Cytogenetic studies in North American minnows (Cyprinidae). XII. Patterns of chromosomal nucleolus organizer region variation among 14 species

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The chromosomal nucleolus organizer region (NOR) phenotypes of eight species of North American cyprinid fish are documented. This brings the total number of cyprinid species examined for chromosomal NORs to 14. At least 10 different NOR chromosome phenotypes are identifiable among the 14 species. These interspecific variations include differences in the (haploid) number of chromosomal NORs, the chromosomal location(s) of the NORs, and the type(s) of chromosomes upon which the NOR is located. Intraspecific variations or heteromorphisms of NOR chromosomes also occur, but are of a qualitatively different nature than the NOR variants observed between species. Arrangement of the interspecies NOR differences into phylogenetic hypotheses yields results which are not discordant with present concepts of North American cyprinid taxonomy, and in fact support the hypothesis of a close relationship between the cyprinid genera *Notropis* and *Pimephales*. These data suggest that NOR chromosome phenotypes will be useful in resolving problems in cyprinid systematics. The data also show that at least one or more chromosomal changes involving the NOR separate most of the species examined, and that at least nine different chromosomal rearrangements have occurred since the 14 species last shared a common ancestor. This suggests that chromosomal changes in cyprinids have been much more frequent than previously thought.

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On trouvera ici des données sur les phénotypes de huit espèces nord-américaines de cyprinidés qui diffèrent par la région de l'organisateur du nucléole (RON) sur les chromosomes. Cette étude porte à 14 le nombre d'espèces de cyprinidés dont les RON chromosomiques ont été étudiées. Au moins 10 phénotypes différents reliés à la RON chromosomique peuvent être reconnus. Parmi les variations interspécifiques, il y a des différences dans le nombre (haploïde) de RON chromosomiques, la position des RON sur les chromosomes et le type (ou les types) de chromosomes sur lesquels sont situés les RON. Il existe aussi des variations intraspécifiques, ou hétéromorphismes, des chromosomes RON, mais ces variations sont de nature différente de celle des différences qui prévalent entre espèces. La formulation d'hypothèses phylogénétiques pour exprimer les différences RON interspécifiques donne des résultats qui ne viennent pas en contradiction avec les concepts actuels de taxonomie des cyprinidés nord-américains et ces résultats appuient l'hypothèse selon laquelle les genres *Notropis* et *Pimephales* sont très apparentés. Ces données indiquent que les phénotypes reliés aux chromosomes RON pourront servir à résoudre certains problèmes en systématique des cyprinidés. Les données indiquent également qu'au moins un changement chromosomique relié à la RON, et peut-être plus d'un, sépare la plupart des espèces examinées et qu'au moins neuf réarrangements chromosomiques différents se sont produits depuis le moment où les 14 espèces se sont séparées de l'ancêtre commun. Il semble donc que les changements chromosomiques chez les cyprinidés aient été beaucoup plus fréquents qu'on ne l'avait cru à ce jour.

[Traduit par la revue]

Introduction

Comparative karyology, particularly that involving differential banding of metaphase chromosomes, has proven a useful and powerful tool in addressing a number of systematic and evolutionary questions in a broad spectrum of vertebrates. As examples, Dutrillaux and colleagues (1981, 1982) have published extensively on chromosome banding comparisons in primates and have erected chromosomal phylogenies for most of the major groups. They also have been able to evaluate differential chromosomal change in separate lineages and to identify exceptional cytogenetic situations where they have occurred. Similar and no less elegant chromosome studies have been carried out by Baker and colleagues on bats and rodents (Yates *et al.* 1979; Haiduk and Baker 1982; Baker *et al.* 1982), and by Schmid (1980, 1982) on amphibians. Numerous other studies have been published on the chromosomes of other mammals, birds, turtles, and snakes (Arnason 1974; Wurster-Hill and Centerwall 1982; Stock and Mengden 1975; Stock and Bunch 1982; Bickham and Baker 1976; Bickham and Rogers 1985; Mengden and Stock 1980).

In fishes, comparative karyology in a systematic context has received far less attention. Until recently, most of the work has centered either on defining the chromosome and (or) chromosome arm numbers of different species or on identifying heteromorphic chromosomes (Gold 1979). A few studies (e.g., Chen 1971; Uyeno and Miller 1973; Scheel 1975; Uyeno *et al.* 1983) have focused on cytotaxonomic aspects of karyotypic differentiation in fishes, but in general the contribution of karyology to fish systematics has been minimal. The limiting factors apparently include obtaining consistently good metaphase preparations from fish tissues and successfully applying the chromosome banding techniques commonly in use in other vertebrates. In addition, most fish complements contain a relatively large number of comparatively small chromosomes (Gold 1979). There are several recent reports of successful nucleolus organizer region (NOR) and (or) heterochromatin (C) banding of fish chromosomes (references in Gold 1984; Moreira-Filho *et al.* 1984; Gold *et al.* 1986), and a few scattered reports of relatively decent serial (e.g., G) banding (Blaxhall 1983; Wiberg 1984; Delany and Bloom 1984). Most of these, however, have involved only one or a few fish species from widely separate groups, and no attempts to evaluate the data in a systematic fashion have been made.

