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Cytogenetic Studies in North American Minnows (Cyprinidae). XX. Chromosomal NOR Variation in the Genus *Luxilus*

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Chromosomal nucleolus organizer region (NOR) phenotypes are documented for all extant taxa of the North American cyprinid genus *Luxilus*. All nine species, including two subspecies of *L. chrysocephalus* (*L. c. chrysocephalus* and *L. c. isolepis*), possess $2n = 50$ chromosomes. Five species and one population of *L. c. isolepis* from the Blue River in Oklahoma possess a single pair of NOR-bearing chromosomes. These included *L. cerasinus* and *L. c. isolepis* from Oklahoma (NOR phenotype *C*), *L. zonistius* (NOR phenotype D_2), and all three species (*L. cardinalis*, *L. pilsbryi*, and *L. zonatus*) of the *L. zonatus* group (NOR phenotype D_1). Four taxa (*L. albeolus*, *L. coccogenis*, *L. c. chrysocephalus*, and *L. cornutus*) and populations of *L. c. isolepis* from Alabama, Mississippi, and Louisiana possess two pairs of NOR-bearing chromosomes (NOR phenotype *C*, D_2). A serial banding procedure (Z-banding) was used to test homology of *C*, D_1, and D_2 NOR chromosomes. The NOR chromosome data support monophyly of the *L. cornutus* group (*L. albeolus*, both subspecies of *L. chrysocephalus*, and *L. cornutus*) and *L. coccogenis*. Assuming the *L. coccogenis* group (*L. coccogenis* and *L. zonistius*) is monophyletic, as suggested by morphological, allozymic, and mtDNA data, the chromosomal data support the hypothesis that the *L. coccogenis* and *L. cornutus* groups are sister taxa and that *L. cerasinus* is sister to the *L. coccogenis* group–*L. cornutus* group clade. Because the D_1 NOR chromosome of all three species of the *L. zonatus* group does not occur elsewhere in *Luxilus*, there is no chromosomal evidence for a phyletic relationship between the *L. zonatus* group and any other species in *Luxilus*. The hypothesis that the *L. coccogenis* group is sister to the *L. cornutus* group suggests that the "missing" species of *Luxilus* in the Ohio River–northern Atlantic slope drainages may have been a member (or ancestor) of the *L. cornutus* group.

STUDIES in our laboratory over the last decade have focused on identification of nucleolus organizer regions (NORs) on the chromosomes of North American cyprinid fishes (Gold and Amemiya, 1986; Amemiya and Gold, 1988, 1990a). Because NORs represent chromosomal sites of nuclear genes that encode 18S, 5.8S, and 28S ribosomal RNAs (Ritossa and Spiegelman, 1965; Gall and Pardue, 1969; Howell, 1982), they have been useful in identifying homologous chromosomes within and among cyprinid complements. This has been critical for studies of cyprinids because most chromosomes in the typical minnow complement are similar in size and centromere position and, hence, difficult to distinguish in standard or nondifferentially stained preparations (Gold et al., 1981; Amemiya, 1987).

To date, nearly 70 North American cyprinid species or subspecies have been assayed for chromosomal NORs (Gold et al., 1990a, unpubl.; Amemiya et al., 1992). Interspecific variation of chromosomal NORs has been used to infer hypotheses of phylogenetic relationships among various cyprinid species or species groups and to test hypotheses inferred from morphological and other data (Amemiya and Gold, 1988, 1990a, 1990b). Interspecies NOR variation and its phylogenetic and taxonomic implications were reviewed by Amemiya et al. (1992).

Our methods for phylogenetic inference using chromosomal NOR data are outlined in Gold and Amemiya (1986) and Amemiya and Gold (1988, 1990a). Briefly, each NOR phenotype is treated as a different state of the same character, rather than a different character. Character state polarity is inferred by outgroup comparison. A central problem in using chromosomal NORs for phylogenetic inference concerns the difficulty in identifying homology of NOR phenotypes (character states). NOR-banding per se does not provide the resolution necessary for

determining whether the same NOR phenotype found in different species represents the same homologous character state. For example, 20 of the cyprinid species surveyed possess a *D* NOR character state, defined as a NOR terminal on the short arm of a medium-sized submetacentric chromosome (Amemiya et al., 1992). Monophyly of all species possessing a *D* NOR state, however, is not supported by morphological data (Amemiya and Gold, 1990a), which is not surprising because there are a number of medium-sized submetacentric chromosomes in minnow complements. In a few instances (e.g., among five species of the genus *Cyprinella*), C-banding was used to test homology of specific NOR chromosomes (Amemiya, 1987; Amemiya and Gold, 1988; Gold, unpubl.) and, in one instance (Gold and Amemiya, 1986), to test homology (and transformation) of a NOR chromosome between two genera. The C-banding approach as a whole, however, appears to be limited because C-bands on most cyprinid chromosomes are small and primarily centromeric (Gold et al., 1986; Y. C. Li and Gold, unpubl.). In the instances cited above, the NOR chromosomes were the largest chromosomes in the complement and also possessed an atypically large C-band.

Recently, we developed methods for resolving serial or fluctuant bands (sensu Sumner, 1977) on cyprinid chromosomes (Gold et al., 1990b). These bands, commonly called G- or R-bands, are lateral or transverse striations along arms of chromosomes that in mammals are thought to represent regions of either differential DNA base sequence composition or differential chemical and/or thermal sensitivity (Comings, 1978; Jorgenson et al., 1978; Holmquist et al., 1982). Although used extensively to address systematic and evolutionary problems in several vertebrate groups (e.g., Haiduk and Baker, 1982; Stock and Bunch, 1982; Dutrillaux et al., 1982), there are no reports, to our knowledge, of comparative serial chromosome banding in fishes.

In this paper, the serial banded phenotypes of the NOR-bearing chromosomes of the 10 nominal taxa in the cyprinid genus *Luxilus* (sensu Mayden, 1989) are documented. We chose to focus our initial study of cyprinid serial bands on *Luxilus* largely because the genus is the most thoroughly studied group in North American Cyprinidae in terms of alternative (systematic) data sets. Briefly, Gilbert (1964), on the basis of morphology, recognized three species groups within *Luxilus*: the *L. coccogenis* group (*L. coccogenis* and *L. zonistius*); the *L. cornutus* group [*L. albeolus*, *L. cerasinus*, two subspecies of *L. chrysocephalus* (*L. c. chrysocephalus* and *L. c. isolepis*), and *L. cornutus*]; and the *L. zonatus* group (*L. pilsbryi* and *L. zonatus*). Gilbert (1964) proposed that the *L. cornutus* group, with *L. cerasinus* as the sister to *L. albeolus*, *L. chrysocephalus*, and *L. cornutus*, was sister to the *L. zonatus* group, with the *L. coccogenis* group being the most distant. Mayden (1988a, 1989) supported Gilbert's (1964) hypothesis of monophyly of *Luxilus* and also described a new species, *L. cardinalis*, which he placed as sister to *L. pilsbryi* in the *L. zonatus* group. Based on protein electrophoretic (allozyme) data, Buth (1979) suggested that *L. cerasinus* constituted its own species group and was most closely related phyletically to the *L. coccogenis* group. Buth (1979) also hypothesized that the *L. cerasinus*–*L. coccogenis* clade was sister to the *L. zonatus* group and that this clade was sister to the *L. cornutus* group. Finally, Dowling et al. (1992) studied restriction enzyme site variation among the mitochondrial (mt)DNAs of all *Luxilus*. Phylogenetic analysis of the mtDNA data appeared to resolve the major species groups (including *L. cerasinus*) but was unable to resolve relationships among the species groups. As will be shown in this paper, the chromosomal data indicate a different set of relationships among the *Luxilus* species groups than have been previously hypothesized.

