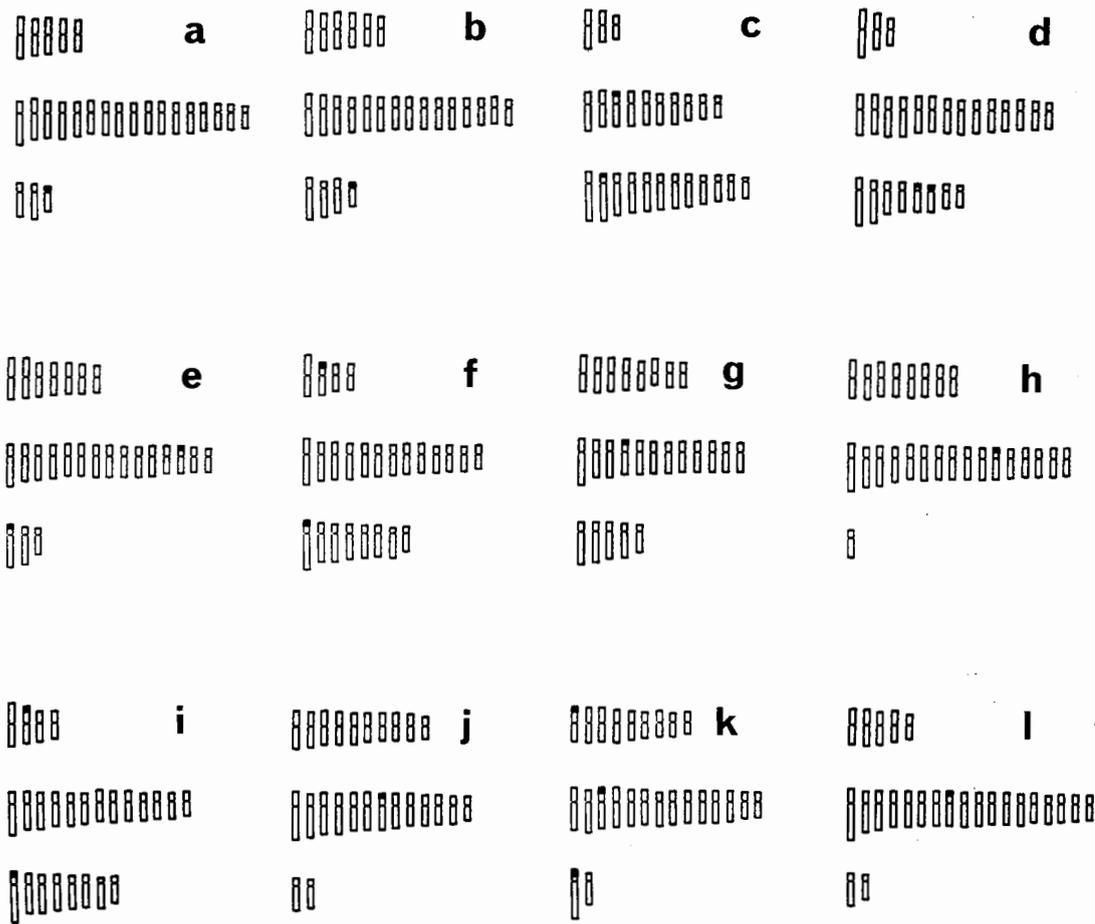


Fig. 3. Haploid idiograms of the twelve taxa examined in this study. Chromosomes are arranged into rows in each karyotype on the basis of centromere position (after LEVAN et al. 1964): metacentric chromosomes are in the top row, submetacentric chromosomes are in the middle row, and acrocentric (subtelocentric) chromosomes are in the bottom row. Within rows, chromosomes are arranged according to relative size. NORs are indicated by darkened areas. **a** *Dionda episcopa*, **b** *Ericymba buccata*, **c** *Hybopsis storeriana*, **d** *Nocomis asper*, **e** *Notropis atherinoides*, **f** *Notropis bellus*, **g** *Notropis chalybaeus*, **h** *Notropis chrosomus*, **i** *Notropis fumeus*, **j** *Notropis girardi*, **k** *Notropis roseipinnis*, and **l** *Notropis stilbius*.

strate the NOR position and relative size and centromere position of NOR-bearing chromosomes within the complements of each species.

Six of the twelve species examined were found to possess a single pair of NOR-bearing chromosomes (Table 1). In four of these (*N. chalybaeus*, *N. chrosomus*, *N. girardi*, and *N. stilbius*), the NOR was located terminally on the short arm of a medium-sized submetacentric chromosome (NOR phenotype D); in the other two (*D. episcopa* and *E. buccata*), the single NOR was located terminally on the short arm of a small-sized acro/subtelocentric

chromosome (NOR phenotype B). The remaining six species examined were found to possess multiple NORs. *Nocomis asper* possessed two pairs of NOR chromosomes with B phenotypes; *H. storeriana* possessed one pair of NOR chromosomes with a D phenotype, and one pair with an F phenotype (NOR terminal on the short arm of a large-sized acro/subtelocentric chromosome). *Notropis atherinoides*, *N. bellus*, *N. fumeus*, and *N. roseipinnis* each possessed a NOR which was terminal on the short arm of a large-sized acro/subtelocentric chromosome, which also was the largest

Table 1. Summary of NOR-stained material examined

Taxon	Number of specimens examined	Number of metaphases examined	Number of (haploid) NOR chromosomes	NOR chromosome phenotypes*
<i>Dionda episcopa</i>	2	46	1	B
<i>Ericymba buccata</i>	3	115	1	B
<i>Hybopsis storeriana</i>	3	55	2	D, F
<i>Nocomis asper</i>	5	100	2	B, B
Genus <i>Notropis</i>				
<i>N. atherinoides</i>	8	312	2	F', J
<i>N. bellus</i>	8	300	2	F', H
<i>N. chalybaeus</i>	2	40	1	D
<i>N. chrosomus</i>	4	47	1	D
<i>N. fumeus</i>	5	217	2	F', H
<i>N. girardi</i>	3	40	1	D
<i>N. roseipinnis</i>	5	124	3	F', H, C
<i>N. stilbius</i>	3	62	1	D

\* NOR chromosome phenotypes: B, terminal on the short arm of a small-sized acro-/subtelocentric; C, terminal on the short arm of a large-sized submetacentric; D, terminal on the short arm of a medium-sized submetacentric; F, terminal on the short arm of a large-sized acro-/subtelocentric; J, terminal on the short arm of a small-sized submetacentric

A prime symbol (') indicates the chromosome is the largest in the complement

chromosome in the complement (NOR phenotype F'). *Notropis atherinoides* possessed a second NOR located terminally on the short arm of a small-sized submetacentric chromosome (NOR phenotype J). *Notropis bellus*, *N. fumeus*, and *N. roseipinnis* each possessed a second NOR located terminally on one arm of a medium-sized metacentric chromosome (NOR phenotype H). *Notropis roseipinnis* possessed a third NOR located terminally on the short arm of a large-sized submetacentric chromosome (NOR phenotype C).