Chromosome studies in our laboratory over the past 10 years have focused on the standard karyology of the North American cyprinid fishes. Briefly, these fishes comprise an essentially

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monophyletic assemblage of more than 200 extant species, most of which are placed in a single subfamily, the Leuciscinae (Hubbs 1955; Miller 1959). The lone exception is the monotypic genus *Notemigonus* (*crysoleucas*) which may belong to the Old World subfamily Abramidinae (Miller 1959). One genus, *Notropis*, contains over 100 living representatives, but most genera contain only one or a few species (Miller 1965; Lee *et al.* 1980). With notable exceptions, most North American cyprinids generally resemble one another in body shape, are less than 15 cm in length, and are found in streams and rivers more often than in lakes or ponds (Lee *et al.* 1980). They also display a wealth of diversity in habitats, adaptations, and behaviors. As might be expected for such a large group of morphologically similar, small forms, the systematics and taxonomy of the group has had a long and storied history and numerous problems abound.

Standard karyotypes documenting chromosome and (estimated) chromosome arm numbers are published for over 60 North American cyprinids (22 in *Notropis*), and unpublished karyotypes exist for another 20 or so species (Gold *et al.* 1980; C. T. Amemiya and J. R. Gold, unpublished data). Of all these, over 90% (including all *Notropis*) have $2n = 50$ chromosomes, and all fall in the range $2n = 48–52$. Estimated (diploid) chromosome arm numbers vary from 80 to 100 (from 92 to 100 in *Notropis*), but only a few species (<6%) have chromosome arm numbers less than 92. As a whole, the data have been uninformative systematically. Alternatively, the apparent conservatism in gross chromosome structure is in striking contrast to the apparently rapid speciation exhibited by the group. Gold *et al.* (1978) estimated the speciation rate within living North American cyprinid genera to be approximately 0.7 new species per lineage (genus) per 10^6 years. This rate is considerably higher than speciation rates estimated for many lower vertebrate taxa (Bush *et al.* 1977), and differs markedly from the apparently low rates of cyprinid gross chromosomal evolution (Gold 1980; Gold *et al.* 1981).

Recently, we developed reliable cytological methods for localizing the nucleolus organizer regions (NORs) to cyprinid chromosomes and examined the NOR chromosomal phenotypes within and between six cyprinid species (Gold and Ellison 1983; Gold 1984). We found that at least one or more chromosomal changes involving the NOR separated all but two of the species examined, and in addition, that the differences corresponded roughly to the established taxonomy of the species under study. Considerable variation in the chromosomal NORs also was found within species, but was of a qualitatively different nature than that observed between species. These data suggested first, that chromosomal changes in cyprinids may occur much more frequently than previously thought, and secondly, that chromosomal NOR phenotypes could ultimately prove useful in resolving systematic problems in the group. In this paper, the chromosomal NOR phenotypes of 8 additional North American cyprinid species are documented, and the NOR data from the 14 species thus far studied are evaluated and discussed.

Materials and methods

The fish examined in the study were collected by seine from natural populations. The species (collection localities) were as follows: *Notropis potteri*, *Notropis shumardi*, *Hybopsis aestivalis*, and *Hybognathus placitus* Brazos River, Brazos County, Texas); *Notropis braytoni* (Pecos River, Val Verde County, Texas); *Notropis longirostris* (W. Thompsons Creek, W. Feliciana Parish, Louisiana); *Notropis ardens* (Scroungeabout Creek, Morgan County, Alabama); and *Notropis* sp. (cf. *longirostris*) (Little Sandy Creek, Bibb County, Alabama). The latter is an undescribed species from the Mobile Bay drainage in Alabama and is most closely related to *N. longirostris* (Heins *et al.* 1980). Specimens collected in Alabama were processed (i.e., kidney and gill tissues removed and fixed) in Dr. W.M. Howell's laboratory at Samford University in Birmingham; all others were returned live and processed in our laboratory in College Station. Voucher specimens for each species were deposited in the Texas Cooperative Wildlife Collection at Texas A&M University.

Chromosome preparations were made either directly from kidney or gill tissue of colcemid- or colchicine-injected specimens (following Gold 1984), or from fibroblast cultures (following Amemiya *et al.* 1984). Slides prepared by either method were stored desiccated at room temperature until used. NOR-banding of prepared slides was carried out by staining either with silver (after Gold and Ellison 1983) or with chromomycin A_3 (CMA_3) or 4,6-diamidino-2-phenylindole (DAPI) (after Amemiya and Gold 1986). Chromosomes stained with silver (including Giemsa counterstaining) were photographed in bright-field using technical pan film at ASA 25–40; those stained with CMA_3 or DAPI were photographed using epifluorescence and either technical pan film at ASA 125–160 or panatomic X film at ASA 100–160. Both films were developed in either Diafine (Acufine) or D19 (Kodak).

Quantitative determinations 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 a program (BANDSCAN) written by one of us (C.T.A.) and Dr. John R. Ellison of the Department of Biochemistry and Biophysics at Texas A&M University. The program essentially (*i*) sorts the chromosomes by length, centromere position, and NOR-band size and position, (*ii*) arranges the chromosomes into pairs on the basis of centromere position and then the pairs on the basis of chromosome length, and (*iii*) provides both hard copy data and idealized haploid and diploid idiograms of each measured metaphase spread. The program is written in BASIC and is available from Dr. Ellison.

Results

Summary data of the NOR-stained material are shown in Table 1 and include the six species studied by Gold (1984). The species are arranged in the Table in accordance with present concepts of North American cyprinid taxonomy (references in Lee *et al.* 1980). All individuals from all species possessed $2n = 50$ chromosomes, typical of most North American cyprinids (Gold *et al.* 1980), and in agreement with the chromosome numbers previously found for these species (Gold *et al.* 1980; Amemiya and Gold 1987).

