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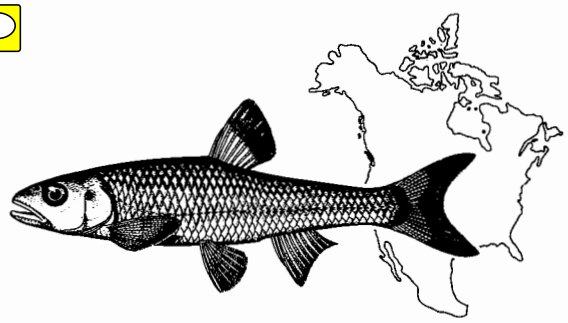

Chromosomal Evolution in North American Cyprinids

Chris T. Amemiya, Patricia K. Powers, and John R. Gold

The Cyprinidae are the most diverse freshwater fishes in North America, encompassing over 250 extant species with a wide range of ecological preferences, adaptive strategies, and morphologies (Miller, 1959; Lee et al., 1980). The earliest known North American cyprinid fossils are from mid-Oligocene and Miocene deposits (Smith, 1981; Cavender, 1986). The majority, however, have been taken from Pliocene and Pleistocene deposits (Miller, 1959, 1965; Kimmel, 1975; Smith, 1975, 1981; Cavender, 1986), suggesting that much of the group is of relatively recent origin and that many species or species-groups have undergone substantial rates of morphological or organismal evolution (Gold et al., 1978). As might be expected for such a large, diverse assemblage as the North American Cyprinidae, taxonomic and systematic problems abound and have prompted a reexamination of phylogenetic relationships within the group (Coburn, 1982; Cavender and Coburn, 1986; Mayden, 1989). Mayden (1989) has recently questioned the monophyly of several North American cyprinid taxa, and has proposed relationships within and among several of the problematic assemblages.

Over the last 10–15 years, our laboratory has carried out extensive cytogenetic investigations on North American cyprinids (Gold and Avise, 1977; Gold et al., 1978, 1979, 1981, 1986, 1988; Gold, 1980, 1984; Gold and Amemiya, 1986; Amemiya and Gold, 1987a, 1988, 1990). Initially, the studies focused on descriptions of standard karyotypes and their variation among cyprinid species. More recently, we have extended our analyses to "banded" or differentially stained chromosomes. The general purposes of our chromosomal investigations in North American cyprinids essentially have been twofold. The first was to document the extent of (standard) karyotype diversity within North American Cyprinidae in order to test the hypothesis that gross karyotypic evolution is positively correlated with organismal evolution and speciation. Briefly, Wilson et al. (1975), Wilson (1976), Levin and Wilson (1976), and Bush et al. (1977), among others, proposed that chromosome structural

rearrangements could facilitate both organismal evolution and speciation by (i) functioning as cytogenetic reproductive isolating mechanisms, (ii) altering patterns of gene expression (regulation), or (iii) creating adaptive super-genes that could not be easily broken up by recombination. The evidence for this hypothesis was that many animal and plant taxa that have undergone rapid speciation and organismal evolution seem also to have undergone rapid chromosomal evolution as measured by differences in standard karyotypes. Because of their apparently rapid evolution and speciation, North American cyprinids appeared to represent an excellent group in which to test this hypothesis. The second purpose was to determine if cyprinid chromosomes would provide informative characters for the inference of phylogenetic hypotheses of species relationships. The use of chromosomal characters in phylogenetic inference was well documented in other vertebrates, primarily mammals (e.g., Dutrillaux et al., 1982; Baker et al., 1982), and it was anticipated that chromosomal characters could resolve at least some of the phylogenetic problems extant in North American cyprinids. In this chapter, we review our progress and discuss the evolution of chromosomal differentiation and variation with respect to the phylogenetic relationships of cyprinid fishes.

FISH CYTOGENETICS

Few cytogenetic studies exist for fishes despite the fact that they represent nearly one-half of all extant vertebrate species (Cohen, 1970). Fishes typically have few mitotically active tissues yet large numbers of relatively small chromosomes. Thus, most cytogenetics work has focused on gross karyotype descriptions and identification of heteromorphic chromosomes (Gold, 1979). Although improvements in fish cell culture (Wolf and Ahne, 1982; Amemiya et al., 1984) have resulted in higher yields of quality metaphase spreads, it is difficult to apply many routine chromosome banding methods to fish chromosomes (Hartley and Horne, 1985). The only staining methods that work consistently on fish chromosomes are those that produce localized bands such as NOR- or C-bands (Amemiya and Gold, 1986, 1987b; Gold et al., 1986; Schmid and Guttenbach, 1988). In particular, methods for visualizing chromosomal NORs or nucleolus organizer regions on fish chromosomes have been used extensively (e.g., Foresti et al., 1981; Sola et al., 1984; Galetti et al., 1984; Takai and Ojima, 1986; Mayr et al., 1987; Amemiya and Gold, 1988, 1990; Thode et al., 1988; Cau et al., 1988; Phillips et al., 1988).