## II. Patterns of interspecific NOR variation among North American Cyprinidae

The chromosomal NOR phenotypes of the twelve cyprinid species documented here brings the total number of North American cyprinids assayed for chromosomal NORs to 50. A compilation of the interspecific chromosomal NOR data for all 50 taxa is presented in Table 2; the various chromosomal NOR phenotypes thus far identified are shown in Fig. 4. With regard to the number of NOR-bearing chromosome pairs per genome, 37 species (74 %) possess only a single NOR pair; 13 species (26 %) possess multiple NORs. Only *C. anomalum* and *N. roseipinnis* possess more than two pairs of NOR chromosomes (Table 2). A total of 12 different NOR chromosome phenotypes occur among the 50 species (Table 2; Fig. 4). The twelve NOR phenotypes are distributed on a minimum of 14 different chromosomes since two different A phenotypes occur in *C. anomalum* and two different B phenotypes

occur in both species examined from the genus *Nocomis* (Table 2). The vast majority of species possessed NORs located terminally on chromosomal short arms (Fig. 4). The exceptions are *N. emiliae* (E' phenotype), where the single NOR is found interstitially on the short arm of a large-sized submetacentric chromosome, and *N. jemezianus* (I' phenotype), where the single NOR is found terminally on the long arm of a large-sized acro-/subtelocentric chromosome (Table 2; Fig. 4). Summed together, the interspecific variations in the number of NOR pairs and NOR chromosome phenotypes result in 19 discrete NOR conditions (Table 2). Eleven different NOR conditions are found among the 37 species with single NORs; whereas eight different NOR conditions are found among the 13 species with multiple NORs.

Comparison of the interspecific chromosomal NOR variation in North American cyprinids with those from a similar study of 22 Asian cyprinid species (TAKAI and OJIMA 1986) reveals some notable similarities. The majority of Asian cyprinids (14 of 22 species — 64 %) possess only a single NOR pair, and all 22 species possess NORs located terminally on chromosomal short arms. Collectively, the types of interspecific NOR differences observed among all cyprinids examined appear similar to those observed in other fish groups (FORESTI et al. 1981; GALETTI et al. 1984; MOREIRA-FILHO et al. 1984; TAKAI and OJIMA 1986; MAYR et al. 1987), although species-specific differences in chromosomal NOR size (sensu FORESTI et al. 1981) have yet to be documented in cyprinids.

Table 2. Summary of chromosomal NOR phenotypes\* and conditions among the 50 North American cyprinid species examined to date<sup>1</sup>

NOR Conditions	Taxa
<b>Single NORs</b>	
A	<i>Notemigonus crysoleucas</i>
B	<i>Dionda episcopa</i> , <i>Ericymba buccata</i> , <i>Hemitemria flammea</i> , <i>Hybognathus placitus</i>
C	<i>Pimephales notatus</i> , <i>Pimephales vigilax</i>
C'	<i>Notropis braytoni</i> , <i>Notropis lepidus</i> , <i>Notropis lutrensis</i> , <i>Notropis spilopterus</i> <i>Notropis venustus</i>
D	<i>Notropis baileyi</i> , <i>Notropis buchanani</i> , <i>Notropis chalybaeus</i> , <i>Notropis chrosomus</i> , <i>Notropis girardi</i> , <i>Notropis longirostris</i> , <i>Notropis sp. cf. longirostris</i> <sup>2</sup> , <i>Notropis maculatus</i> , <i>Notropis nubilus</i> , <i>Notropis pilsbryi</i> , <i>Notropis stilbius</i> , <i>Notropis stramineus</i> , <i>Notropis texanus</i> , <i>Notropis volucellus</i>
E'	<i>Notropis emiliae</i>
F	<i>Notropis hubbsi</i>
F'	<i>Notropis amabilis</i> , <i>Notropis potteri</i> , <i>Notropis shumardi</i>
H	<i>Notropis camurus</i> , <i>Notropis galacturus</i> , <i>Notropis proserpinus</i> , <i>Notropis whipplei</i>
I'	<i>Notropis jemezianus</i>
J	<i>Notropis oxyrhynchus</i>
<b>Multiple NORs</b>	
A, A, G	<i>Camptostoma anomalum</i>
B, B	<i>Nocomis asper</i> , <i>Nocomis leptocephalus</i>
B, F	<i>Hybopsis aestivalis</i>
D, F	<i>Hybopsis storeriana</i> , <i>Notropis signipinnis</i> , <i>Notropis welaka</i>
F', C	<i>Notropis ardens</i>
F', H	<i>Notropis bellus</i> , <i>Notropis fumeus</i> , <i>Notropis umbratilis</i>
F', H, C	<i>Notropis roseipinnis</i>
F', J	<i>Notropis atherinoides</i>

<sup>1</sup> Data from GOLD (1984), GOLD and AMEMIYA (1986), AMEMIYA and GOLD (1990), GOLD et al. (1988), and this paper.

\* NOR chromosome phenotypes (cf. Fig. 3) are as follows: A, terminal on short arm of medium-sized acro-/subtelocentric; B, terminal on short arm of small-sized acro-/subtelocentric; C, terminal on short arm of large-sized submetacentric; D, terminal on short arm of medium-sized submetacentric; E, interstitial on short arm of large-sized submetacentric; F, terminal on short arm of large-sized acro-/subtelocentric; G, terminal on one arm of large-sized metacentric; H, terminal on one arm of medium-sized metacentric; I, terminal on long arm of large-sized acro-/subtelocentric; J, terminal on short arm of small sized submetacentric. A prime symbol (') indicates the chromosome is the largest in the complement.

<sup>2</sup> An undescribed species from the Mobile Bay drainage (HEINS et al. 1980).

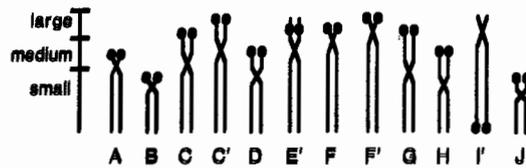


Fig. 4. The different NOR chromosome phenotypes observed among North American cyprinids. The scale to the left indicates approximate chromosome size (relative to the largest chromosome in the complement). Letters beneath drawings indicate NOR chromosome phenotypes (cf Table 2).