Methods

Specimens examined in this study were collected by seine from natural populations. Collection localities and voucher material are listed in Material Examined. Most of the specimens were returned live to the laboratory in College Station, Texas, and maintained in aerated aquaria until sacrificed. For a few specimens, primarily those from Virginia, fibroblast cultures were seeded in the field following procedures outlined in Gold et al. (1990b).

Metaphase chromosomes were prepared either directly from solid tissues (after Gold, 1984) or from cultured fibroblasts (after Amemiya et al., 1984; following modifications in Gold et al., 1990b). Silver-staining for chromosomal NORs was carried out using the one-step method of Howell and Black (1980) as modified by Gold and Ellison (1983). Serial banding was carried out by a procedure we call Z-banding. As noted by Gold et al. (1990b), a comparison of Z-bands

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[a]
L. cerasinus group				
1. *L. cerasinus*	6	55	1	*C*
L. coccogenis group				
2. *L. coccogenis*	2	12	2	*C*, D_2
3. *L. zonistius*	2	10	1	D_2
L. cornutus group				
4. *L. albeolus*	2	23	2	*C*, D_2
5. *L. c. chrysocephalus*	8	137	2	*C*, D_2
6. *L. c. isolepis*[b]	15	127	2	*C*, D_2
7. *L. c. isolepis*[c]	13	82	1	*C*
8. *L. cornutus*	2	22	2	*C*, D_2
L. zonatus group				
9. *L. cardinalis*	10	78	1	D_1
10. *L. pilsbryi*	12	59	1	D_1
11. *L. zonatus*	6	62	1	D_1

[a] NOR chromosome phenotypes (states): *C*, terminal on short arm of large-sized submetacentric; *D*, terminal on short arm of medium-sized submetacentric. Subscripts indicate homologous NOR chromosomes (see text).
[b] From Alabama, Mississippi, and Louisiana
[c] From Oklahoma

on human chromosomes with those produced by trypsin indicated that Z-bands are the same as trypsin G-bands. A full description of the procedures used in Z-banding may be found in Gold et al. (1990b). Briefly, prepared slides are dried overnight at 65–70 C on a slide warmer or in an incubator and incubated for 15 min, on ice, in a coplin jar containing absolute ethanol. The absolute ethanol should be stored at least 24 h in a −70 C ultracold freezer prior to use. Slides are removed from the alcohol, blown dry, and placed into humidity chambers (after Arrighi and Hsu, 1974) containing 0.01M phosphate buffer at pH 6.8. Approximately 100–150 µl of working solution is pipetted onto each slide, a coverslip is added, and the chamber is incubated for 2 h at 37 C. The working solution is a 1:10 dilution (in sterile, distilled water) of a stock solution made fresh each week of equal volumes of 0.1M $MgCl_2$ (pH 8.6) and 0.5M Tris-HCl (pH 8.4). Following incubation, coverslips are gently washed off with distilled water, and slides stained 2–3 min in 3–5 ml of Giemsa stain in 0.01M phosphate buffer at pH 6.8. Following identification and microphotography (see below) of appropriately Z-banded preparations, slides are destained with freshly prepared 3:1 (methanol : acetic acid) fixative, rinsed with distilled water, air dried, and silver-stained using the procedures noted above to identify the NOR-bearing chromosomes. Bright field photomicroscopy followed procedures outlined in Gold and Amemiya (1986).

Determinations of NOR-band size and position(s), serial band size and position(s), and relative size of NOR-bearing and other chromosomes were made from positive prints using a digitizer, a small laboratory computer, and the BANDSCAN program described in Gold and Amemiya (1986). The letter designations used for NOR chromosome phenotypes follow Gold and Amemiya (1986) and Amemiya and Gold (1988) and are based on the position of the NOR on the chromosome (terminal, subterminal, etc.), the centromere position of that chromosome (median, submedian, etc.), and the relative size of the chromosome within the complement.

RESULTS AND DISCUSSION

Summary data of the silver-stained material from *Luxilus* are shown in Table 1 and include three entries for *L. chrysocephalus* (see below). The taxa are arranged in the table by species group (sensu Buth, 1979). All individuals from all species possessed $2n = 50$ chromosomes, typical of most North American cyprinids (Gold et al., 1980; Amemiya et al., 1992). The chro-