North American Cyprinidae: Standard Karyotypes

The standard or gross karyotype provides basic information on chromosome number (2n) and chromosome arm or fundamental number (FN). Standard karyotypes are known for over 100 North American cyprinid species (Gold et al., 1980, 1988, unpubl. data; Amemiya and Gold, 1987a, 1988, 1990). In contrast to their high rates of speciation and morphological evolution, gross karyotypic change in North American cyprinids appears to have been minimal: over 90% (including all taxa previously placed in "*Notropis*") possess diploid chromosome numbers of 50 (range = 48–52), and estimated (diploid) chromosome arm numbers between 92 and 100 (80–100). Heteromorphic sex chromosomes, although suspected in some taxa, have yet to be unequivocally demonstrated. The typical North American cyprinid karyotype

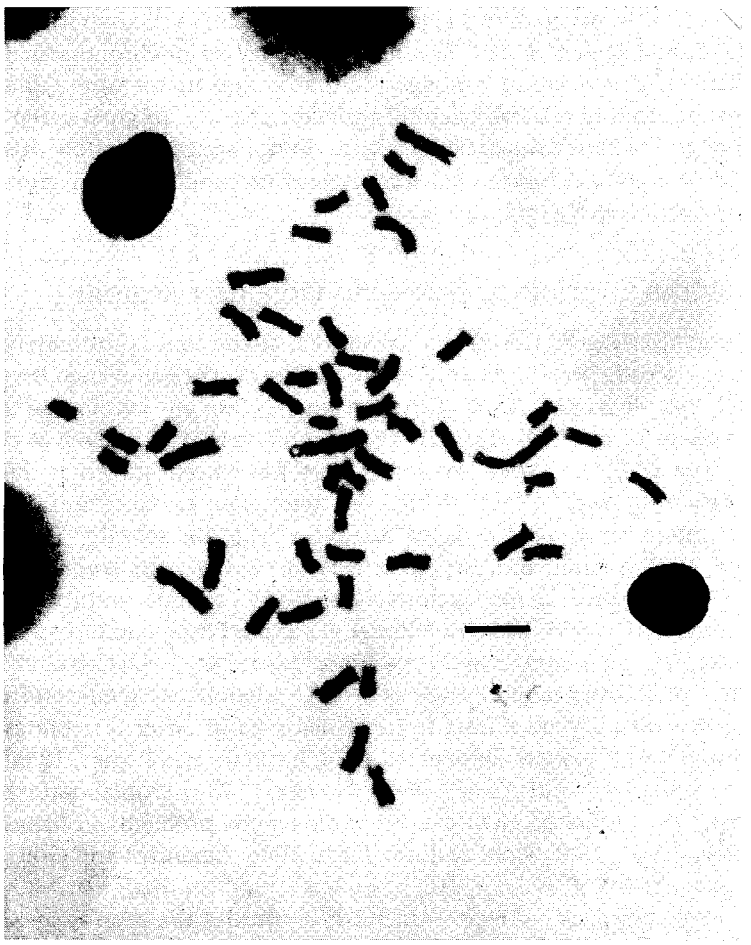

Figure 1.
Metaphase chromosomes of *Cyprinella lutrensis*. Scale bar is the equivalent of 5 μm.

(Fig. 1) is comprised of a continuous series of chromosomes ranging in length from ~1.5 μm to ~5.5 μm in a metaphase plate. Approximately one-third of the typical North American cyprinid karyotype consists of medium-sized meta-/submetacentric chromosomes (*sensu* Levan et al., 1964); uniarmed (subtelo-/acrocentric) chromosomes generally comprise a small fraction of the complement.

The homogeneity of cyprinid gross karyotypes is striking. The conservation in chromosome number implies that Robertsonian-type rearrangements (centric fusions or fissions) have not contributed significantly to chromosomal evolution in the North American minnows. Variation in arm number has been interpreted more cautiously in view of the difficulties in measuring the small cyprinid chromosomes and in establishing chromosome homologies within and between taxa. Types of chromosomal changes that may have contributed to the arm number variation include pericentric inversions, chromatin additions/deletions, transpositions, and non-Robertsonian translocations.

The overall homogeneity in gross karyotypes suggests that chromosomal rearrangement *per se* is infrequent, and that rates of chromosomal evolution are not commensurate with the apparently high rates of speciation and morphological

evolution experienced by the group. This suggestion runs counter to the hypothesis of Wilson et al. (1975) and Bush et al. (1977) which states that major chromosomal changes are correlated with speciation episodes and that groups undergoing rapid evolutionary divergence should generally exhibit concomitant rates of gross karyotypic change. Although the North American cyprinids do not appear to follow this trend, the presence of cryptic chromosomal rearrangements (undetected in gross karyotypes) has not yet been fully investigated.

North American Cyprinidae: Nucleolus Organizer Regions

The nucleolus organizer region (NOR) is a chromosomal site that is intimately involved with nucleolus formation during interphase and houses the genes encoding the 18S, 5.8S, and 28S ribosomal RNAs. Chromosomal NORs are cytologically detected using a variety of methods (Howell, 1982), most readily via silver (Ag) staining (Fig. 2a). The NORs of lower vertebrates also have been shown to be differentiable via staining with either of the GC base pair enhancing DNA fluorochromes chromomycin A_3 (CMA) or mithramycin (Fig. 2b; Schmid, 1982; Amemiya and Gold, 1986; Schmid and Guttenbach, 1988). Cytogenetic studies on the NORs of fishes have focused almost exclusively on NOR variation within and among species (Foresti et al., 1981; Moreira-Filho et al., 1984; Phillips and Ihssen, 1985; Gold and Amemiya, 1986; Takai and Ojima, 1986; Mayr et al., 1987; Cau et al., 1988; Phillips et al., 1988). Intra- and interspecific NOR variations are common in fishes and patterns of variability are similar to those observed in other vertebrates (Howell, 1982; Schmid, 1982; Moreira-Filho et al., 1984; Bickham and Rogers, 1985; Gold and Amemiya, 1986; Takai and Ojima, 1986; Gold et al., 1988). Our laboratory was the first to apply chromosomal NOR data to phylogenetic problems in fishes (Gold, 1984; Amemiya et al., 1986; Gold and Amemiya, 1986; Amemiya and Gold, 1988, 1990; Gold et al., 1988).