The degree of interspecific variation in chromosomal NORs observed among North American cyprinids appears at odds with their apparent conservatism in gross karyotypes (GOLD et al. 1978; GOLD 1980). The 19 different NOR conditions observed among the 50 species (Table 2) suggest that a minimum of 18 different chromosomal rearrangements involving a NOR have occurred since the 50 species last shared a common ancestor. Although NOR-banding per se does not provide sufficient resolution to determine the qualitative nature of NOR chromosome rearrangements, C-banding has been used in previous studies (GOLD and AMEMIYA 1986; AMEMIYA and GOLD 1988) to demonstrate that at least a few of the interspecific NOR chromosome differences observed in North American cyprinids stem from classical chromosomal rearrangements such as inversions or translocations. Further, if the frequencies of rearrangement for all the chromosomes in the genome are similar to that for the NOR chromosomes, the rate of chromosomal evolution within North American Cyprinidae may have been considerably higher than previously believed.

### III. Systematic/phylogenetic implications of the chromosomal NOR data in North American Cyprinidae

Over the last few years, there has been a renewed interest in the systematics of North American cyprinids, which has been precipitated, in large part, by the extensive researches of COBURN (1982), MAYDEN (1987, 1989), and CAVENDER and COBURN (1985, 1986). These researchers have independently surveyed a large number of North American cyprinid species for a variety of morphological (including osteological) characters and from these data inferred hypotheses of species relationships, using a cladistic or Hennigian approach. The importance of these investigations has been the generation of phy-

logenetic hypotheses, which can now be tested by alternative data bases.

In Table 3, the chromosomal NOR phenotypes of the 50 North American cyprinid species thus far assayed are listed according to the monophyletic groups to which the taxa have been putatively assigned on the basis of morphology (LEE et al. 1980; COBURN 1982; MAYDEN 1989). Inspection of the data in Table 3 reveals several phenetic similarities in NOR chromosome phenotypes, most of which appear to corroborate present concepts of North American cyprinid taxonomy. These phenetic groups include the two *Hybopsis* species with two NOR pairs and F NOR phenotypes; the two *Nocomis* species with two pairs of B NOR chromosomes; the four *Notropis* (*Cyprinella*) species with H NOR phenotypes; the four *Notropis* (*Cyprinella*) species with C' NOR phenotypes; the five *Notropis* (*Lythrurus*) species with multiple NORs and F' NOR phenotypes; the three *Notropis* (*Pteronotropis*) species with F NOR phenotypes; and the three species each in *Notropis* (*Hydrophlox*) and the *N. volucellus* species-group as well as the two species in the *N. texanus* species-group with D NOR phenotypes. These observations demonstrate that chromosomal NORs are taxonomically informative in North American Cyprinidae, and suggest that there is a phylogenetic component to the chromosomal NOR variation.

In the following, each NOR condition is treated as a "character state". For example, *Notemigonus crysoleucas*, a species with a single NOR pair, has an A NOR character state; whereas *Hybopsis aestivalis*, a species with two NOR pairs, has a B, F NOR character state (cf. Table 3). The choice of outgroups for cladistic considerations (WATROUS and WHEELER 1981) was based on the phylogenetic hypotheses of COBURN (1982) and MAYDEN (1989). Both researchers are in agreement that a single morphological character (the presence of an open posterior myodome) defines many of the "eastern" North American cyprinid assemblages. The two taxa examined for chromosomal NORs which do not possess an open posterior myodome, and hence could potentially serve as outgroups to the remainder, are *Notemigonus*, the only endemic North American cyprinid not thought to belong to the subfamily Leuciscinae (MILLER 1959), and *Hemitremia*. *Notemigonus*, however, may not be an appropriate outgroup since its relationship to other cyprinid subgroups is not well established (HOWES 1981).

A cladogram for the North American cyprinid genera examined for chromosomal NORs and based

Table 3. Summary of NOR conditions of all North American cyprinids examined to date<sup>1</sup> with regard to putative monophyletic assemblages defined on the bases of morphology<sup>2</sup>

Taxon	Number of (haploid) NOR chromosomes	NOR conditions
Family Cyprinidae		
Subfamily Abramidinae		
1. <i>Notemigonus crysoleucas</i>	1	A
Subfamily Leuciscinae		
2. <i>Hemitremia flammea</i>	1	B
Chubs		
3. <i>Campostoma anomalum</i>	3	A, A, G
4. <i>Dionda episcopa</i>	1	B
5. <i>Hybognathus placitus</i>	1	B
6. <i>Hybopsis aestivalis</i>	2	B, F
7. <i>Hybopsis storeriana</i>	2	D, F
8. <i>Nocomis asper</i>	2	B, B
9. <i>Nocomis leptocephalus</i>	2	B, B
Genus <i>Notropis</i>		
Subgenus <i>Alburnops</i>		
10. <i>N. girardi</i>	1	D
11. <i>N. potteri</i>	1	F'
Subgenus <i>Cyprinella</i>		
12. <i>N. camurus</i>	1	H
13. <i>N. galacturus</i>	1	H
14. <i>N. lepidus</i>	1	C'
15. <i>N. lutrensis</i>	1	C'
16. <i>N. proserpinus</i>	1	H
17. <i>N. spilopterus</i>	1	C'
18. <i>N. venustus</i>	1	C'
19. <i>N. whipplei</i>	1	H
Subgenus <i>Hydrophlox</i>		
20. <i>N. baileyi</i>	1	D
21. <i>N. chrosomus</i>	1	D
22. <i>N. nubilus</i>	1	D
Subgenus <i>Luxilus</i>		
23. <i>N. pilsbryi</i>	1	D
Subgenus <i>Lythrurus</i>		
24. <i>N. ardens</i>	2	F', C
25. <i>N. bellus</i>	2	F', H
26. <i>N. fumeus</i>	2	F', H
27. <i>N. roseipinnis</i>	3	F', H, C
28. <i>N. umbratilis</i>	2	F', H
Subgenus <i>Notropis</i>		
29. <i>N. amabilis</i>	1	F'
30. <i>N. atherinoides</i>	2	F', J
31. <i>N. jemezianus</i>	1	I'
32. <i>N. oxyrhynchus</i>	1	J
33. <i>N. shumardi</i>	1	F'
34. <i>N. stilbius</i>	1	D
Subgenus <i>Pteronotropis</i>		
35. <i>N. hubbsi</i> <sup>3</sup>	1	F
36. <i>N. signipinnis</i>	2	F, D
37. <i>N. welaka</i>	2	F, D

Table 3. Continued.