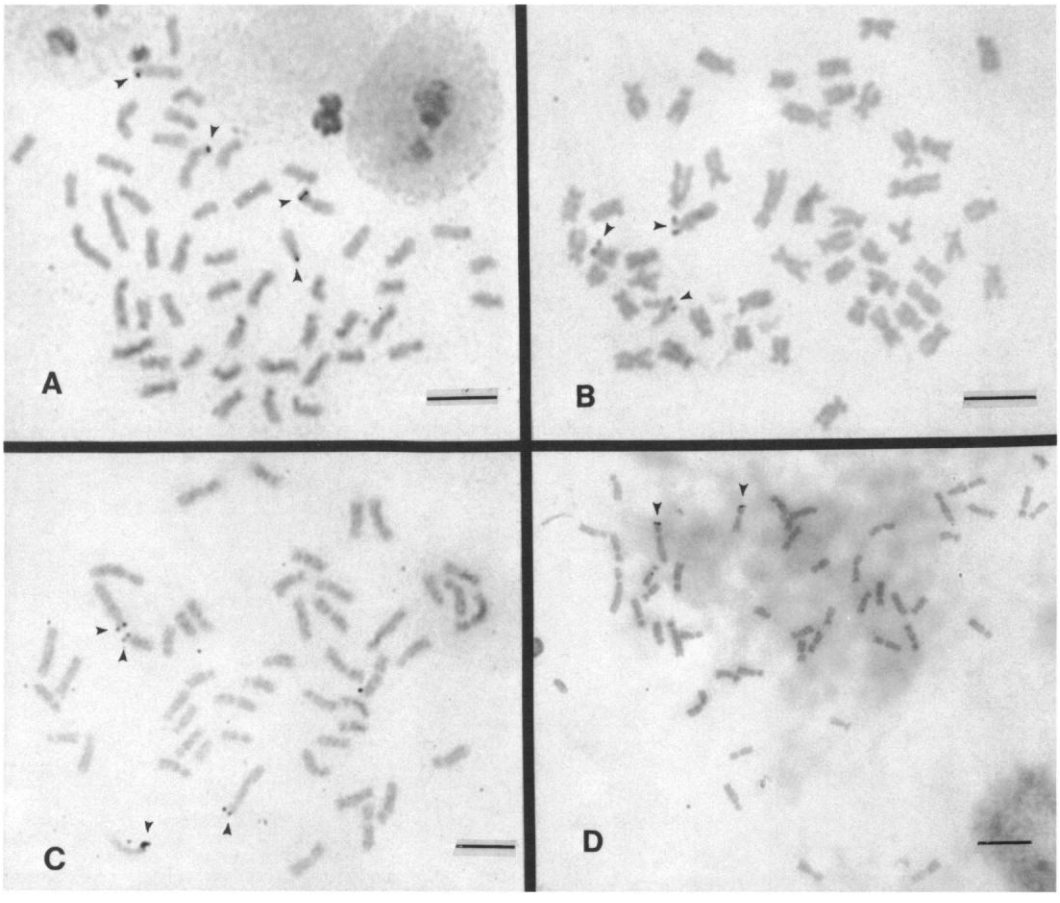

Fig. 1. Representative NOR karyotypes of (A) *Luxilus albeolus*, (B) *L. c. chrysocephalus*, (C) *L. c. isolepis*, and (D) *L. cornutus*. Chromosomal NORs are indicated by arrowheads. The individuals in B and D have activity heteromorphisms (see Gold and Amemiya, 1986). Chromosomal NORs in A and D were accentuated by overexposing the negatives. Bars are the equivalent of 5 μm.

mosome numbers of *L. albeolus*, *L. cardinalis*, *L. cerasinus*, *L. coccogenis*, *L. zonatus*, and *L. zonistius* are reported for the first time.

Representative silver-stained metaphases of *Luxilus* are shown in Figures 1–3. Based on visual inspection of individual, silver-stained metaphases and on haploid idiograms generated from computer-assisted measurements of the relative size of NOR-bearing chromosomes (Fig. 4), three NOR chromosome phenotypes were tentatively identified (Table 1). These included (1) the *C* NOR phenotype found in *L. cerasinus* and *L. c. isolepis* from the Blue River in Oklahoma; (2) the *D* NOR phenotype found in *L. cardinalis*, *L. pilsbryi*, *L. zonatus*, and *L. zonistius*; and (3) the *C*, *D* NOR phenotype found in *L. albeolus*, *L. c. chrysocephalus*, *L. c. isolepis* from Alabama, Mississippi, and Louisiana, *L. coccogenis*, and *L. cornutus*. Scrutiny of the computer-assisted idiograms suggested that the *D* NOR chromosomes found in species of the *L. zonatus* group were larger than the *D* NOR chromosomes found in species of both the *L. coccogenis* and *L. cornutus* groups. However, distinguishing among the *D* NOR chromosomes on the basis of relative size alone was not possible, because (1) size differences via computer-assisted measurement are only valid within (not among) idiograms, and (2) relative length differences per se could be a result of differential contraction of individual chromosomes during mitosis.

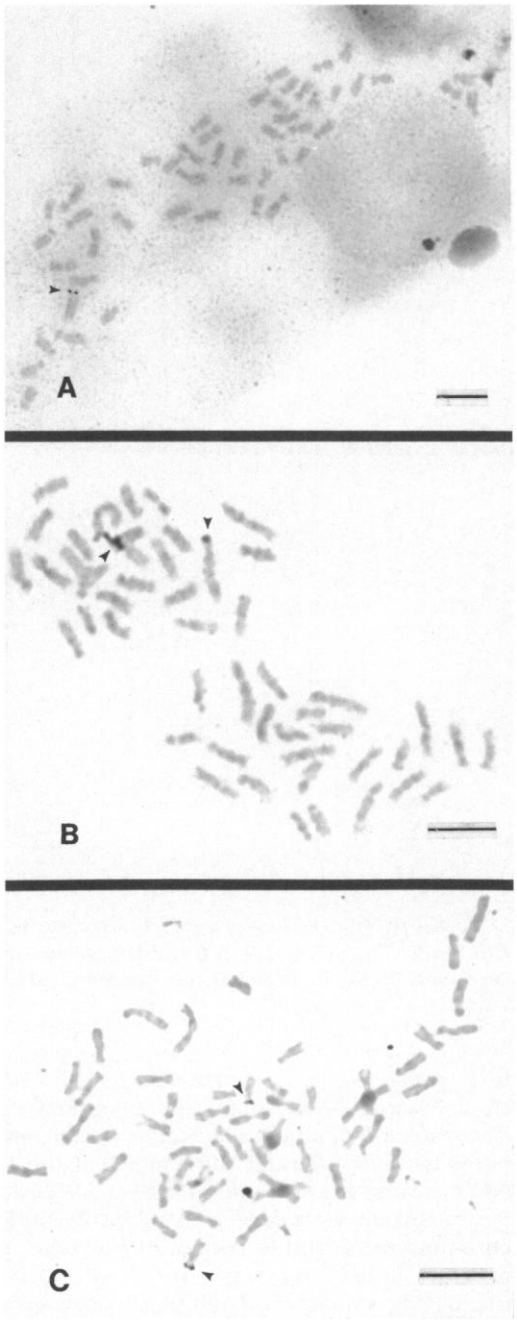

Fig. 2. Representative NOR karyotypes of (A) *Luxilus cerasinus*, (B) *L. coccogenis*, and (C) *L. zonistius*. Chromosomal NORs are indicated by arrowheads. The individual in A has an activity heteromorphism. The metaphase in B shows a NOR association of three NOR chromosomes. Chromosomal NORs in A and C were accentuated by overexposing the negatives. Bars are the equivalent of 5 μm.