To date, 65 species or subspecies of North American cyprinids have been assayed for chromosomal NORs. Interspecific variation includes the number and type of NOR-bearing chromosomes and NOR positions. NOR phenotypes used herein refer to these types: the criteria for delineation and nomenclature are given in Gold and Amemiya (1986), Amemiya (1987), and Amemiya and Gold (1988). The thirteen chromosomal NOR phenotypes thus far observed in North American cyprinids are illustrated in Figure 3.

A compilation of interspecific NOR data for the 65 taxa examined to date is presented in Table 1. The taxa are listed (with noted exceptions) according to the putatively monophyletic groups to which they have been assigned on the basis of morphology (Mayden, 1989). With regard to the number of NOR-bearing chromosome pairs per genome, 47 species (72%) possess only a single NOR pair; whereas 18 species (28%) possess multiple NORs. Only *Campostoma anomalum* and *Lythrurus roseipinnis* have been found to possess more than two pairs of NOR chromosomes. The 13 NOR phenotypes are distributed on a minimum of 15 different chromosomes since two different *A* phenotypes were found in *C. anomalum* and two different *B* phenotypes were found in both species of *Nocomis*. The vast majority of North American cyprinids possess NORs located terminally on chromosomal short arms. The exceptions are (i) *Opsopoeodus emiliae* (*E'* phenotype), where the single NOR is found interstitially on the short arm of a large-sized submetacentric chromosome,

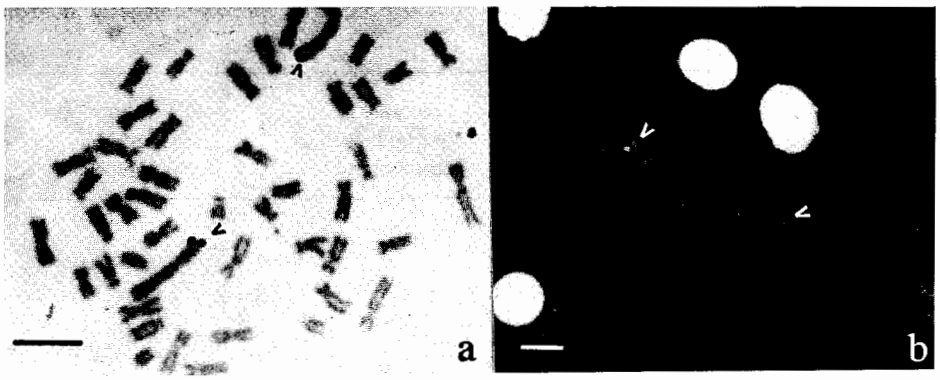

Figure 2.
NOR-banding of *Cyprinella lutrensis* metaphase chromosomes: (a) Ag staining; (b) CMA staining. NORs are indicated by arrowheads. Scale bars are the equivalent of 5 μm. (From Amemiya and Gold, 1986, with permission of Allen Press.)

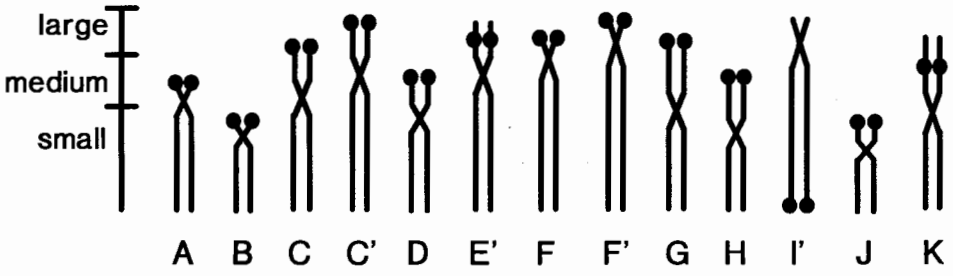

Figure 3.
The different NOR chromosome phenotypes observed to date in North American cyprinids. The scale to the left indicates approximate chromosome size relative to the largest chromosome in the complement. Letters beneath drawings indicate NOR chromosome phenotypes (cf. Table 1).

(ii) *Notropis jemezanus* (*I'* phenotype), where the single NOR is found terminally on the long arm of a large-sized acro-/subtelocentric chromosome, and (iii) *Cyprinella gibbsi* (*K* phenotype), where the single NOR is found interstitially on one arm of a large-sized metacentric chromosome (Fig. 3). The interspecific variations in the number of NOR pairs and NOR chromosome phenotypes results in a minimum of 21 discrete NOR conditions or character states. Twelve different NOR conditions are found among the 47 species with single NORs; whereas nine different NOR conditions are found among the 18 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

Table 1. NOR chromosome phenotypes of all North American cyprinids examined to date. Taxa are listed according to putative monophyletic assemblages defined on the basis of morphology.

Taxon	Number of (haploid) NOR chromosomes	NOR chromosome phenotype(s)*
Family Cyprinidae		
Subfamily Abramidinae		
1. *Notemigonus crysoleucas*	1	*A*
Subfamily Leuciscinae		
2. *Hemitremia flammea*	1	*B*
Chubs		
3. *Campostoma anomalum*	3	*AAG*
4. *Dionda episcopa*	1	*B*
5. *Extrarius aestivalis*	2	*BF*
6. *Hybognathus placitus*	1	*B*
7. *Macrhybopsis storeriana*	2	*DF*
8. *Nocomis asper*	2	*BB*
9. *Nocomis leptocephalus*	2	*BB*
"*Notropis*"-like shiners		
Genus *Cyprinella*		
10. *C. camura*	1	*H*
11. *C. formosa*	1	*C'*
12. *C. galactura*	1	*H*
13. *C. gibbsi*	1	*K*
14. *C. lepida*	1	*C'*
15. *C. lutrensis*	1	*C'*
16. *C. proserpina*	1	*H*
17. *C. spiloptera*	1	*C'*
18. *C. venusta*	1	*C'*
19. *C. whipplei*	1	*H*
Genus *Luxilus*§		
20. *L. albeolus*	2	*CD2*
21. *L. cardinalis*	1	*D1*
22. *L. cerasinus*	1	*C*
23. *L. chrysocephalus chrysocephalus*	2	*CD2*
24. *L. chrysocephalus isolepis*	2	*CD2*
25. *L. coccogenis*	2	*CD2*
26. *L. cornutus*	2	*CD2*
27. *L. pilsbryi*	1	*D1*
28. *L. zonatus*	1	*D1*
29. *L. zonistus*	1	*D2*
Genus *Lythrurus*		
30. *L. ardens*	2	*F'C*
31. *L. bellus*	2	*F'H*
32. *L. fumeus*	2	*F'H*
33. *L. roseipinnis*	2	*F'HC*
34. *L. umbratilis*	2	*F'H*