Taxon	Number of (haploid) NOR chromosomes	NOR conditions
<i>N. dorsalis</i> species-group		
38. <i>Ericymba buccata</i>	1	B
39. <i>N. longirostris</i>	1	D
40. <i>N. sp. cf. longirostris</i> <sup>4</sup>	1	D
<i>N. texanus</i> species-group		
41. <i>N. chalybaeus</i>	1	D
42. <i>N. texanus</i>	1	D
<i>N. volucellus</i> species-group		
43. <i>N. buchanani</i>	1	D
44. <i>N. maculatus</i>	1	D
45. <i>N. volucellus</i>	1	D
Unknown affinities		
46. <i>N. braytoni</i>	1	C'
47. <i>N. emiliae</i> <sup>5</sup>	1	E'
48. <i>N. stramineus</i>	1	D
Genus <i>Pimephales</i> <sup>6</sup>		
49. <i>P. notatus</i>	1	C
50. <i>P. vigilax</i>	1	C

<sup>1</sup> Data from GOLD (1984), GOLD and AMEMIYA (1986), AMEMIYA and GOLD (1988, 1990), GOLD et al. (1988), and this paper

<sup>2</sup> Systematic placements essentially follow LEE et al. (1980) and MAYDEN (1989), with notable exceptions as indicated below

<sup>3</sup> *Notropis hubbsi* is placed here in the subgenus *Pteronotropis* following the suggestions of BAILEY and ROBISON (1978) and COBURN (pers. commun.), and on the basis of chromosomal NOR data (AMEMIYA and GOLD 1990); however, this view is not shared by MAYDEN (1989) or DIMMICK (1987)

<sup>4</sup> An undescribed species from the Mobile Bay drainage (HEINS et al. 1980)

<sup>5</sup> MAYDEN (1989) placed *Notropis emiliae* in the *N. volucellus* species-group. More recent evidence (CAVENDER and COBURN 1986; MAYDEN and MATSON 1988; AMEMIYA and GOLD 1990) suggests that *N. emiliae* is more closely related to the genus *Pimephales* and the *Notropis* subgenus *Cyprinella*.

<sup>6</sup> The genus *Pimephales* is now considered to be most closely related to lineages within *Notropis* (CAVENDER and COBURN 1986; MAYDEN and MATSON 1988; AMEMIYA and GOLD 1990).

solely on the condition of the posterior myodome is shown in Fig. 5. NOR chromosome conditions found in each of the genera are indicated in the Figure. As shown, the B NOR character state, as found in the outgroup (*Hemitremia*), is observed in five of eight ingroup genera. This suggests, both by outgroup comparison and commonality (WATROUS and WHEELER 1981), that a single NOR located terminally on the short arm of a small acrocentric chromosome (NOR phenotype B) represents the primitive or plesiomorphic NOR character state for the open posterior myodome assemblage. This inference (hypothesis) is further supported by the findings of TAKAI and OJIMA (1986) that fifteen of twenty-two Asian cyprinids examined also possessed a NOR which was terminal on the short arm

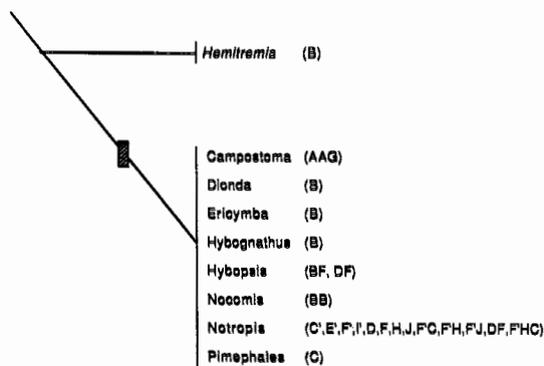
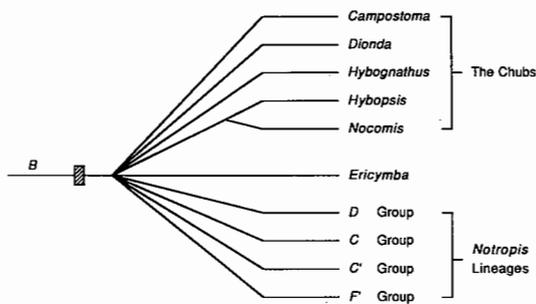


Fig. 5. Phylogenetic hypothesis used to infer the plesiomorphic NOR condition for the open posterior myodome assemblage. The crosshatched bar represents the synapomorphy of an opening of the floor of the posterior myodome as discussed by COBURN (1982) and MAYDEN (1989). NOR conditions within each genus are shown in parentheses (cf Table 2).

of a small acrocentric chromosome, and is in agreement with the suggestions of HSU et al. (1975) and SCHMID (1978) that a single pair of chromosomal NORs is primitive for most vertebrates.

Treatment of the interspecific NOR chromosome data in a cladistic fashion yields the phylogenetic hypothesis shown in Fig. 6. By outgroup comparison, the plesiomorphic NOR character state for the open posterior myodome assemblage is a single NOR chromosome pair of the B NOR phenotype (see above); all other NOR conditions, including multiple NORs, interstitially located NORs, NORs found on submetacentric and metacentric chromosomes, and NORs found on large chromosomes are, by definition, derived or apomorphic. The open posterior myodome assemblage consists of an unresolved polychotomy, which includes the five "chub" genera (*Campostoma*, *Dionda*, *Hybognathus*, *Hybopsis*, and *Nocomis*), *Ericymba buccata*, and each of four phenetic groups defined by D, C, C', and F' NOR chromosome phenotypes. These four phenetic groups are referred to hereafter as the *Notropis* lineages. Specific character state changes for the chub taxa and for the *Notropis* lineages are discussed below. In brief, three different types of NOR character state changes were inferred from the chromosomal NOR data. These include: (i) an addition of a NOR chromosome (denoted by "+", e.g., +B, +H); (ii) a loss of a NOR chromosome (denoted by "-", e.g., -D, -C); and (iii) a NOR chromosome transformation (denoted by "→", e.g., C' → H, C' → J).