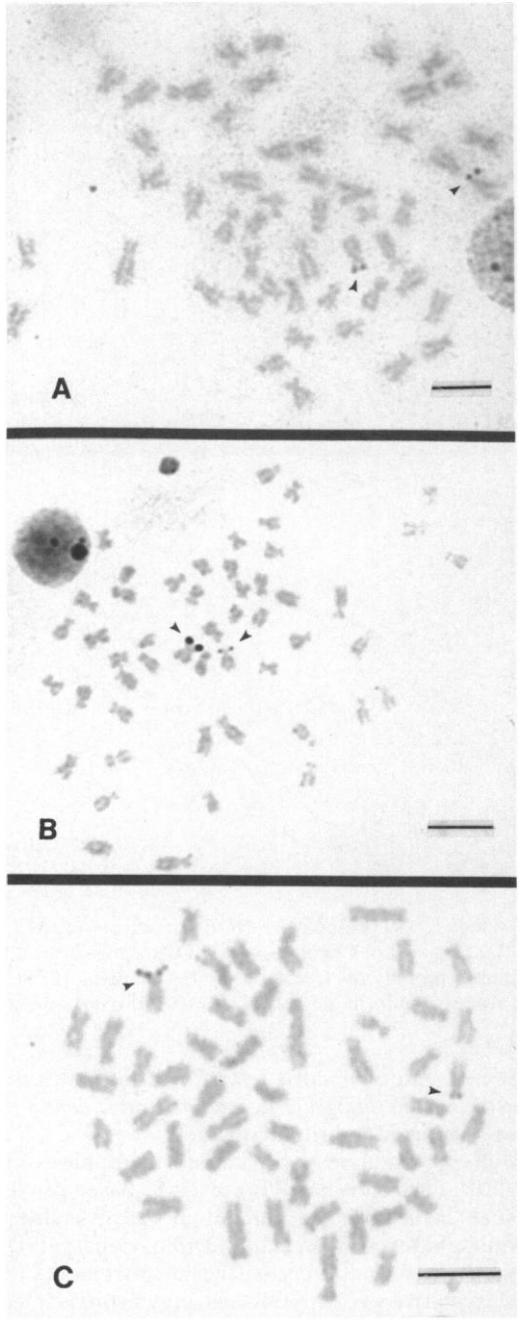

Fig. 3. Representative NOR karyotypes of (A) *Luxilus cardinalis*, (B) *L. pilsbryi*, and (C) *L. zonatus*. Chromosomal NORs are indicated by arrowheads. One NOR chromosome in C shows a NOR size heteromorphism (see Gold and Amemiya, 1986). Bars are the equivalent of 5 μm.

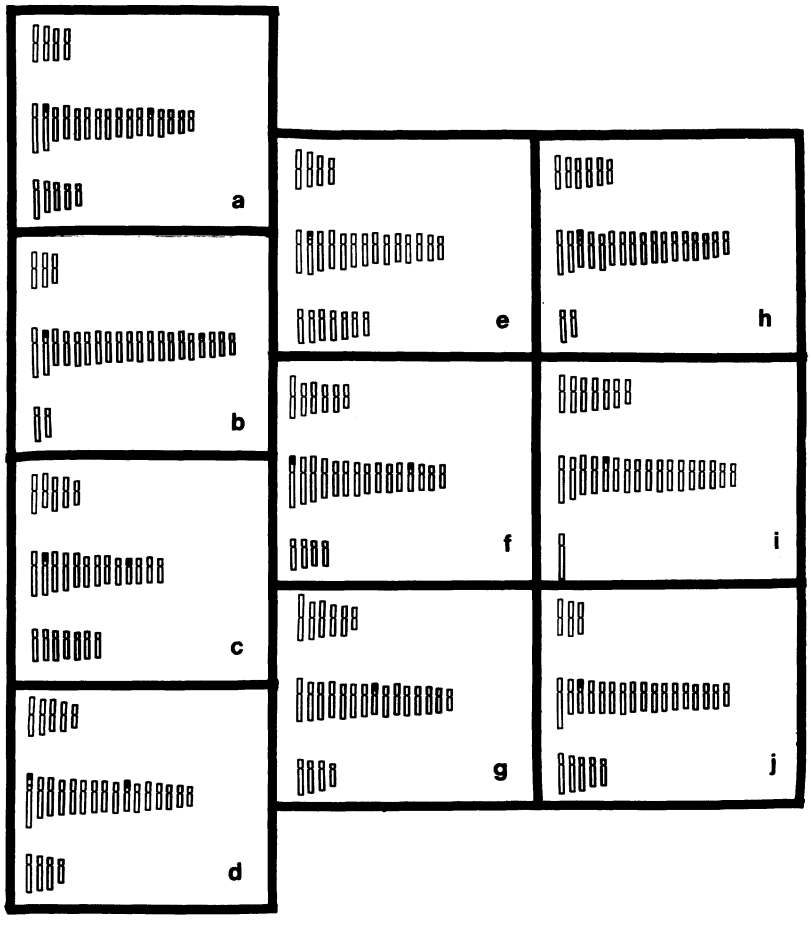

Fig. 4. Haploid idiograms of NOR karyotypes of all *Luxilus*. Chromosomes are arranged into rows in each karyotype on the basis of centromere position (after Levan et al., 1964): metacentric chromosomes are in the top row, submetacentric chromosomes are in the middle row, and acrocentric (subtelocentric) chromosomes are in the bottom row. Within rows, chromosomes are arranged according to relative size. NORs are indicated by darkened areas. (a) *L. albeolus*, (b) *L. c. chrysocephalus*, (c) *L. c. isolepis*, (d) *L. cornutus*, (e) *L. cerasinus*, (f) *L. coccogenis*, (g) *L. zonistius*, (h) *L. cardinalis*, (i) *L. pilsbryi*, and (j) *L. zonatus*.

A comparison of the Z-banding patterns of all NOR chromosomes is shown in Figure 5. As shown, the *C* NOR chromosomes in *L. albeolus*, *L. cerasinus*, *L. c. chrysocephalus*, *L. c. isolepis*, *L. coccogenis*, and *L. cornutus* appear to be homologous to one another, as do the *D* NOR chromosomes in *L. cardinalis*, *L. pilsbryi*, and *L. zonatus*, and the *D* NOR chromosomes in *L. albeolus*, *L. c. chrysocephalus*, *L. c. isolepis* from Alabama, Mississippi, and Louisiana, *L. coccogenis*, *L. cornutus*, and *L. zonistius*. The *D* NOR chromosomes found in species of the *L. zonatus* group, however, do not appear to be homologous to the *D* NOR chromosomes found elsewhere in *Luxilus* (Fig. 5). The *D* NOR chromosome in species of the *L. zonatus* group differs by the occurrence of a third Z-band on the long arm and is defined as the D_1 NOR chromosomes; the *D* NOR chromosome in the remaining species is defined as the D_2 NOR chromosome (Table 1).