At least 10 different NOR chromosome phenotypes are identifiable among the 14 species. These are shown by letter designations under the appropriate column in Table 1. Note that a minimum of two different A phenotypes must exist since *C. anomalum* possesses two pairs of NOR chromosomes with the A phenotype, and that at least two different C and F phenotypes must exist on the basis of differences in relative chromosome size (see below). Note also that identical NOR phenotypes between species does not necessarily imply chromosomal homology. The NOR chromosome phenotypes were assigned on the basis of 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. The first two criteria were relatively easy to assess in most preparations; the third was more equivocal, especially when differentiating the large submetacentric or acrocentric NOR chromosomes found in *H. aestivalis*, *P. vigilax*, and all the *Notropis* species examined. We differentiated these on the basis of whether the chromosome was medium- or large-sized in the complement, or

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*
Family Cyprinidae				
Subfamily Abramidinae				
1. *Notemigonus crysoleucas*†	9	112	1	A
Subfamily Leuciscinae				
2. *Campostoma anomalum*†	6	43	3	A,A,G
3. *Hybopsis aestivalis*	6	137	2	B,F
4. *Hybognathus placitus*	1	200	1	B
5. *Pimephales vigilax*†	10	114	1	C
Genus *Notropis*				
Subgenus *Alburnops*				
6. *N. longirostris*	2	48	1	D
7. *N.* sp. (cf. *longirostris*)	4	51	1	D
8. *N. potteri*	2	38	1	C′
Subgenus *Cyprinella*				
9. *N. lutrensis*†	31	329	1	C′
10. *N. venustus*†	32	325	1	C′
Subgenus *Lythrurus*				
11. *N. ardens*	2	46	2	C,F′
Subgenus *Notropis*				
12. *N. shumardi*	5	112	1	C′
Unknown affinities				
13. *N. braytoni*	4	255	1	C′
14. *N. emiliae*†	3	123	1	E′

NOTE: A prime symbol (′) indicates the chromosome is the largest in the complement.

*NOR chromosome phenotypes: A, terminal on the short arm of a medium-sized acrocentric; B, terminal on the short arm of a small-sized acrocentric; C, terminal on the short arm of a large-sized submetacentric; D, terminal on the short arm of a medium-sized submetacentric; E, subterminal on the short arm of a large-sized submetacentric; F, terminal on the short arm of a large-sized acrocentric; G, terminal on one arm of a large-sized metacentric.

†Data in part from Gold (1984).

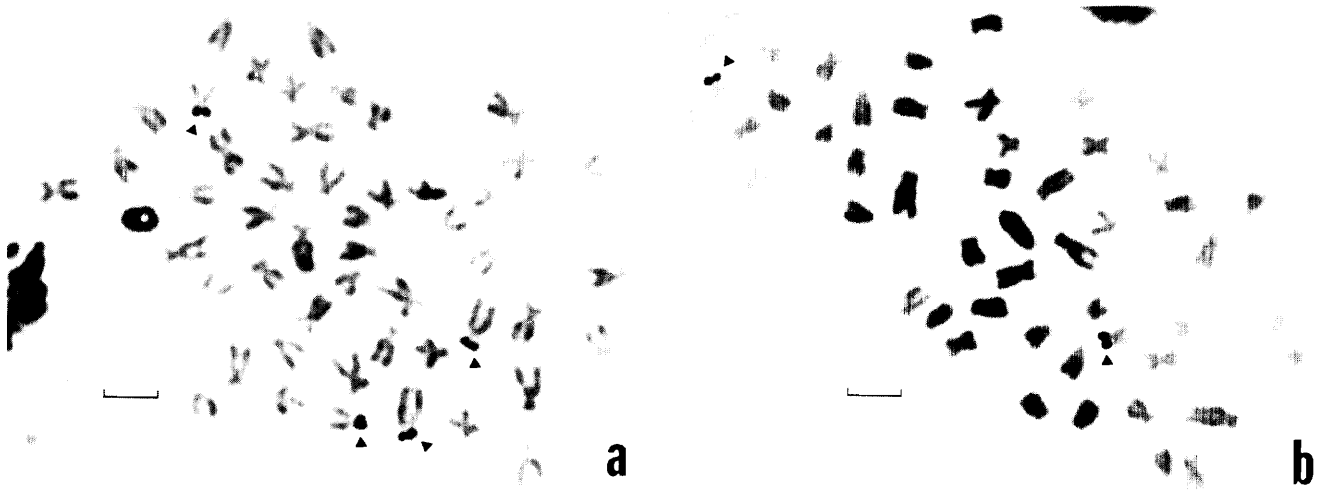

FIG. 1. Silver-stained metaphases of (*a*) *Hybopsis aestivalis* and (*b*) *Hybognathus placitus*. Chromosomal NORs are indicated by arrowheads. Bars equal 5 μm.

whether the chromosome was obviously the largest in the complement. The latter is indicated in the Table by a prime symbol (′) following the letter designation. NOR-stained metaphases or karyograms of the eight species examined in the study are shown in Figs. 1–4. Haploid idiograms generated from computer-assisted measurements of NOR karyotypes of the 14 species are shown in Fig. 5. These idiograms are intended only to demonstrate the NOR position and the relative size and centromere position(s) of NOR-bearing chromosomes within the complements of each species.