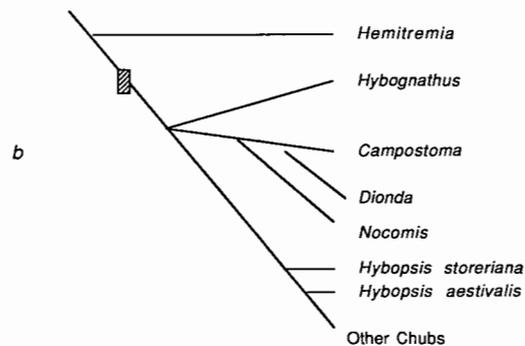
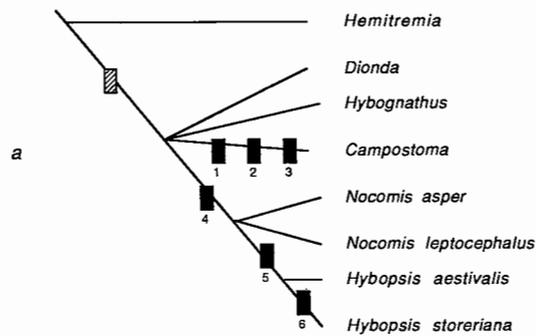


**Fig. 6.** Phylogenetic hypothesis of North American cyprinid relationships based on chromosomal NOR data. The crosshatched bar represents the synapomorphic character state of an opening of the posterior myodome (MAYDEN 1989). *Pimephales* is included in the *Notropis* lineage (C group).

### The Chubs

The chub genera *Campostoma*, *Dionda*, *Hybognathus*, *Hybopsis*, and *Nocomis* were placed in an unresolved polychotomy, which included *Ericymba buccata* and the various chromosomal groups of the *Notropis* lineages (Fig. 6). The placements of the chub taxa in Fig. 6 are based solely on chromosomal NOR data, and are conservative assignments necessitated by the absence of a chromosomal synapomorphy uniting the chubs. Both MAYDEN (1989) and CAVENDER and COBURN (1988), however, concur on the monophyly (on morphological grounds) of the chub species examined for chromosomal NORs. If monophyly of the chub species is accepted on this basis, then the plesiomorphic NOR condition for the chubs (using *Hemitremia* as the out-group) would be a single NOR of phenotype B and yield the phylogenetic hypothesis shown in Fig. 7a. For comparison, the phylogenetic hypothesis (relative to the taxa studied) of MAYDEN (1989) is shown in Fig. 7b.

The salient assumption used to generate the chromosomal hypothesis (Fig. 7a) is that the B NOR chromosomes in *Dionda*, *Nocomis* (one pair), and *Hybopsis* are homologous to one another and to the B NOR chromosome of *Hemitremia*. The hypothesis has six character state changes and supports monophyly of the two species of *Hybopsis* (defined by the inferred transformation of a B to an F NOR chromosome) as well as a *Nocomis-Hybopsis* clade (defined by the inferred addition of a B NOR chromosome). The chromosomal hypothesis differs from MAYDEN's (1989) morphological hypothesis primarily in the placement of the genus *Nocomis*.



**Fig. 7a and b.** Phylogenetic hypothesis of relationships within the chubs. The cladogram in a is based on chromosomal NOR data and assumes the chubs are monophyletic. The cladogram in b is from MAYDEN (1989) and is based on morphology. The crosshatched bar delimits the open posterior myodome assemblage. Character state changes in a are: (1) B→A (or G); (2) +A; (3) +G (or A); (4) +B; (5) B→F; and (6) B→D.

In his view (Fig. 7b), *Nocomis* is a sister assemblage to a *Dionda-Campostoma* clade; whereas the chromosomal hypothesis suggests that *Nocomis* belongs to a monophyletic assemblage which includes the two species of *Hybopsis*. *Nocomis* and *Hybopsis* have had a long history of taxonomic and systematic confusion, and the taxonomic status of *Nocomis* has vacillated between genus, subgenus, and species-group for over 100 years (LACHNER and JENKINS 1981). *Nocomis* is now regarded as a good genus, although it clearly has affinities with certain species of *Hybopsis* (JENKINS and LACHNER 1971; LACHNER and JENKINS 1971). The NOR chromosome data lend further evidence for this relation-

ship. One last point to note is that MAYDEN's (1989) hypothesis also indicates a sister-group relationship between *Campostoma* and *Dionda* which is not shown (but not falsified) by the chromosomal hypothesis.

#### *Ericymba buccata*

The possession of a B NOR chromosome in *Ericymba* is somewhat troublesome. Based on the outgroup comparison with *Hemitremia* (and assuming the B NOR chromosomes are homologous), the inference is that *Ericymba* has maintained the plesiomorphic and hence phylogenetically uninformative NOR character state. However, both MAYDEN (1989) and COBURN (pers. commun.), on the basis of morphology, concur that *Ericymba* properly belongs in the *Notropis dorsalis* species-group. We have examined two members of this species-group (*N. longirostris* and *N. sp. cf. longirostris*), and both possess a single NOR chromosome with a D phenotype (Table 2). This might suggest that the *N. dorsalis* species-group, as presently constituted, is not monophyletic. Alternatively, *Ericymba* could indeed belong to the *N. dorsalis* species-group, and its B NOR chromosome could represent a non-homology or homoplasy with respect to the B NOR chromosomes found in other cyprinid taxa. This hypothesis, however, is less parsimonious than the arrangement shown in Fig. 6.

#### The *Notropis* lineages

As might be expected, the situation in *Notropis* is rather complex. Inspection of the NOR data (Table 3) reveals at least four phenetic chromosomal assemblages (Fig. 6) encompassing the forty nominal *Notropis* species (including *Pimephales*) thus far examined. We have defined these *Notropis* assemblages as (i) the D group, comprising seventeen species and defined by the presence (or inferred past presence) of the D NOR character state, (ii) the C group, comprising two species and defined by the C NOR character state; (iii) the C' group, comprising ten species and defined by the presence of the C' NOR character state or of its presumed derivatives; and (iv) the F' group, comprising eleven species and defined by the presence of the F' NOR character state or its presumed derivatives.