The situation among samples of *L. c. isolepis* was discussed fully in Gold and Zoch (1990). In brief, *L. c. isolepis* from Alabama, Mississippi, and Louisiana possessed both *C* and D_2 NOR chromosomes, whereas *L. c. isolepis* from the Blue River in Oklahoma possessed only a *C* NOR chromosome. We interpreted the chromosomal NOR phenotype of *L. c. isolepis* from the Blue River to represent an intraspecific NOR poly-

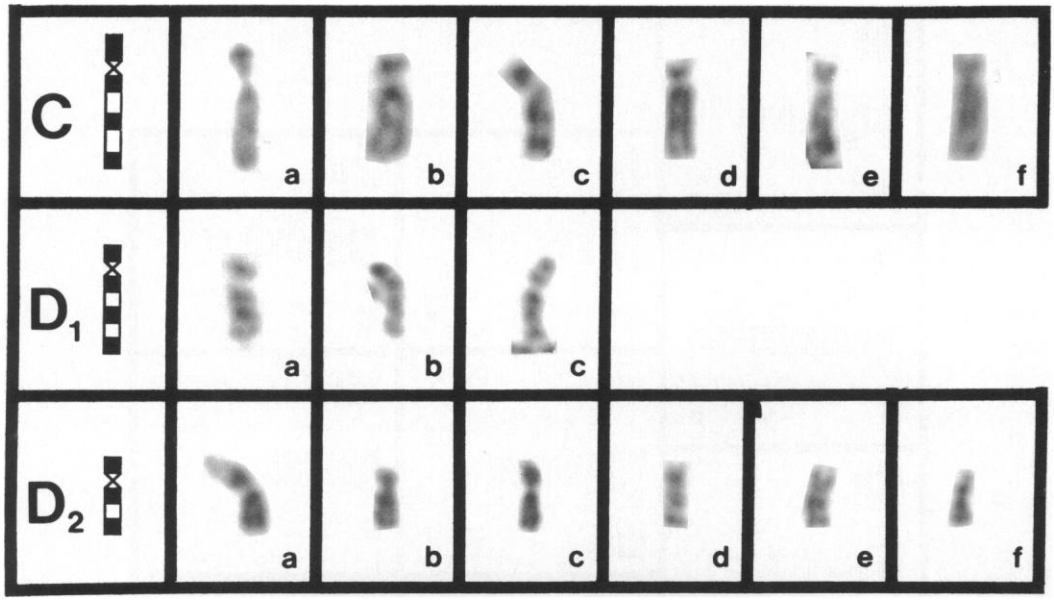

Fig. 5. Z-banded NOR chromosomes of all *Luxilus*. *C* NOR chromosomes are from (a) *L. albeolus*, (b) *L. c. chrysocephalus*, (c) *L. c. isolepis*, (d) *L. coccogenis*, (e) *L. cornutus*, and (f) *L. cerasinus*. D_1 NOR chromosomes are from (a) *L. cardinalis*, (b) *L. pilsbryi*, and (c) *L. zonatus*. D_2 NOR chromosomes are from (a) *L. albeolus*, (b) *L. c. chrysocephalus*, (c) *L. c. isolepis*, (d) *L. coccogenis*, (e) *L. cornutus*, and (f) *L. zonistius*. Computer-assisted drawings of the Z-band patterns are shown on the left.

morphism that became fixed in that population (Gold and Zoch, 1990). We noted, however, that one could argue that the loss of a *D* NOR pair in Blue River *L. c. isolepis* could represent an autapomorphy and that Blue River *L. c. isolepis* could merit consideration as a separate species. Recent mtDNA data (Dowling et al., 1992) suggest that the latter is a distinct possibility. Given the similarity in morphology between *L. c. isolepis* from the Blue River and *L. c. isolepis* from elsewhere (Gilbert, 1964), the Blue River form is best considered as derived from an ancestor of *L. c. isolepis* that possessed a *C*, D_2 NOR state.

Cytosystematic considerations.—Phenetically, similarities in NOR chromosomes suggest an alignment among the *L. coccogenis* group, the *L. cornutus* group, and *L. cerasinus*. All seven taxa in these three groups (including *L. c. isolepis*) possess homologous *C* or D_2 (or both) NOR chromosomes. Similarly, the three species in the *L. zonatus* group appear to be phenetically similar to one another (i.e., all three possess a homologous D_1 NOR chromosome) but not to other members of *Luxilus*.

The four most parsimonious phylogenetic hypotheses of relationships based on chromosomal NORs among *Luxilus* with *C* and D_2 NORs are shown in Figure 6. For comparison, hypotheses of relationships among all *Luxilus* taxa of Gilbert (1964), Buth (1979), and Dowling et al. (1992) are shown in Figure 7. These hypotheses are based on morphology (Gilbert, 1964), allozymes (Buth, 1979), and mtDNAs (Dowling et al., 1992). In the chromosomal hypotheses, a single pair of NORs is hypothesized to be the plesiomorphic state, and the taxa have been assumed to be interrelated by virtue of possessing either *C* or D_2 (or both) NOR chromosomes. The assumption that a single pair of NORs is plesiomorphic for *Luxilus* was based on both outgroup comparison and commonality (Watrous and Wheeler, 1981). Briefly, Mayden's (1989, pers. comm.) hypothesis of relationships among his "*Notropis*"-like shiners suggests the appropriate outgroups to *Luxilus* would be members of either a *Cyprinella-Pimephales-Opsopoeodus* clade or the genus *Lythrurus*. The plesiomorphic NOR state in these two clades has been hypothesized by Amemiya and Gold (1990a) and Amemiya et al. (1992) to be either *C'* (*Cyprinella-Pimephales-Opsopoeodus*) or *F'* (*Lythrurus*), neither of which is found in *Luxilus*.

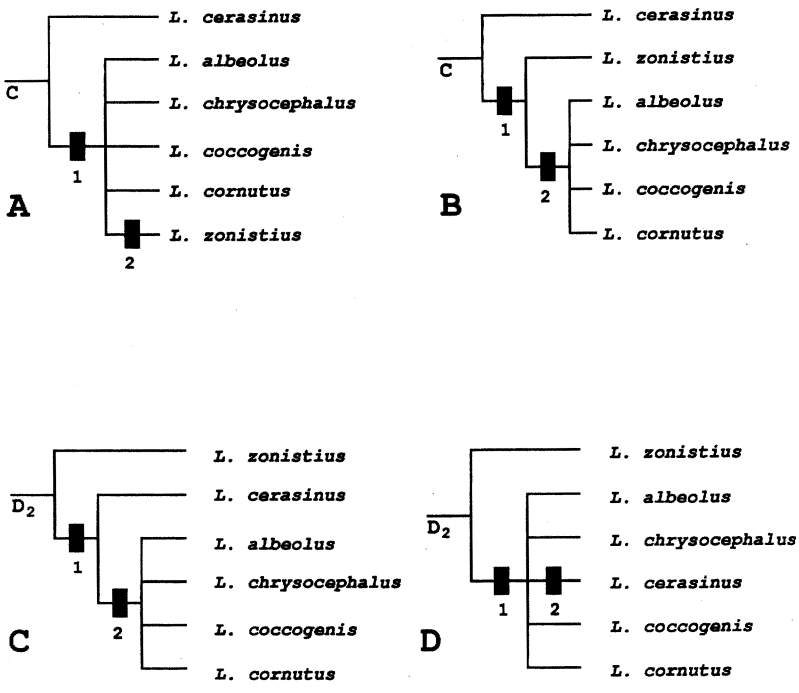

Fig. 6. Four equally parsimonious hypotheses of relationships among six species of *Luxilus* based on NOR chromosome data. Proposed chromosomal NOR state changes are as follows: (A) [1] + D_2, [2] − C; (B) [1] $C \rightarrow D_2$, [2] + C; (C) [1] $D_2 \rightarrow C$, [2] + D_2; and (D) [1] + C, [2] − D_2.