Intraspecific variations or heteromorphisms in NOR chromosome phenotypes were observed in over 10% of all specimens examined regardless of species. These variants were identified by criteria outlined in Amemiya and Gold (1986), and included either (*i*) consistent differences between chromosomally homologous NOR sites in the size of silver- or CMA_3-stained NORs,

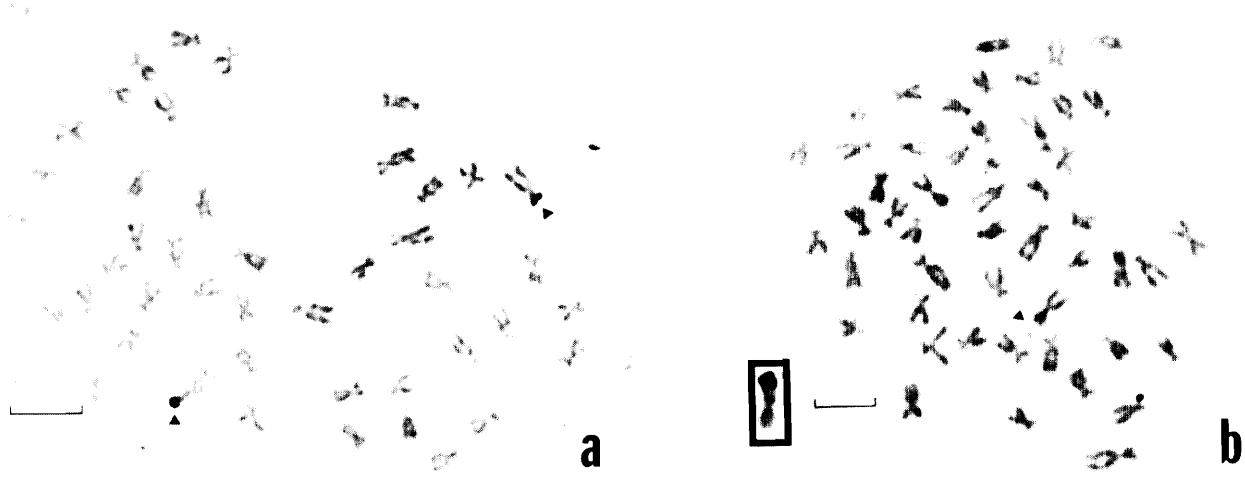

FIG. 2. (*a*) Silver-stained metaphase of *Notropis shumardi*, and (*b*) Giemsa-stained metaphase of *Notropis* sp. (cf. *longirostris*). The latter shows a prominent satellite at the NOR. Chromosomal NORs are indicated by arrowheads. The individual in Fig. 2*b* has an activity heteromorphism (cf. text) and only the satellited NOR chromosome could be identified by silver-staining (insert). Bars equal 5 μm.

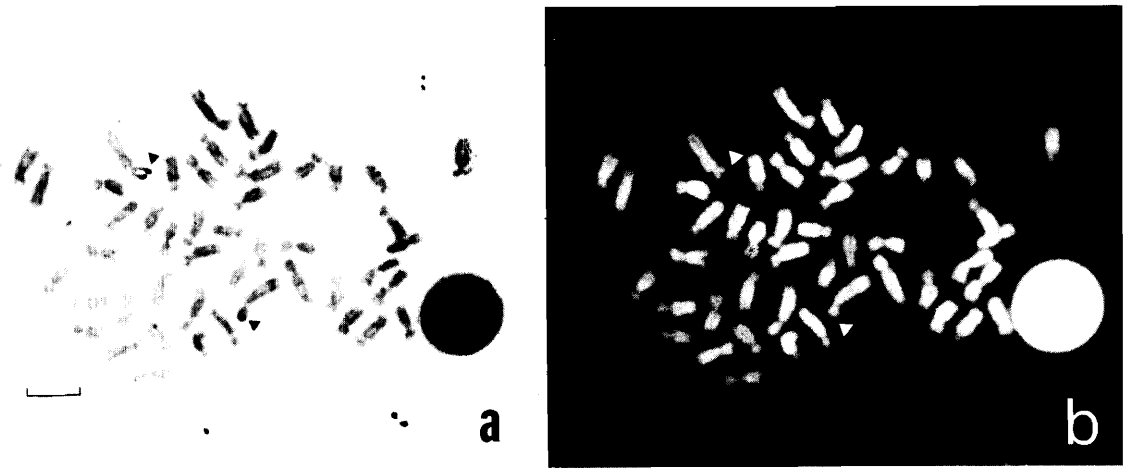

FIG. 3. (*a*) Silver-stained metaphase of *Notropis potteri*. (*b*) The same metaphase stained with DAPI. Note the reduced fluorescence of the NOR sites in Fig. 3*b*. Chromosomal NORs are indicated by arrowheads. Bar equals 5 μm.

or (*ii*) the absence of silver (but not CMA_3) stainability at one of two chromosomally homologous NOR sites. The former is considered to reflect amplification(s) of ribosomal gene sequences at an individual NOR site; the latter is thought to reflect loss of NOR transcriptional activity at a preceding interphase (references in Gold 1984). These types of NOR heteromorphisms appear to be relatively common in fishes (Foresti *et al.* 1981; Gold 1984; Moreira-Filho *et al.* 1984), and in cyprinids at least are qualitatively different from the NOR variants found between species. The latter have included only the (haploid) number or chromosomal location of NORs; whereas the former have included primarily variations in size and (or) apparent transcriptional activity between chromosomally homologous NOR sites. The only exceptions we have found were one individual of *P. vigilax* which apparently was heterozygous for an inversion involving the NOR (Fig. 2*a* in Gold 1984), and one individual of *N. lutrensis* in which one of the two (diploid) NOR sites was apparently deleted (Fig. 5*a* in Gold 1984). The importance of these observations is that (*i*) interspecies NOR variants in cyprinids appear to represent distinct character states indicative of species-specific differences, and (*ii*) they appear to have arisen via chromosomal changes not typically found within species.