By outgroup comparison to *Hemitremia*, the D, C, C', and F' NOR chromosomes are all apomorphic. Exactly which (if any) of these NOR chromosomes might be plesiomorphic for the *Notropis* line-

ages is uncertain. With one exception (AMEMIYA and GOLD 1990), the C' and F' NOR chromosomes have not been found outside of *Notropis*, and only *Hybopsis storeriana* (Table 2) possesses a D NOR chromosome. The latter, however, was inferred by parsimony to be autapomorphic within *Hybopsis* (Fig. 7a). The exception (AMEMIYA and GOLD 1990) is a species of *Hybognathus* (*H. nuchalis*) which appears to possess a C' NOR chromosome. Thus far, we have been unable to determine whether the C' NOR chromosome found in a few *H. nuchalis* specimens represents a past hybridization between *H. nuchalis* and a *Notropis* species with a C' NOR chromosome, or whether a C' NOR chromosome represents a true chromosomal state within *Hybognathus*. Regardless, a definitive chromosomal synapomorphy for the *Notropis* lineages has yet to be identified, with the result that the four NOR chromosomes (D, C, C', and F') defining each group have been treated as autapomorphies (Fig. 6). Compositions and relationships within and among the four groups are briefly discussed below.

1) *The D group*. — The D group is defined by the occurrence (or inferred past occurrence) of a D NOR character state and comprises seventeen species (Fig. 8). Monophyly of all of the D group taxa is not supported by morphology. MAYDEN (1989) places *N. stramineus* and the two *N. longirostris* species outside of a large "Notropis" clade, and there are at least nine putative morphological assemblages represented among the seventeen D group species (Table 3). These include the *Notropis* subgenera *Pteronotropis* (*N. hubbsi*, *N. signipinnis*, *N. welaka*), *Hydrophlox* (*N. baileyi*, *N. chrosomus*, *N. nubilus*), *Alburnops* (*N. girardi*), *Luxilus* (*N. pilsbryi*), and *Notropis* (*N. stilbius*), as well as the *N. texanus* species-group (*N. chalybaeus*, *N. texanus*), the *N. volucellus* species-group (*N. buchanani*, *N. maculatus*, *N. volucellus*), the *N. dorsalis* species-group (*N. longirostris*, *N. sp. cf. longirostris*), and *N. stramineus*. It also is possible that some of the D NOR chromosomes are not homologous or are homoplasious since the typical North American cyprinid genome consists of large numbers of medium-sized submetacentric chromosomes (GOLD et al. 1979, 1981). This problem awaits further examination using alternative chromosome banding methods. However, monophyly of the three *Pteronotropis* species (defined by the addition of an F NOR chromosome) is suggested by the chromosome data (Fig. 8), and AMEMIYA and GOLD (1990) have shown via RHG-banding that the F NOR chromosome in all three species is homolog-

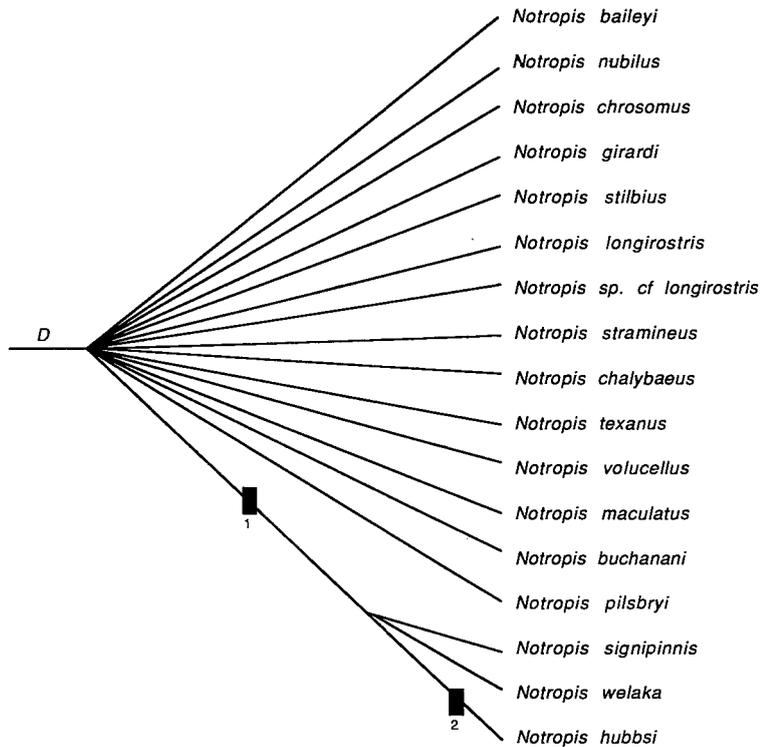


Fig. 8. Phylogenetic hypothesis of relationships within the D NOR chromosome group. Character state changes are: (1) +F; and (2) -D.

ous. Finally, given MAYDEN's (1989) hypothesis that *N. stramineus* and the two *N. longirostris* species may not belong in *Notropis*, and that the subgenus *Pteronotropis* is a basal lineage in a large *Notropis* clade, it could be argued that the D NOR character state is the plesiomorphic NOR condition for *Notropis*.

2) *The C group*. — The C group is defined by the C NOR character state and comprises the two species of *Pimephales* thus far examined (Table 2). C NOR chromosomes also occur in *N. ardens* and *N. roseipinnis* of *Lythrurus*, but were considered by parsimony (see below) to be apomorphic within that subgenus. Monophyly of the two *Pimephales* species is supported by morphological data (MAYDEN 1987). Recent evidence (CAVENDER and COBURN 1986; MAYDEN and MATSON 1988) indicates that *Pimephales* is closely related phylogenetically to certain lineages (specifically *N. emiliae* and the subgenus *Cyprinella*) within the genus *Notropis*. Both *N. emiliae* and *Cyprinella* were placed in the C' NOR chromosome group, and it is possible that the C NOR chromosome could be derived from the C'

NOR chromosome by a simple translocation. Work to test this hypothesis is currently in progress in our laboratory.

3) *The C' group*. — The C' group is defined by the presence of the C' NOR character state or its presumed derivatives and comprises ten species (Fig. 9). Except for *N. braytoni* (a species whose affinities are essentially unknown), monophyly of the C' group is supported by morphology. The remaining C' group species except for *N. emiliae* are placed in the putatively monophyletic *Notropis* subgenus *Cyprinella* (COBURN 1982; MAYDEN 1989), and recent evidence (CAVENDER and COBURN 1986; MAYDEN and MATSON 1988) supports a close phyletic relationship between *N. emiliae* and *Cyprinella*. In addition, C-banding (GOLD and AMEMIYA 1986; AMEMIYA and GOLD 1988; unpublished) has shown that (i) all of the C' NOR chromosomes are homologous; (ii) the E' NOR chromosome in *N. emiliae* is homologous to and easily derived by a paracentric inversion from a C' NOR chromosome; and (iii) the H NOR chromosome in *N. proserpinus* was