Table 1. *Continued*

Taxon	Number of (haploid) NOR chromosomes	NOR chromosome phenotype(s)*
Genus *Notropis*		
Subgenus *Alburnops*		
35. *N. girardi*	1	*D*
36. *N. potteri*	1	*F′*
Subgenus *Hydrophlox*		
37. *N. chrosomus*	1	*D*
38. *N. nubilus*	1	*D*
Subgenus *Notropis*		
39. *N. amabilis*	1	*F′*
40. *N. atherinoides*	2	*F′J*
41. *N. jemezanus*	1	*I′*
42. *N. oxyrhynchus*	1	*J*
43. *N. shumardi*	1	*F′*
44. *N. stilbius*	1	*D*
Notropis dorsalis species-group[1] (including *Ericymba*)		
45. *Ericymba buccata*	1	*B*
46. *N. dorsalis*	1	*D*
47. *N. longirostris*	1	*D*
48. *N. ammophilus*	1	*D*
Notropis texanus species-group		
49. *N. boops*	1	*D*
50. *N. chalybaeus*	1	*D*
51. *N. texanus*	1	*D*
Notropis volucellus species-group		
52. *N. buchanani*	1	*D*
53. *N. maculatus*	1	*D*
54. *N. volucellus*	1	*D*
Genus *Opsopoeodus*[2]		
55. *Opsopoeodus emiliae*	1	*E′*
Genus *Pimephales*[2]		
56. *P. notatus*	1	*C*
57. *P. promelas*	1	*C*
58. *P. vigilax*	1	*C*
Genus *Pteronotropis*		
59. *P. hubbsi*[3]	1	*F*
60. *P. signipinnis*	2	*FD*
61. *P. welaka*	2	*FD*
Unknown affinities[4]		
62. *Notropis baileyi*	1	*D*
63. *Notropis braytoni*	1	*C′*
64. *Notropis greenei*	1	*D*
65. *Notropis ludibundus*	1	*D*

* NOR chromosome phenotypes 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 →

Table 1. *Continued*

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; *K*, subterminal (interstitial) on one arm of large-sized metacentric. A prime (') symbol indicates that the chromosome is the largest in the complement. Data are from Gold (1984), Gold and Amemiya (1986), Gold et al. (1988), Zoch and Gold (1988), Amemiya and Gold (1990), and Powers and Gold (unpubl. data)

Taxonomic placements essentially follow Mayden (1989) with the exceptions indicated below.

[1] The *Notropis dorsalis* species-group and *Ericymba buccata* are placed in the *"Notropis"*-like shiner assemblage based on recent evidence of Coburn and Cavender (pers. comm.).

[2] *Opsopoeodus emiliae* and the genus *Pimephales* are now considered to belong to the *"Notropis"*-like shiner assemblage (Cavender and Coburn, 1986; Mayden and Matson, 1988; Amemiya and Gold, 1990).

[3] *Pteronotropis hubbsi* is considered to belong to the genus *Pteronotropis* on the basis of morphological and chromosomal evidence (Coburn, pers. comm.; Amemiya and Gold, 1990).

[4] *Notropis baileyi* may not belong to the subgenus *Hydrophlox* (of *Notropis*) where it is currently placed (Mayden and Matson, 1988); *Notropis greenei* shares affinities with other members of the genus *Notropis* (*sensu* Mayden, 1989); *Notropis ludibundus* (replacement name for *Notropis stramineus*, cf. Mayden and Gilbert, 1989) is possibly related to the *N. dorsalis* species-group and *Ericymba buccata* (Coburn, pers. comm.).

§ The *D*1 NOR chromosomes in *L. cardinalis*, *L. pilsbryi*, and *L. zonatus* are putatively homologous to one another, as are the *D*2 NOR chromosomes in *L. albeolus*, *L. c. chrysocephalus*, *L. c. isolepis*, *L. coccogenis*, *L. cornutus*, and *L. zonistius*. The *D*1 and *D*2 NOR chromosomes are not homologous. See text for further details.

differences in chromosomal NOR size as described by Foresti et al. (1981) have yet to be documented in cyprinids.

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 21 different NOR conditions observed among the 65 species suggests that a minimum of 20 different chromosomal rearrangements or alterations involving a NOR have occurred since the 65 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 employed by Gold and Amemiya (1986) and 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 be considerably higher than previously believed, or at least as indicated by consideration of standard karyotypes.