The distribution of the NOR chromosome phenotypes among the 14 species (cf. Table 1) reveals a minimum of nine species-specific NOR differences. These are referred to hereafter as NOR character states. Eleven of the species possess only a single (homologous) pair of NOR chromosomes representing a minimum of six NOR character states. These include A (*N. crysoleucas*), B (*H. placitus*), C (*P. vigilax*), D (*N. longirostris* and *N.* sp. [cf. *longirostris*]), C′ (*N. potteri*, *N. lutrensis*, *N. venustus*, *N. shumardi*, and *N. braytoni*), and E′ (*N. emiliae*). Three of the species possess multiple NORs (i.e., more than one homologous pair) representing three distinct NOR character states. These include A,A,G (*C. anomalum*), B,F (*H. aestivalis*), and C,F′ (*N. ardens*).

Arrangement of these NOR character states into a systematic or phylogenetic hypothesis depends on the identification of

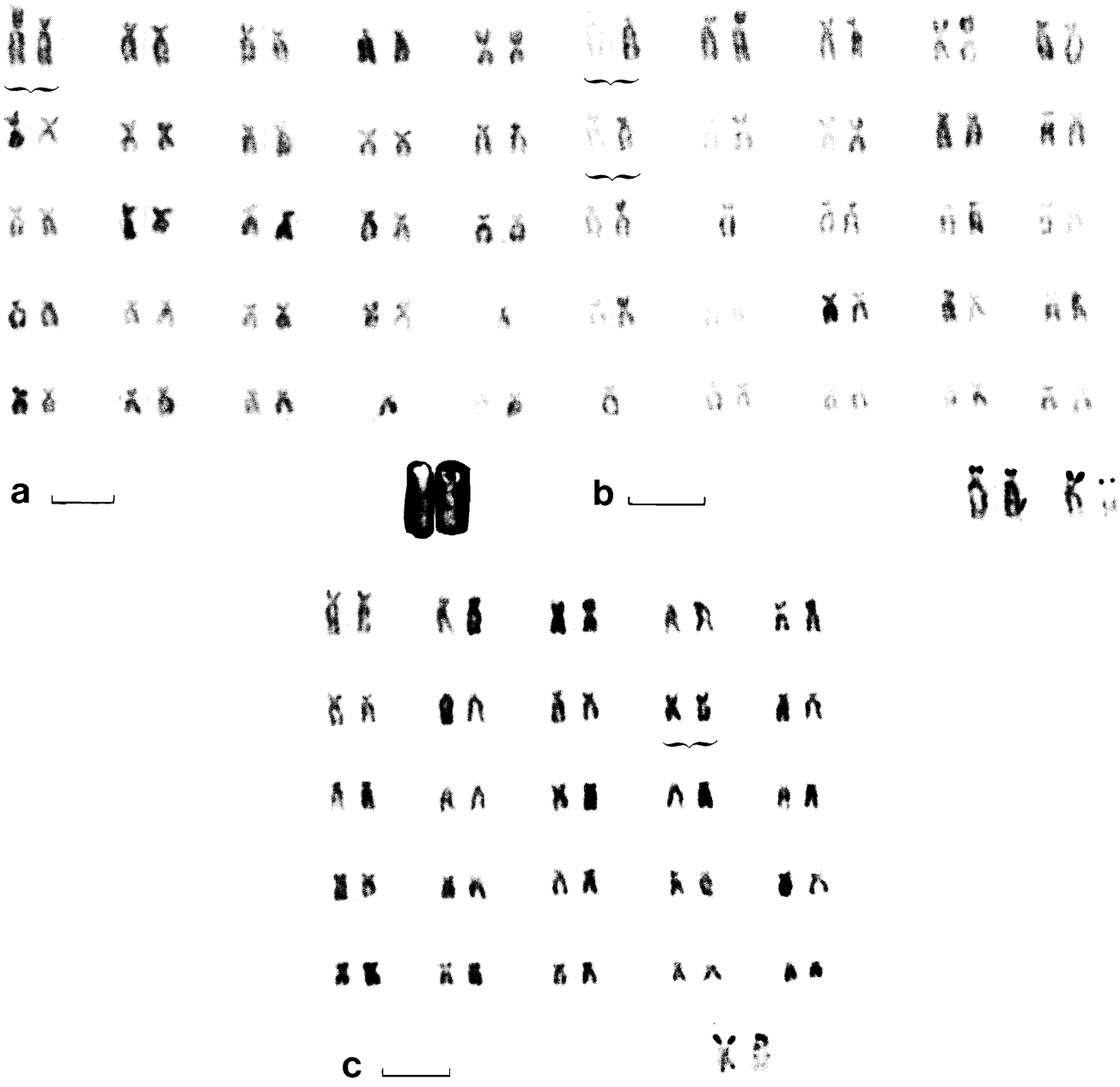

FIG. 4. Karyograms showing the NOR chromosomes of (*a*) *Notropis braytoni*, (*b*) *Notropis ardens*, and (*c*) *Notropis longirostris*. Horizontal parentheses underscore NOR chromosomes. At the bottom of each karyogram, the CMA_3- or silver-stained NOR chromosomes from the same metaphase are shown. Bars equal 5 μm.