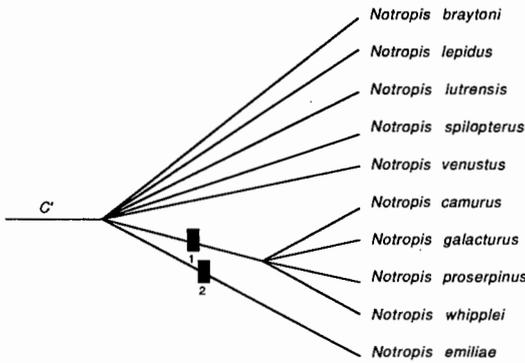


Fig. 9. Phylogenetic hypothesis of relationships within the C' NOR chromosome group. Character state changes are: (1) C'→H; and (2) C'→E'.

probably derived from a C' NOR chromosome via a non-reciprocal translocation. The chromosome data therefore suggest that the C' NOR chromosome represents both a chromosomal synapomorphy which unites the ten C' group species and a chromosomal symplesiomorphy for the subgenus *Cyprinella*. The H NOR chromosome would then appear to represent a chromosomal synapomorphy uniting the four species which possess this character state.

4) *The F' group.* — The F' group is defined by the presence of the F' NOR chromosome or of its pre-

sumed derivatives and comprises eleven species. Of these, nine possess an F' NOR chromosome, which by commonality is inferred to be the symplesiomorphic NOR character state within the F' group. *Notropis jemezianus* is provisionally placed in the F' group since its I' NOR chromosome (cf Table 2) could easily be derived from an F' NOR chromosome by a pericentric inversion. *Notropis oxyrhynchus* is also included in the F' group, in part because it is considered on morphological grounds to be the sister species of *N. atherinoides* (COBURN, pers. commun.), and in part because its NOR chromosome condition can be derived from that of *N. atherinoides* by a single event (loss of a NOR site). The phylogenetic relationships inferred from the chromosomal NOR data are shown in Fig. 10.

Inferred synapomorphies (based on parsimony) support monophyly of *N. atherinoides* and *N. oxyrhynchus* (defined by the inferred addition of a J NOR chromosome), the five species (*N. ardens*, *N. bellus*, *N. fumeus*, *N. roseipinnis*, *N. umbratilis*) of the subgenus *Lythrurus* (defined by the addition of an H NOR chromosome), and *N. ardens* and *N. roseipinnis* (defined by the addition of a C NOR chromosome). Monophyly of *N. atherinoides* and *N. oxyrhynchus* and of the five *Lythrurus* species is consistent with current hypotheses based on morphological data (COBURN 1982, pers. commun.; MAYDEN 1989). A chromosomal NOR synapomorphy uniting the members of the subgenus *Notropis* (viz., *N. amabilis*, *N. atherinoides*, *N. jeme-*

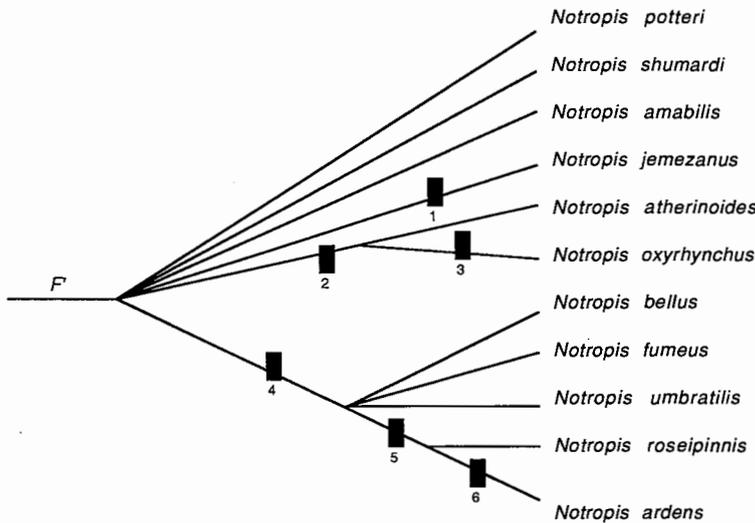


Fig. 10. Phylogenetic hypothesis of relationships within the F' NOR chromosome group. Character state changes are: (1) F'→I'; (2) +J; (3) -F; (4) +H; (5) +C; and (6) -H.

zanus, *N. oxyrhynchus*, and *N. shumardi*) was not identified.

The F' group comprises species from at least three morphological assemblages: the *Notropis* subgenera *Lythrurus* and *Notropis* (see above, Table 3) and the *Notropis* subgenus *Alburnops* (*N. potteri*). Monophyly of the F' group, and of the three *Notropis* subgenera, is largely dependent on whether the F' NOR chromosome is homologous among the various taxa. Similar C-banding patterns have been observed on the F' NOR chromosomes of *N. amabilis* and *N. shumardi* (*Notropis*), *N. roseipinnis* (*Lythrurus*), and *N. potteri* (*Alburnops*) (AMEMIYA 1987), which suggests that all three subgenera are closely related phylogenetically. This hypothesis, however, is not strongly supported by morphological data. MAYDEN (1989) and COBURN (pers. commun.) are of the opinion that *Lythrurus* may be more closely related to the subgenus *Cyprinella*, although both acknowledge that proposed relationships among the various *Notropis* subgenera and species-groups are more than a bit equivocal. One other point to note is that both *N. girardi* (currently placed in *Alburnops*) and *N. stilbius* (currently placed in *Notropis*) possess a single pair of NOR chromosomes of the D NOR phenotype (Table 3). Assuming these D NOR chromosomes are not homoplasious, the inference is that neither *Alburnops* or *Notropis* as presently constituted are monophyletic.