Intraspecific variations (heteromorphisms) in chromosomal NORs have been detected in approximately 13% of North American cyprinids examined (N > 500) regardless of species. The majority of these heteromorphisms include either enlargements (amplifications) of known NOR sites, or NOR sites that are either deleted or functionally inactive. Both of these intraspecific heteromorphisms appear commonly in fishes (Foresti et al., 1981; Moreira-Filho et al., 1984; Takai and Ojima, 1986), and examples of both in North American species may be found in Gold

(1984), Gold and Amemiya (1986), Amemiya (1987), and Gold et al. (1988). Importantly, these types of intraspecific heteromorphisms differ qualitatively from the differences found interspecifically (viz., differences in number, position, and chromosomal types of NORs), and reinforce the observations made by Gold and Amemiya (1986) and Amemiya and Gold (1988) that interspecies NOR differences can serve as valid taxonomic and systematic characters.

We have, however, found seven instances where the types of NOR variants typically found among cyprinid species apparently occur within a species. Six of these (listed in Gold and Zoch, 1990) involved either a change in the chromosomal position of a NOR, a change in the type of chromosome on which a NOR was located, or an addition of a single NOR-bearing chromosome, and were detected in single individuals from populations where all other individuals examined had chromosomal NOR phenotypes characteristic of the species under study. The seventh was an apparent loss of a pair of *D* NOR chromosomes from a population of the striped shiner, *Luxilus chrysocephalus isolepis*, in the Blue River in Oklahoma, which presumably became fixed via a founder-type or bottleneck event (Gold and Zoch, 1990).

SYSTEMATIC/PHYLOGENETIC IMPLICATIONS OF THE CHROMOSOMAL NOR DATA IN THE NORTH AMERICAN CYPRINIDS

The chromosome data shown in Table 1 reveal several phenotype similarities that suggest phenetic relationships concordant with present concepts of North American cyprinid taxonomy. Some examples include the two *Nocomis* species with two pairs of *B* phenotypes, the four *Cyprinella* species with *H* phenotypes and the four *Cyprinella* species with *C'* phenotypes, the four members of the *L. cornutus* species-group with *C* and *D* phenotypes, the five *Lythrurus* species with multiple NORs and *F'* phenotypes, and the three *Pimephales* species with *C* phenotypes. In addition, 13 of 20 species (including *N. greenei* and the *N. dorsalis* species-group) assayed from the genus *Notropis* possess a *D* NOR chromosome. These similarities in NOR chromosome phenotypes within putatively monophyletic groups do indicate that chromosomal NORs are taxonomically informative in the North American Cyprinidae, and further suggest that chromosomal NORs could be phylogenetically informative.

Our methodology for inferring phylogenetic relationships from chromosomal NOR data is outlined in Gold and Amemiya (1986) and Amemiya and Gold (1988). Briefly, each different NOR phenotype was treated as a different state of the same character as opposed to different characters. As examples, *Notemigonus crysoleucas*, a species with a single NOR pair, has an *A* character state; whereas *Extrarius aestivalis*, a species with two NOR pairs, has a *BF* character state. We used this approach primarily because of difficulties encountered in recognizing or identifying transformations (and their mechanisms) from one chromosomal NOR phenotype to another. Thus far, differences in chromosomal NOR phenotypes among cyprinid species essentially have been of three types: (i) differences in the type (e.g., metacentric vs. submetacentric, etc.) and/or size (e.g., large vs. small) of NOR chromosomes; (ii) differences in the chromosomal position (e.g., terminal vs. subterminal or long-arm vs. short-arm) of a NOR; and (iii) differences in the number of non-homologous NOR chromosomes. Differences in the type and/or size of a NOR chromosome could ostensibly stem from

one of several "classical" chromosomal alterations within or between chromosomes. Examples of "within" chromosome alterations include inversions, heterochromatic additions/deletions, and euchromatic additions/deletions; examples of "between" chromosome alterations include primarily translocations and/or transpositions since there is little evidence (based on chromosome numbers) that Robertsonian fusions/fissions have been common in cyprinids. Differences in the position of a chromosomal NOR site could also stem from alterations within and between chromosomes, although the former would possibly be more likely (*a priori*) if the chromosomes involved were of the same relative size. Differences in the number of non-homologous NOR chromosomes would obviously involve interchromosomal events if there were an increase in the number of chromosomal NORs. Possible mechanisms here might include a translocation involving a break-point within a NOR followed by amplification of NOR DNA on the translocation products. This seems unlikely, however, to the extent that most cyprinid NORs are found in terminal chromosomal positions. Another possible mechanism to explain an increase in the number of non-homologous NOR chromosomes could be transposition (followed by amplification) of NOR DNA from one chromosome to another. Decreases in the number of non-homologous NOR chromosomes (if they occur) would most easily be explained by simple deletions.

Except for transpositions, examples of the above cited chromosomal alterations are well documented, in a general sense, among animals (White, 1973). In North American cyprinids, we have tentatively identified two "classical" chromosomal alterations involving a NOR (see below), both adduced by obtaining C-banding patterns of the NOR chromosomes involved. The central problem is that to identify a NOR transformation, and the particular chromosomal alteration involved, *all* (or nearly all) of the unique (homologous) chromosomes both within and between species must be unequivocally differentiated. In the two cyprinid examples, we were fortunate in that one of the NOR chromosomes involved in both cases was the *C'* NOR chromosome. The *C'* NOR chromosome has a distinctive C-banding pattern (Amemiya, 1987; Amemiya and Gold, 1988) that allowed easy identification of the alteration products. The majority of cyprinid chromosomes, however, have rather similar C-banding patterns (Gold et al., 1986; Amemiya, 1987), which means that to identify individual cyprinid chromosomes it will be necessary to use a serial banding methodology. We have recently developed serial banding procedures for cyprinid chromosomes (Gold et al., 1990), and used them to test homologies of NOR chromosomes among species of the genus *Luxilus* (see below).