homology of character states between species and the determination of character-state polarity. Thus far, we have been able to demonstrate homology of the C′ NOR chromosome in the five species (see above) which possess this character state (Amemiya 1985; Gold *et al.* 1986; C. T. Amemiya and J. R. Gold, unpublished data). We also have shown (Amemiya 1985) that the E′ NOR chromosome in *N. emiliae* is homologous to the C′ NOR chromosome except for a (presumed) small inversion of the short arm. Our evidence is based on the C-band patterns exhibited by the C′ and E′ NOR chromosomes (Fig. 6), although the size and shape of these chromosomes are very distinctive (e.g., Figs. 2*a*, 3*a*, 3*b*, 4*a*, and 5*a*–5*e* of this paper, and Figs. 1*a*–1*c* in Gold 1984). One other (putative) homology, i.e., the D NOR character state in *N. longirostris* and *Notropis* sp. (cf. *longirostris*), may be inferred since these two species are very close relatives and have only recently diverged from one another (Heins *et al.* 1980). The remaining NOR chromosome phenotypes shared by different species include A (*N. cryso-leucas* and *C. anomalum*), B (*H. placitus* and *H. aestivalis*), and C (*P. vigilax* and *N. ardens*). Homology of any of these three (A, B, or C) would be of interest since very little is known regarding intergeneric relationships in North American Cyprinidae. Homology of the A phenotype(s) between *Notemigonus* and *Campostoma* would be especially critical since the former is a logical outgroup to all the other genera (see below). However, in the absence of C-banding or other data to test these possible