Coburn and Cavender's (1992) hypothesis of relationships among their notropin clade suggests the appropriate outgroups to *Luxilus* would be members of either a *Cyprinella-Pimephales-Opsopoeodus-Lythrurus* clade or a clade containing, among others, species belonging to the *Alburnops, Notropis, N. texanus, N. dorsalis,* and *N. volucellus* species groups as well as *N. greenei* and *N. ludibundus* [formerly *N. stramineus* (Mayden and Gilbert, 1989)]. The latter clade is largely, but not exclusively, Mayden's (1989) genus *Notropis.* Within this clade, 21 of 22 assayed species possess a single pair of NOR chromosomes (Amemiya et al., 1992). Interestingly, 15 of the 22 species in this clade possess a single pair of *D* NOR chromosomes (Amemiya et al., 1992), although only one (in *N. greenei*) has been Z-banded (Li and Gold, unpubl.). The Z-band pattern of the *D* NOR chromosome in *N. greenei,* however, differs from both the D_1 or D_2 NOR chromosomes in *Luxilus* (Li and Gold, unpubl.). Taken together, these observations support the hypothesis that a single pair of NOR chromosomes is plesiomorphic for *Luxilus,* assuming that *Luxilus* is monophyletic.

In all four chromosomal hypotheses (Fig. 6), monophyly of the *L. cornutus* group (sensu Buth, 1979, Fig. 7B) and *L. coccogenis* is supported. In three of the four chromosomal hypotheses (Fig. 6B–D), monophyly of the *L. coccogenis* group (*L. coccogenis* and *L. zonistius*) is not supported, and in only one of the four (Fig. 6D) is *L. cerasinus* included within the putative *L. coccogenis–L. cornutus* clade. A summary of the information deduced from these four topologies is as follows: (1) *L. coccogenis* belongs in a clade with *L. albeolus,* both subspecies of *L. chrysocephalus,* and *L. cornutus*; (2) the *L. coccogenis* group, as presently constituted, may be paraphyletic; and (3) either *L. zonistius* or *L. cerasinus* (but not both) is sister to the *L. coccogenis–L. cornutus* clade. Alternatively, if the *L. coccogenis* group (comprised of *L. coccogenis* and *L. zonistius*) is monophyletic, as suggested by morphological, allozyme, and mtDNA data (Fig. 7), the chromosomal hypothesis shown in Figure 6A would be preferred because it is the only one that does not falsify the hypothesis of monophyly of the *L. coccogenis* group. On this basis, we suggest that (1) a single pair of *C* NOR chromosomes is plesiomorphic for *Luxilus* (exclusive of the *L. zonatus* group species), (2) the addition of a D_2 NOR chro-

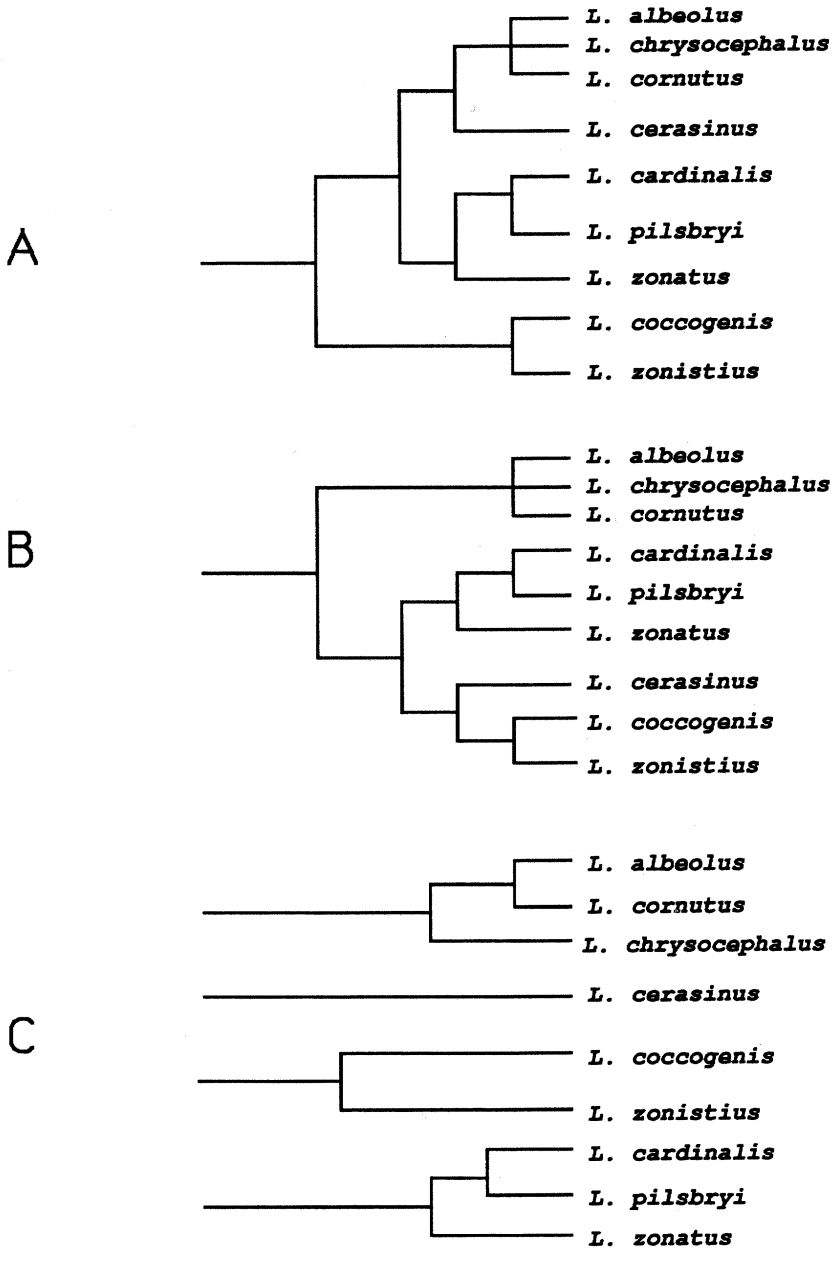

Fig. 7. Phylogenetic hypotheses of relationships among species of *Luxilus*. (A) Hypothesis of Gilbert (1964) based on morphology. (B) Hypothesis of Buth (1979) based on allozymes. (C) Hypothesis of Dowling et al. (1992) based on restriction site variation among mtDNAs. The location of *L. cardinalis* in hypotheses A and B is based on Mayden (1988a).

mosome is a synapomorphy uniting the *L. coccogenis* and *L. cornutus* groups, and (3) the single D_2 NOR chromosome in *L. zonistius* represents the autapomorphic loss of a *C* NOR chromosome. Assuming both the *L. coccogenis* and *L. cornutus* groups to be monophyletic (based on nonchromosomal data), the phylogenetic inference is that the *L. coccogenis* and *L. cornutus* groups are sister to one another and that *L. cerasinus* is sister to the *L. coccogenis–L. cornutus*

clade. One final point to note is that superimposition of the NOR chromosome data from the species with *C* and D_2 NOR chromosomes (i.e., excluding the *L. zonatus* group) onto the hypotheses of either Gilbert (1964, Fig. 7A) or Buth (1979, Fig. 7B) requires a minimum of three character state changes regardless of whether *C* or D_2 NOR chromosomes are considered as plesiomorphic.