With regard to character coding of NOR chromosome phenotypes, it seems likely that many, if not most, NOR chromosome differences among cyprinid species will turn out to be the result of single, non-independent chromosomal alterations. Thus, the conservative approach is to treat each different NOR chromosome phenotype as a different state of the same character rather than as separate characters coded as present or absent.

A second problem in using chromosomal NOR data to infer hypotheses of phylogenetic relationships is the identification of appropriate outgroup taxa. Consequently, we have relied on the phylogenetic hypotheses of Coburn (1982) and Mayden (1989) for choice of outgroups relative to the taxa examined. According to Mayden (1989), a single morphological character (the presence of an open posterior myodome) may define many of the "eastern" North American cyprinid assemblages. The two taxa examined for chromosomal NORs that do not possess an open posterior

myodome, and hence could serve as outgroups to this clade, are *Notemigonus* and *Hemitremia*. *Notemigonus*, however, may not be an appropriate outgroup since its relationship to other cyprinid subgroups is not well established (Howes, 1981).

The *B* chromosome NOR phenotype, as found in *Hemitremia*, is observed in five of the fourteen (ingroup) genera placed by Mayden (1989) in the eastern North American clade. Gold and Amemiya (1986) inferred that a single NOR located terminally on the short arm of a small acrocentric chromosome (phenotype *B*) represents the plesiomorphic state for the open posterior myodome clade. This inference (hypothesis) is further supported by the findings of Takai and Ojima (1986) that fifteen of the twenty-two Asian cyprinids examined also possessed a NOR that was terminal on the short arm of a small acrocentric chromosome. Comparison of the interspecific NOR data among the North American Cyprinidae suggests that 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, apomorphic in a relative sense, i.e., within the group some NOR conditions may be plesiomorphic to others.

Based strictly on chromosomal NOR data, relationships among *all* of the species assayed from the open posterior myodome assemblage remain unresolved. This is due, in large part, to our present inability to definitively establish chromosome homologies (including many NOR chromosomes) among taxa in order to accurately establish character state transformation series. Nonetheless, the chromosomal NOR data are, in some instances, informative and supportive of certain systematic inferences (Gold and Amemiya, 1986; Amemiya and Gold, 1988, 1990; Gold et al., 1988).

Genus *Cyprinella*

There are three NOR chromosome phenetic groups among the ten species of *Cyprinella* thus far examined (Table 1): (i) the *C'* group (*C. formosa*, *C. lepida*, *C. lutrensis*, *C. spiloptera*, and *C. venusta*); (ii) the *H* group (*C. camura*, *C. galactura*, *C. proserpina*, and *C. whipplei*); and (iii) the *K* group (*C. gibbsi*). The NOR chromosomes of the five species in the *C'* group exhibit identical C-banding patterns (Amemiya, 1987; Amemiya and Gold, 1988; Gold, unpubl. data) and are presumed to be homologous. For reasons discussed elsewhere (Gold et al., 1988; Amemiya and Gold, 1990), a *C'* NOR state is hypothesized to be plesiomorphic for *Cyprinella*. The *K* NOR state in *C. gibbsi* is thus hypothesized to represent an autapomorphy; whereas the *H* NOR state is hypothesized to represent a synapomorphy uniting *C. camura*, *C. galactura*, *C. proserpina*, and *C. whipplei*. Monophyly of these four species is not entirely supported by morphological data (Mayden, 1989), and it remains to be shown via C- or alternative chromosome banding methods that all four *H* NOR chromosomes are homologous. The C-banding pattern of the *C. proserpina* karyotype, however, suggests that the *H* NOR chromosome in this species could have arisen from a *C'* NOR chromosome by a non-Robertsonian whole-arm translocation (Amemiya and Gold, 1988).

Genus *Pteronotropis*

The genus *Pteronotropis* is generally defined to include three species (*Pteronotropis euryzonus*, *Pteronotropis hypselopterus*, and *Pteronotropis signipinnis*) from the southeastern United States (Bailey and Suttkus, 1952; Suttkus, 1955). Mayden (1989)

considered *Pteronotropis welaka* to be the sister to the above three species, and Bailey and Robison (1978) considered *Pteronotropis hubbsi* to be closely related to *P. welaka*. Mayden (1989, pers. comm.) examined *P. hubbsi*, but did not include the species in his concept of *Pteronotropis*. The three species (*P. hubbsi*, *P. signipinnis*, and *P. welaka*) now karotyped (Amemiya and Gold, 1990) were found to possess virtually identical standard karyotypes, and more importantly, a pair of *F* NOR chromosomes which by RHG-banding appeared to be homologous. By outgroup comparison, and with the assumption that the *D* NOR chromosomes in *P. signipinnis* and *P. welaka* (Table 1) are homologous, the addition of an *F* NOR chromosome was hypothesized to represent a synapomorphy uniting *P. hubbsi*, *P. signipinnis*, and P. *welaka* into a monophyletic assemblage (Amemiya and Gold, 1990). The single *F* NOR condition in *P. hubbsi* was inferred to represent the autapomorphic loss of a *D* NOR chromosome. Although other interpretations exist (Amemiya and Gold, 1990), the occurrence of the putatively homologous *F* NOR chromosome in all three species suggests that *P. hubbsi*, *P. signipinnis*, and *P. welaka* comprise a monophyletic group and that *P. hubbsi* should be included in *Pteronotropis*.