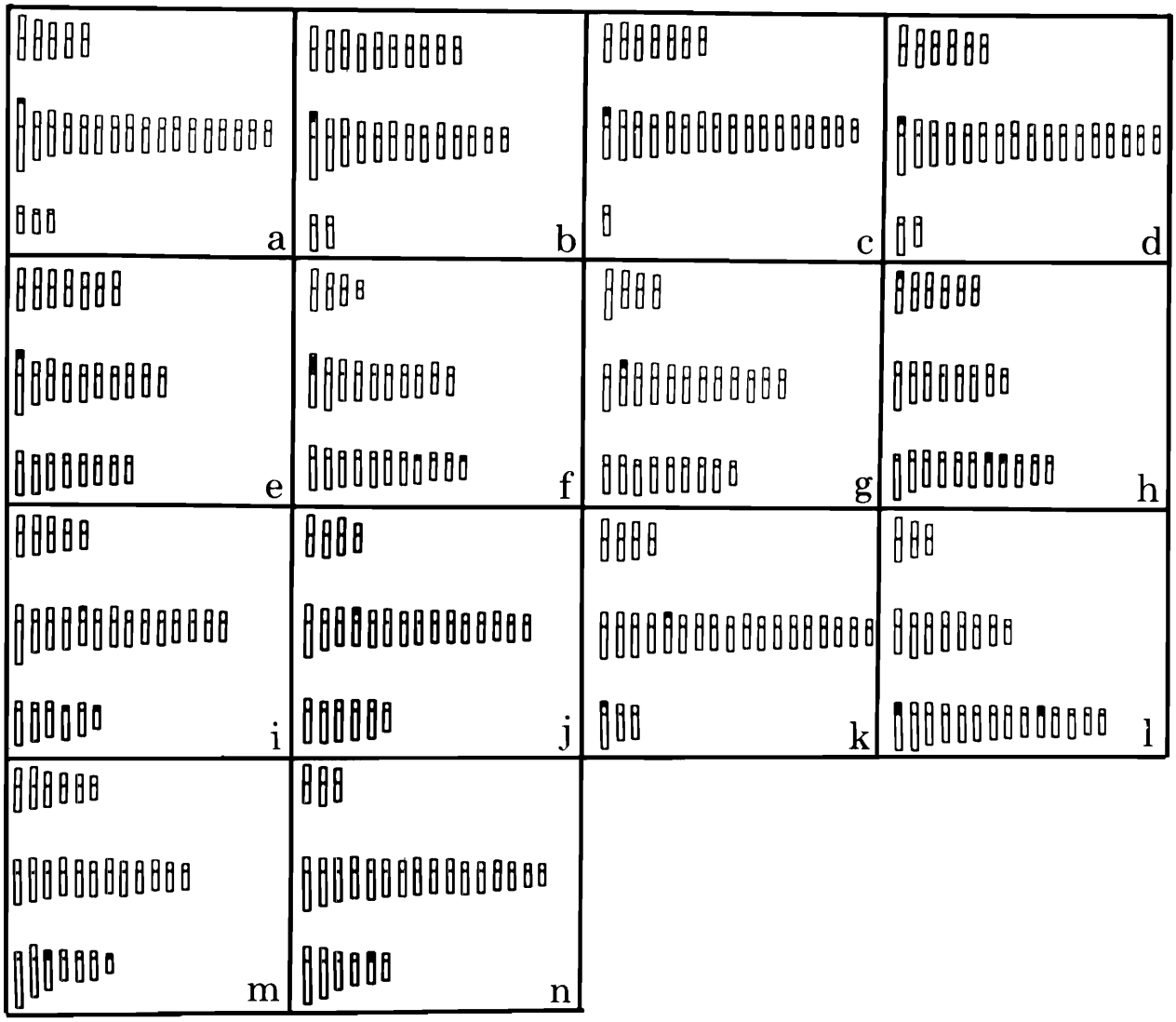

FIG. 5. Haploid idiograms of NOR karyotypes of the 14 species. Chromosomes are arranged into rows in each karyotype on the basis of centromere position (after Levan *et al.* 1964): metacentric chromosomes are in the top row, submetacentric chromosomes are in the middle row, and acrocentric (subtelocentric) chromosomes are in the bottom row. Within rows, chromosomes are arranged according to relative size. NORs are indicated by darkened areas. (*a*) *Notropis lutrensis*; (*b*) *Notropis venustus*; (*c*) *Notropis shumardi*; (*d*) *Notropis potteri*; (*e*) *Notropis braytoni*; (*f*) *Notropis emiliae*; (*g*) *Pimephales vigilax*; (*h*) *Campostoma anomalum*; (*i*) *Notropis longirostris*; (*j*) *Notropis* sp. (cf. *longirostris*); (*k*) *Notropis ardens*; (*l*) *Hybopsis aestivalis*; (*m*) *Notemigonus crysoleucas*; and (*n*) *Hybognathus placitus*.

homologies, the safest (and conservative) course at present is to treat them as nonhomologies or homoplasies (i.e., convergences).

Determination of character-state polarity depends in large part on the present taxonomy of the group since this affects the choice of appropriate outgroup comparisons. The pertinent taxonomic information relative to the present data set is given in Table 1. Simplistically, *Notemigonus* (subfamily Abramidinae) could be considered an outgroup to all other genera (subfamily Leuciscinae), and each of the leuciscine genera (excepting *Pimephales*, see below) could be considered as possible outgroups to *Notropis*. The equivocation regarding *Pimephales* is based on the early suggestion by Hubbs and Black (1947) that *Pimephales* might be a specialized derivative of a *Notropis* lineage, and on recent work (Cavender and Coburn 1985) which does suggest an intimate relationship between the two genera.

These outgroup comparisons, although limited, lead to three general hypotheses regarding the directions of NOR character-state evolution in North American Cyprinidae. The first is that possession of a single (haploid) NOR is plesiomorphic (primitive), whereas multiple NORs are apomorphic (derived). This follows in part from the observation that *Notemigonus* and three of the leuciscine genera (i.e., *Hybognathus*, *Pimephales*, and *Notropis*) have species with single NORs, and in part from the commonality principle (Watrous and Wheeler 1981) in that the majority of cyprinid species examined to date possess only a single NOR (Table 1). This is in accord with the suggestion of Hsu *et al.* (1975) and Schmid (1978) that a single homologous pair of NORs is primitive for most vertebrates. The second hypothesis is that a terminal position of the (single) NOR site is plesiomorphic, whereas nonterminal or interstitial NORs are apomorphic. This follows from both the outgroup comparisons and the commonality principle. The third and most tentative hypothesis is that the plesiomorphic condition for the (single, terminal) NOR is to be situated on the short arm of an acrocentric chromosome. This follows from the observations that *Notemi-*

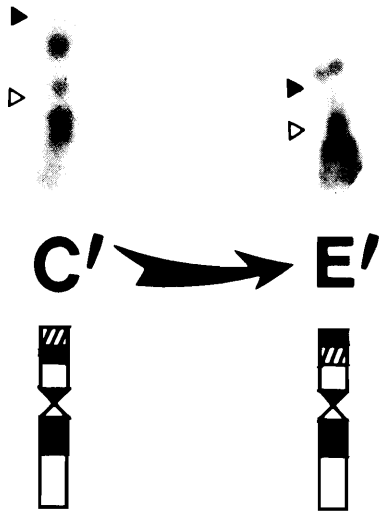

FIG. 6. C-banded preparations and schematic diagrams of the C′ and E′ NOR chromosomes from *Notropis lutrensis* and *Notropis emiliae*, respectively. Open arrowheads indicate centromere position; filled-in arrowheads indicate NOR position. Methods for C-banding followed Gold *et al.* (1986). Note that the E′ chromosome differs from the C′ chromosome by only a single (presumed) paracentric inversion in the short arm.

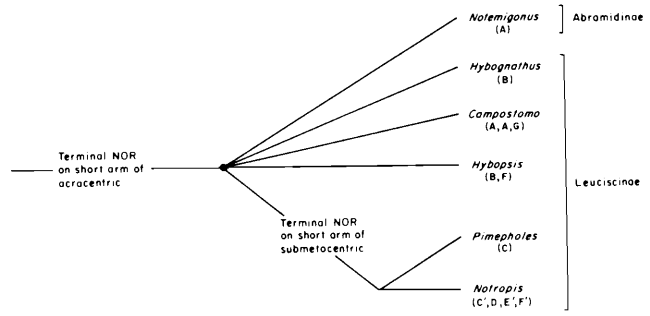

FIG. 7. Cladogram for genera of North American Cyprinidae. NOR character states (letters) are defined in Table 1. The subfamilial placements are based on suggestions from the literature (cf. text).