All three species of the *L. zonatus* group possess a D_1 NOR state (Table 1). Because the D_1 NOR chromosome was not found in any other species currently placed in *Luxilus,* it is not possible to infer the phylogenetic relationship between the *L. zonatus* group and any of the other species of *Luxilus.* If one assumes, as indicated by most of the published evidence (Gilbert, 1964; Buth, 1979), that *Luxilus* is monophyletic and that a single pair of NOR chromosomes is plesiomorphic for *Luxilus,* there are two possible alternatives. First, the D_1 NOR state could be plesiomorphic in which case there would be no chromosomal evidence for monophyly of the *L. zonatus* group. The remaining *Luxilus* taxa would then form a monophyletic clade regardless of which hypothesis shown in Figure 6A–D is correct. The second alternative is that the D_1 NOR state is autapomorphic for the *L. zonatus* group. The transition to a D_1 state is problematic. In all likelihood, the ancestor to a monophyletic *L. zonatus* group possessed a single pair of NOR chromosomes, because transition from a *C,* D_2 NOR state would necessitate two character state changes, i.e., loss of a chromosomal NOR plus transition to a D_1 NOR state. A more parsimonious solution would be a single character state change from the (presumed) plesiomorphic *C* NOR state to a D_1 NOR state. In this case, divergence of a monophyletic *L. zonatus* group could have occurred either before or after divergence of *L. cerasinus,* as shown in Figure 6A. A third alternative is that *Luxilus* is not monophyletic. Coburn and Cavender (1992) noted that the scale morphology of members of the *L. zonatus* group is very similar to that of *N. leuciodus* and *N. nubilus* of the subgenus *Hydrophlox.* The possibility that the *L. zonatus* group may have closer relatives elsewhere among the "*Notropis*"-like shiners merits further investigation.

The phylogenetic inference, based on the chromosomal data, that the *L. coccogenis* group is sister to the *L. cornutus* group may resolve a biogeographic problem involving species of *Luxilus.* Mayden (1987, 1988b), based on Buth's (1979) hypothesis that the *L. coccogenis* group was sister to the *L. zonatus* group, proposed that a now extinct form of *Luxilus* was once present in the Ohio River and northern Atlantic slope drainages. His hypothesis was necessitated on the basis of current distributions of *Luxilus* and presumed vicariant events which, in sequence, involved the separation of the Tennessee River system from the ancestral Teays-Interior Highland drainages, followed by the separation of the Teays system from the Interior Highlands–northwest Central Lowlands drainages. If, in fact, the *L. coccogenis* group is sister to the *L. cornutus* group, as indicated by the chromosomal data, the "missing" species in the Ohio River–northern Atlantic slope drainages could have been a member (or ancestor) of the *L. cornutus* group because *L. chrysocephalus* or *L. cornutus* presently occur in much of the upper Ohio River and northern Atlantic slope drainages (Gilbert, 1964).

Material Examined

Collection localities of *Luxilus* examined are listed below including catalog numbers of voucher specimens deposited in the Texas Cooperative Wildlife Collections (TCWC) at Texas A&M University. *L. albeolus* and *L. cerasinus*: Roanoke River, Roanoke County, Virginia (6869.02 and 6869.01); *L. cardinalis*: Lee Creek, Crawford County, Arkansas (6864.01); *L. c. chrysocephalus*: Martin Creek, Sharp County, Arkansas; West Fork White River, Washington County, Arkansas; *L. c. isolepis*: unnamed tributary of the Talapoosa River, Randolph County, Alabama (6865.01); Little River, LaSalle Parish, Louisiana (6866.01); Okatoma Creek, Covington County, Mississippi; Blue River, Johnston County, Oklahoma (6867.01); *L. coccogenis*: North Fork Holston River, Scott County, Virginia (6868.01); *L. cornutus*: Saline River, Washtenaw County, Michigan (6871.01); *L. pilsbryi*: Hock Creek, Madison County, Arkansas; West Fork White River, Washington County, Arkansas (6872.01); Jenkyns Creek, Jasper County, Missouri (6873.01); *L. zonatus*: Martin Creek, Sharp County, Arkansas (6870.01); Spring River, Sharp County, Arkansas; and *L. zonistius*: unnamed tributary of the Talapoosa River, Randolph County, Alabama (6865.02).

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Literature Cited

Amemiya, C. T. 1987. Cytogenetic and cytosystematic studies on the nucleolus organizer regions of North American cyprinid fishes. Unpubl. Ph.D. diss., Texas A&M Univ., College Station.

———, and J. R. Gold. 1988. Chromosomal NORs as taxonomic and systematic characters in North American cyprinid fishes. Genetica 76:81–90.

———, and ———. 1990a. Cytogenetic studies in North American minnows (Cyprinidae). XVII. Chromosomal NOR phenotypes of 12 species with comments on cytosystematic relationships among 50 species. Hereditas 112:231–247.

———, and ———. 1990b. Chromosomal NOR phenotypes of seven species of North American Cyprinidae, with comments on cytosystematic relationships of the *Notropis volucellus* species-group, *Opsopoeodus emiliae,* and the genus *Pteronotropis.* Copeia 1990:68–78.

———, J. W. Bickham, and J. R. Gold. 1984. A cell culture technique for chromosome preparation in cyprinid fishes. Copeia 1984:232–235.

———, P. K. Powers, and J. R. Gold. 1992. Chromosomal evolution in North American cyprinids. *In:* Systematics, historical ecology, and North American freshwater fishes. R. L. Mayden (ed.). Stanford Univ. Press, Palo Alto, California, In press.

Arrighi, F. E., and T. C. Hsu. 1974. Staining constitutive heterochromatin and Giemsa crossbands of mammalian chromosomes, p. 59–71. *In:* Human chromosome methodology. J. J. Yunis (ed.). Academic Press, New York, New York.

Buth, D. G. 1979. Biochemical systematics of the cyprinid genus *Notropis.* I. The subgenus *Luxilus.* Biochem. Syst. Ecol. 7:69–79.

Coburn, M. M., and T. M. Cavender. 1992. Interrelationships of North American cyprinid fishes. *In:* Systematics, historical ecology, and North American freshwater fishes. R. L. Mayden (ed.). Stanford Univ. Press, Palo Alto, California, In press.

Comings, D. E. 1978. Mechanisms of chromosome banding and implications for chromosome structure. Ann. Rev. Genet. 12:25–46.