Genus *Lythrurus*

Chromosomal NOR phenotypes of five of the eight species currently placed in *Lythrurus* are now known (Table 1), and three equally parsimonious chromosomal hypotheses of relationships within *Lythrurus* are shown in Figure 4a–c. In all three, a single pair of *F'* NOR chromosomes is hypothesized to be the plesiomorphic state based on the following. First, a single pair of NOR chromosomes is found in 43 of 51 species (excluding *Lythrurus*) examined from the "*Notropis*"-like shiner assemblage (Table 1). This includes nearly all of the groups that might serve as putative outgroups to *Lythrurus* according to the hypothesis of Mayden (1989). Secondly, an *F'* NOR state is found in other "*Notropis*"-like shiners (e.g., *N. potteri* of the subgenus *Alburnops*, and *N. amabilis* and *N. shumardi* of the subgenus *Notropis*, Table 1). Thirdly, although not yet tested in a species of *Lythrurus*, the long arm of the *F'* NOR chromosomes in *N. amabilis* and *N. shumardi* is homologous in C-banding pattern to the long arms of the *C'* and *E'* NOR chromosomes in species of *Cyprinella* and *O. emiliae*, respectively (Amemiya, 1987), suggesting that an *F'* NOR state may be plesiomorphic for "*Notropis*"-like shiners. Finally, an *F'* NOR chromosome is present in all species of *Lythrurus* thus far examined (Table 1). Assuming an *F'* NOR state is plesiomorphic for *Lythrurus*, all three chromosomal hypotheses (Fig. 4a–c) indicate that *Lythrurus* is monophyletic. In Figure 4a and 4c, the presumed synapomorphy is the addition of an *H* NOR chromosome; whereas in Figure 4b, the presumed synapomorphy is the addition of a *C* NOR chromosome. The current phylogenetic hypothesis relative to these five species of *Lythrurus* is shown in Figure 4d. The hypothesis is based on both morphological and allozyme evidence (Snelson, 1972, 1973; Stein et al., 1985; Mayden, 1989). Of the three chromosomal hypotheses, only the one shown in Figure 4c is compatible with the hypothesis shown in Figure 4d. The key question at present is whether the *C* NOR chromosomes in *L. ardens* and *L. roseipinnis* are homologous. Should they be non-homologous, the chromosomal hypotheses shown in Figure 4a and 4b would be falsified. One last point to note is that the *H* NOR chromosome in *L. roseipinnis* does not appear to be homologous in C-banding pattern to the *H* NOR chromosome in *Cyprinella proserpina* (Amemiya, 1987).

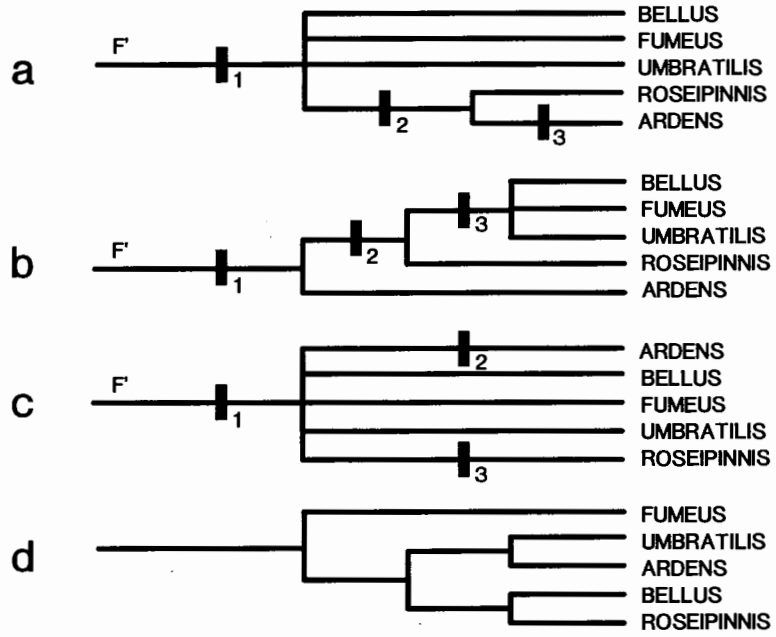

Figure 4.
Phylogenetic hypotheses of relationships among five species of *Lythrurus*. a–c are equally parsimonious hypotheses based on NOR chromosome data. Proposed NOR state changes are as follows: a, (1) +*H*, (2) +*C*, (3) -*H*; b, (1) +*C*, (2) +*H*, (3) -*C*; and c, (1) +*H*, (2) *H*→*C*, (3) +*C*. The phylogeny in d is based on morphological and allozyme data (cf text).

Opsopoeodus emiliae

This species has been the subject of considerable taxonomic confusion, having been placed either in *Notropis* or in the monotypic genus *Opsopoeodus* (Gilbert and Bailey, 1972; Campos and Hubbs, 1973). Phylogenetically, Cavender and Coburn (1986) hypothesized that *O. emiliae* was closely related to the genus *Pimephales*, and that a clade including *O. emiliae* and *Pimephales* represented the sister group to the genus *Cyprinella*. Mayden (1989), however, hypothesized that *O. emiliae* was closely related to *Notropis maculatus* and that both species belonged in the *Notropis volucellus* species-group of *Notropis*. Our chromosomal hypothesis regarding *O. emiliae* is summarized in Amemiya and Gold (1990). Briefly, *O. emiliae* possesses a highly distinctive (and apomorphic) *E'* NOR chromosome (Fig. 3), which has been shown by C-banding (Gold and Amemiya, 1986; Amemiya and Gold, 1988) to be putatively homologous to (and easily derived by a single paracentric inversion from) the *C'* NOR chromosome found in several species of *Cyprinella*. Given nearly any outgroup perspective, the phylogenetic inferences are that (1) the *N. volucellus* species-group (*sensu* Mayden, 1989) is not monophyletic, (2) *O. emiliae* is not the sister to *N. maculatus* (which possesses a *D* NOR state, Table 1), and (3) *O. emiliae* belongs in an assemblage that includes at least some members of the genus *Cyprinella*. Interestingly, we have now examined NOR chromosomes from three species of *Pimephales*, all of which possess a *C* NOR state (Table 1). The NOR chromosome in all three species of *Pimephales*, however, is definitely not the largest chromosome in the

complement, nor is it homologous in C-banding pattern to the *C'* and *E'* NOR chromosomes (Gold et al., unpubl. data). Thus, while the chromosome data support Cavender and Coburn's (1986) hypothesis of a clade involving *O. emiliae* and *Cyprinella*, they do not support the contention of a relationship among *O. emiliae*, *Cyprinella*, and *Pimephales*. The chromosome data, however, do not conflict with Cavender and Coburn's (1986) hypothesis if the *C* NOR state of *Pimephales* is an autapomorphy.