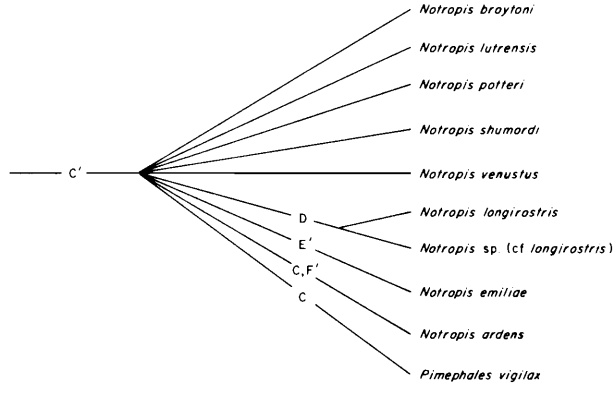

FIG. 8. Cladogram for *Notropis* lineages (including *Pimephales*). NOR character states (letters) are defined in Table 1.

gonus and three of the leuciscine genera (i.e., *Campostoma*, *Hybopsis*, and *Hybognathus*) possess NORs on the short arms of acrocentric chromosomes, and from our recent (and unpublished) findings that three other leuciscine genera (i.e., *Dionda*, *Hemitremia*, and *Nocomis*) also possess NORs which are terminal on the short arms of acrocentric chromosomes. Summarizing the above, we propose that a single (haploid) NOR situated terminally on the short arm of an acrocentric chromosome represents the plesiomorphic NOR condition in North American Cyprinidae; all others, including multiple NORs, interstitially located NORs, and NORs found on submetacentric and metacentric chromosomes are by definition apomorphic. A simple cladogram depicting the above and reflecting intergeneric relationships in North American Cyprinidae is shown in Fig. 7. The only synapomorphy suggested is the possession of a NOR on a submetacentric chromosome which is found in *Pimephales* and *Notropis*. This would appear to corroborate the close relationship between these two genera suggested by Hubbs and Black (1947) and Cavender and Coburn (1985). The NOR character states in the remaining genera are all autapomorphies generating an unresolved polychotomy within the subfamily Leuciscinae. Of interest for future work will be tests of homology of the A NOR chromosome phenotypes in *Notemigonus* and *Campostoma*, and the B NOR chromosome phenotypes in *Hybopsis* and *Hybognathus*.

A simple cladogram depicting relationships within *Notropis* (including *Pimephales*) is shown in Fig. 8. As noted previously, the C-band patterns of the C′ NOR chromosomes appear homologous in the five species which possess this NOR character state. Since these five species represent four distinct lineages within *Notropis* (cf. Table 1), the most parsimonious inference is that the C′ NOR character state is the plesiomorphic condition within *Notropis*. The remaining NOR character states are thus apomorphic and include the synapomorphic D character state which unites the two *longirostris* species and the autapomorphic E′, C, and C,F′ character states found in *N. emiliae*, *P. vigilax*, and *N. ardens*, respectively. Since only the E′ character state has presumably been derived directly from the plesiomorphic C′ character state (Amemiya 1985), all four apomorphies must be treated as an unresolved polychotomy. Of interest for future work will be tests of homology of the C NOR chromosome phenotype in *P. vigilax* and *N. ardens* as well as the determination of which (if either) of the two NOR chromosomes in *N. ardens* is homologous to the plesiomorphic C′ NOR chromosome.

Discussion

From the foregoing, there are two points which merit brief discussion. The first is that interspecies differences in chromosomal NORs would appear to be of potential use in North American cyprinid systematics. Thus far we have identified nine different, species-specific NOR character states among the 14 species examined. These include differences in the (haploid) number of chromosomal NORs per genome,the chromosomal location(s) of the NORs, and the type(s) of chromosomes upon which the NORs are located. Moreover, all of the interspecies NOR differences appear to have arisen via chromosomal changes which are not typically found within species. In addition, we have demonstrated that viable phylogenetic hypotheses can be generated using the NOR data by applying the standard methods of outgroup comparison and commonality. The approach is somewhat limited at present because of the difficulty in obtaining other types of metaphase chromosome banding (e.g., G-bands) on fish chromosomes, i.e., there are limitations in homologizing NOR chromosomes between species. Nonetheless, the phylogenetic hypotheses thus far generated on the

bases of NOR data are not at all discordant with present concepts of North American cyprinid taxonomy, and in fact appear to corroborate the suggested close relationship between the genus *Pimephales* and the genus *Notropis* as well as the obviously close relationship between the two *N. longirostris* species.

The second point to be made is that the observed differences in chromosomal NORs among the 14 species suggest that North American cyprinids are far less conservative in terms of chromosomal evolution than previously believed. As noted in the Introduction, our studies over the past decade on standard karyotypes have documented little evident change in either chromosome or chromosome arm numbers among the more than 80 or so cyprinid species examined. This pattern of chromosome conservatism seemed at odds with the apparently rapid rate of cyprinid speciation (Gold 1980; Gold *et al.* 1978), especially when compared with the positive correlation between speciation and chromosomal evolution exhibited by other vertebrates (Wilson *et al.* 1975; Bush *et al.* 1977). Given that we have identified at least 10 different NOR chromosome phenotypes among the 14 cyprinids examined, it would appear that one or more chromosomal changes involving the NOR separate most of the species, and that at least nine different chromosomal rearrangements have occurred since the 14 species last shared a common ancestor. This assumes, of course, that the NOR variants are derived from classical chromosomal rearrangements (e.g., as in Elder 1980). However, as noted by Gold (1984), it is possible that NORs could be moved from chromosome to chromosome or site to site by mechanisms not involving classical chromosomal rearrangement (e.g., by transposition). Moreover, there are no *a priori* reasons to assume that changes in NOR number or position are associated with classical chromosomal alteration. Regardless, the present data demonstrate that significant chromosomal change has occurred during the evolutionary history of North American Cyprinidae. Whether or not this change has been intimately associated with speciation episodes remains to be seen.

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