#### IV. Working hypothesis of North American Cyprinid relationships based on chromosomal NORs

A working hypothesis of relationships among the North American cyprinids examined for chromosomal NORs is shown in Fig. 11. A few of the presumed synapomorphies (i.e., B → D, D → largest chromosome in the complement) are based on circumstantial evidence and the assumption that most of the *Notropis* species (including *Pimephales*) comprise a monophyletic assemblage. With the exception of a few species (viz., the two species of *N. longirostris*, *N. stramineus*, and possibly *N. braytoni*), this assumption is supported by current morphological data (COBURN 1982, pers. commun.;

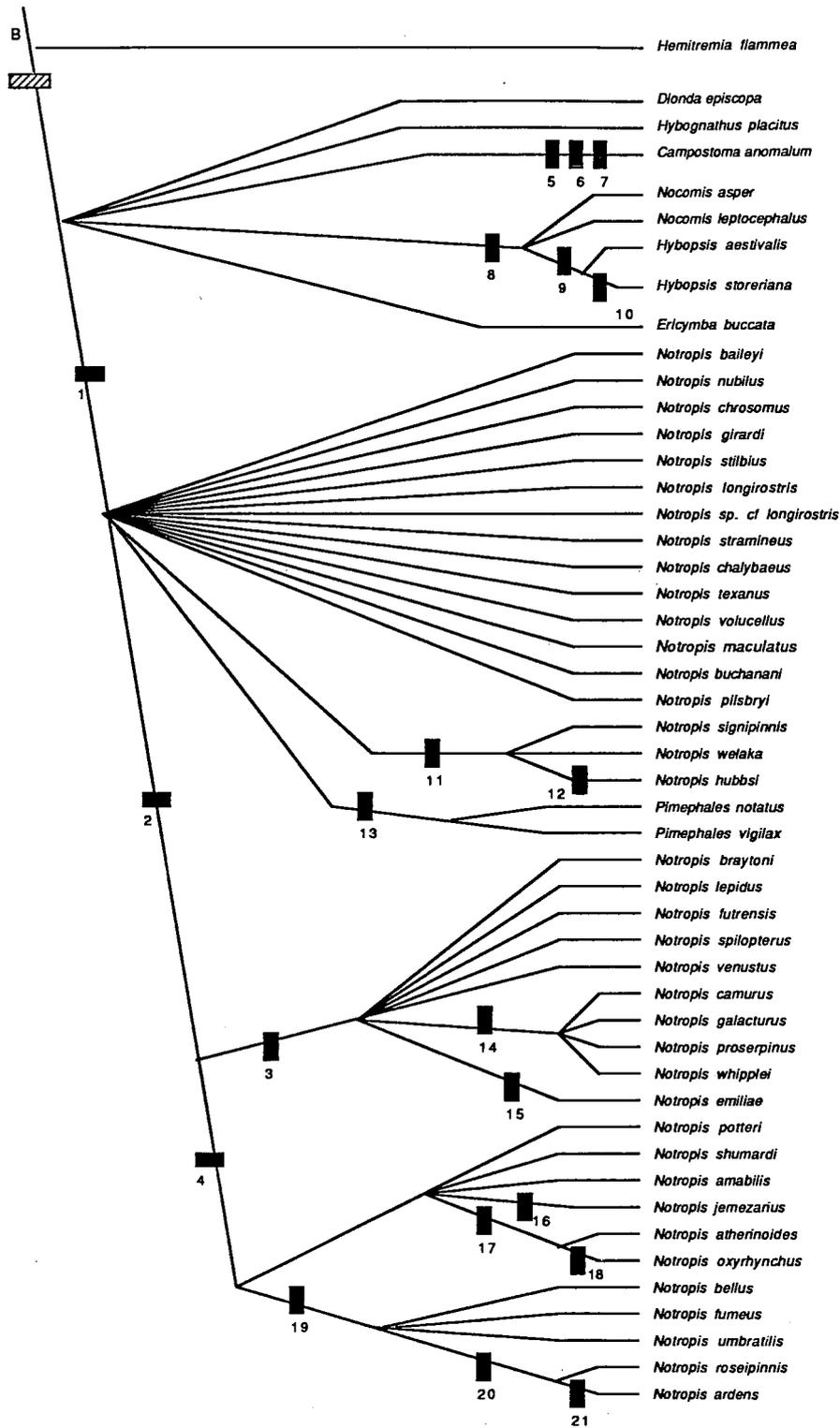
MAYDEN 1989, pers. commun.; CAVENDER and COBURN 1985, 1986, 1988). In the cladogram, the D NOR character state is considered plesiomorphic for the *Notropis* lineages, and the C group (*Pimephales*) is shown to be derived from the putatively plesiomorphic D NOR character state. As noted previously, however, *Pimephales* is now thought to be allied with species placed in the C' group. The sole intention of the working hypothesis is to establish a broad framework or hypothesis which can be tested by future cytosystematic and other phylogenetic studies of North American Cyprinidae.

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#### Appendix I. Taxa examined and collection localities

Taxon	Locale (Drainage)
<i>Dionda episcopa</i>	Nueces R., Real County, Texas (Nueces R.)
	Pecos R., Val Verde County, Texas (Rio Grande R.)
<i>Ericymba buccata</i>	Lees Cr., Washington Parish, Louisiana (Bogue Chitto R.)
<i>Hybopsis storeriana</i>	Brazos R., Brazos County, Texas (Brazos R.)
	Brazos R., Robertson County, Texas (Brazos R.)
	W. Pearl R., Orleans Parish, Louisiana (Pearl R.)
<i>Nocomis asper</i>	Blue R., Johnston County, Oklahoma (Red R.)
<i>Notropis atherinoides</i>	S. Canadian R., Seminole County, Oklahoma (Arkansas R.)
	Red R., Cooke County, Texas (Red R.)
	Lake Texoma, Marshall County, Oklahoma (Red R.)

**Fig. 11.** Working hypothesis of North American cyprinid relationships based on chromosomal NOR data. The cross hatched bar represents the synapomorphic character state of an opening of the posterior myodome (MAYDEN 1989). Character state changes are: (1) B→D; (2) D→NOR site on the largest chromosome in the complement ("x"); (3) "x"→C'; (4) "x"→F'; (5) B→A (or G); (6) +A; (7) +G (or A); (8) +B; (9) B→F; (10) B→D; (11) +F; (12) -D; (13) D→C; (14) C'→H; (15) C'→E'; (16) F'→I'; (17) +J; (18) -F'; (19) +H; (20) +C; and (21) -H.



<i>Notropis bellus</i>	Shades Cr., Jefferson County, Alabama (Cahaba R.)
<i>Notropis chalybaeus</i>	Big Cypress Bayou, Harrison County, Texas (Red R.)
<i>Notropis chrosomus</i>	Cahaba R., Jefferson County, Alabama (Cahaba R.)
<i>Notropis fumeus</i>	Amite R., Livingston Parish, Louisiana (Amite R.)
<i>Notropis girardi</i>	S. Canadian R., Seminole County, Oklahoma (Arkansas R.)
<i>Notropis roseipinnis</i>	Talisheek Cr., St. Tammany Parish, Louisiana (Pearl R.)
<i>Notropis stilbius</i>	Cahaba R., Jefferson County, Alabama (Cahaba R.) Mariot Cr., Cullman County, Alabama (Black Warrior R.)

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