Ericymba buccata

The occurrence of a *B* NOR state in *Ericymba buccata* is somewhat troublesome given our hypothesis (see above) that the *B* NOR state is plesiomorphic for North American cyprinids with an open posterior myodome. The presence of the *B* NOR state in *Ericymba* suggests that this species should be placed in a basal, unresolved polychotomy with other North American cyprinid lineages that also possess a *B* NOR state (Table 1). Both Mayden (1989) and Coburn and Cavender (pers. comm.), however, place *Ericymba* as closely allied on morphological grounds to the four species of the *N. dorsalis* species-group. All three species now examined from the *N. dorsalis* species-group possess *D* NOR states (Table 1), suggesting that either *Ericymba* is misplaced (i.e., does not belong in a putatively monophyletic assemblage with the *N. dorsalis* species-group), or that the *B* NOR chromosome in *Ericymba* is non-homologous with *B* NOR chromosomes found elsewhere in North American Cyprinidae. Distinguishing among these alternatives using C- or other types of chromosome banding will be difficult given the small size of *B* NOR chromosomes.

Genus *Luxilus*

The genus *Luxilus* is the most thoroughly studied group phylogenetically within the "*Notropis*"-like shiners in terms of alternative data sets, viz., morphology (Gilbert, 1964; Mayden, 1988, 1989), allozymes (Buth, 1979), and mitochondrial DNAs (Dowling, unpubl. data). For that reason, we have focused recent efforts on *Luxilus* including the development of a serial or G-banding procedure (Gold et al., 1990) to test the homologies of NOR chromosomes found in *Luxilus* species. In brief, both *C* and *D* NOR chromosomes are found among all ten nominal *Luxilus* taxa (Table 1). The *C* NOR chromosomes of the *L. cornutus* species-group (i.e., *L. albeolus*, *L. c. chrysocephalus*, *L. c. isolepis*, and *L. cornutus*) and in *L. cerasinus* and *L. coccogenis* are all identical in serial banding patterns, as are the *D* NOR chromosomes found in all members of the *L. cornutus* species-group, *L. coccogenis*, and *L. zonistius*. The *D* NOR chromosomes in the *L. zonatus* species-group (i.e., *L. cardinalis*, *L. pilsbryi*, and *L. zonatus*), however, although identical to one another in serial banding patterns, differ in serial banding pattern from the *D* NOR chromosomes in the *L. cornutus* species-group, *L. coccogenis*, and *L. zonistius*. For convenience, we refer to the *D* NOR chromosome in the *L. zonatus* species-group as *D*1 and the *D* NOR chromosome in the *L. cornutus* species-group, *L. coccogenis*, and *L. zonistius* as *D*2. Of importance is that the *D*1 and *D*2 NOR chromosomes are non-homologous.

Phenetically, the similarities in NOR chromosomes suggest an alignment among the *L. coccogenis* species-group (*L. coccogenis* and *L. zonistius*), the *L. cornutus* species-group, and *L. cerasinus*. Similarly, the three species in the *L. zonatus* species-group

appear to be phenetically similar to one another, but not to other members of *Luxilus*. The phylogenetic inferences based on these data are to be discussed fully elsewhere. In brief, the inferences are: (i) the *L. coccogenis* and *L. cornutus* species-groups form a monophyletic assemblage (phylogenetically, *L. cerasinus* is hypothesized to be sister to a monophyletic clade comprising the *L. coccogenis* and *L. cornutus* species-groups), and (ii) there is no chromosomal evidence that the *L. zonatus* species-group is closely related phyletically to other members of *Luxilus*. These inferences are based on the assumption that a single pair of chromosomal NORs is plesiomorphic for *Luxilus* species, and differ from the hypotheses proposed by Gilbert (1964), Buth (1979), and Mayden (1989) where the *L. coccogenis* species-group was not proposed to be sister to the *L. cornutus* species-group and where the *L. zonatus* species-group was considered to be a valid member of *Luxilus*.

SUMMARY

Studies of chromosome evolution and/or cytosystematics in fishes historically have been hampered by inherent difficulties in working with fish chromosomes. As discussed in this chapter, consideration of variation in standard karyotypes (i.e., chromosome and chromosome arm numbers) in cyprinids suggested that gross chromosomal change has not kept pace with the apparent rapid speciation and evolution of the group. Resolution of differences in chromosomal NORs, however, has suggested that chromosomal change in cyprinids may have been more widespread than previously believed. Chromosomal NORs also appear to be useful phylogenetically in cyprinids, at least insofar as testing phylogenetic hypotheses derived from alternative data bases. The major problem, at present, in using chromosomal NORs alone to infer hypotheses of species relationships regards the inability to unequivocally identify the discrete transformations involved in the change from one chromosomal NOR phenotype to another. This problem, as well as the identification of other chromosomal alterations (i.e., those not involving NOR chromosomes) will only be fully resolved when metaphase banding procedures permitting differentiation of *all* the chromosomes within a complement are developed.

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