Dowling, T. E., W. R. Hoeh, G. R. Smith, and W. M. Brown. 1992. Phylogenetic relationships of minnows in the genus *Luxilus* (Cyprinidae) as determined by analysis of mitochondrial DNA. Copeia 1992:306–322.

Dutrillaux, B., J. Couturier, M. Muleris, M. Lombard, and G. Chauvier. 1982. Chromosomal phylogeny of forty-two species or subspecies of cercopithecoids (Primates, Catarrhini). Ann. Genet. 25:96–109.

Gall, J. G., and M. L. Pardue. 1969. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc. Natl. Acad. Sci. U.S. 63:378–383.

Gilbert, C. R. 1964. The American cyprinid fishes of the subgenus *Luxilus* (genus *Notropis*). Bull. Florida State Mus. Biol. Sci. 8:95–194.

Gold, J. R. 1984. Silver-staining and heteromorphism of chromosomal nucleolus organizer regions in North American cyprinid fishes. Copeia 1984: 133–139.

———, and C. T. Amemiya. 1986. Cytogenetic studies in North American minnows (Cyprinidae). XII. Patterns of chromosomal nucleolus organizer region variation among 14 species. Can. J. Zool. 64: 1869–1877.

———, ———, and J. R. Ellison. 1986. Chromosomal heterochromatin differentiation in North American cyprinid fishes. Cytologia 51:557–566.

———, and J. R. Ellison. 1983. Silver-staining for nucleolus organizer regions of vertebrate chromosomes. Stain Technol. 58:51–55.

———, J. D. Jenkin, and P. K. Powers. 1990a. Cytogenetic studies in North American minnows (Cyprinidae). XVIII. Chromosomal NOR variation among eight species. Cytologia 55:483–492.

———, W. J. Karel, and M. R. Strand. 1980. Chromosome formulae of North American fishes. Prog. Fish-Cult. 42:10–23.

———, Y. C. Li, N. S. Shipley, and P. K. Powers. 1990b. Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. J. Fish Biol. 37:563–575.

———, W. D. Womac, F. H. Deal, and J. A. Barlow. 1981. Cytogenetic studies in North American minnows (Cyprinidae). VII. Karyotypes of thirteen species from the southern United States. Cytologia 46: 105–115.

———, and P. K. Zoch. 1990. Intraspecific variation in chromosomal nucleolus organizer regions in *Notropis chrysocephalus* (Pisces: Cyprinidae). Southwestern Nat. 35:211–215.

Haiduk, M. W., and R. J. Baker. 1982. Cladistical analysis of G-banded chromosomes of nectar feeding bats (Glossophaginae: Phyllostomidae). Syst. Zool. 31:252–265.

Holmquist, G., M. Gray, T. Porter, and J. Jordan. 1982. Characterization of Giemsa dark- and light-band DNA. Cell 31:121–129.

Howell, W. M. 1982. Selective staining of nucleolus organizer regions (NORs). Cell Nucleus 11:89–142.

———, and D. A. Black. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 31:260–262.

Jorgenson, K. F., J. H. Van de Sande, and C. C. Lin. 1978. The use of base pair specific DNA binding agents as affinity labels for the study of mammalian chromosomes. Chromosoma 68:287–302.

Levan, A., K. Fredga, and A. A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52:201–220.

Mayden, R. L. 1987. Pleistocene glaciation and historical biogeography of North American central-highland fishes, p. 141–151. *In:* Quaternary en-

vironments of Kansas. W. C. Johnson (ed.). Kansas Geol. Surv. Guidebook Ser. 5.

———. 1988a. Systematics of the *Notropis zonatus* species group, with description of a new species from the Interior Highlands of North America. Copeia 1988:153–173.

———. 1988b. Vicariance biogeography, parsimony, and evolution in North American freshwater fishes. Syst. Zool. 37:329–355.

———. 1989. Phylogenetic studies of North American minnows, with emphasis on the genus *Cyprinella* (Teleostei: Cypriniformes). Misc. Publ. 80:1–189, Publ. Mus., Univ. Kansas Mus. Nat. History, Lawrence.

———, AND C. R. GILBERT. 1989. *Notropis ludibundus* (Girard) and *Notropis tristis* (Girard), replacement names for *N. stramineus* (Cope) and *N. topeka* (Gilbert) (Teleostei: Cypriniformes). Copeia 1989:1084–1089.

RITOSSA, F. M., AND S. SPIEGELMAN. 1965. Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. 53:737–745.

STOCK, A. D., AND T. D. BUNCH. 1982. The evolutionary implications of chromosome banding pattern homologies in the bird order Galliformes. Cytogenet. Cell Genet. 34:136–148.

SUMNER, A. T. 1977. Banding as a level of chromosome organization, p. 17–22. *In:* Current chromosome research. K. Jones and P. E. Brandham (eds.). Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands.

WATROUS, L. E., AND Q. D. WHEELER. 1981. The out-group comparison method of character analysis. Syst. Zool. 30:1–11.

DEPARTMENT OF WILDLIFE AND FISHERIES SCIENCES, TEXAS A&M UNIVERSITY, COLLEGE STATION, TEXAS 77843. PRESENT ADDRESS (PKP): SOUTHWEST FOUNDATION FOR BIOMEDICAL RESEARCH, 7620 NW LOOP 410, SAN ANTONIO, TEXAS 78227–5301. Accepted 6 March 1991.

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Effects of Body Mass and Temperature on Standard Metabolic Rates for Two Australian Varanid Lizards (*Varanus gouldii* and *V. panoptes*)

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Standard metabolic rates (SMR) for two varanid lizards (*Varanus gouldii* and *V. panoptes*) were measured from 2400 and 0800 h, after the lizards had rested for at least 8 h. The relationship between SMR ($\dot{V}O_2$; ml/h) and body mass for varanids at 20 C is 0.04 $g^{0.95}$; at 30 C, 0.030 $g^{1.15}$: at 35 C, 0.089 $g^{1.05}$ and at 40 C, 0.144 $g^{1.04}$. The relationship between SMR ($\dot{V}CO_2$; ml/h) and body mass at 20 C is 0.017 $g^{1.04}$; at 30 C, 0.028 $g^{1.12}$; at 35 C, 0.045 $g^{1.10}$ and at 40 C, 0.107 $g^{1.04}$. There were no significant differences in the mass exponent between species at any temperature (20–40 C), with the pooled slope for *V. gouldii* of 1.12, for *V. panoptes* 1.10, and an overall pooled slope of 1.11. No plateau in $\dot{V}O_2$ was found between the T_b of 30–40 C as previously reported for *V. gouldii* and *V. rosenbergi*.

LARGER lizards consume more oxygen than smaller lizards but less oxygen per gram body mass (see reviews of Bennett and Dawson 1976, and Bennett 1982). The allometric relationship between standard metabolic rate (SMR, ml O_2/h) and body mass (M; g) is SMR = aM^b, where a is the mass coefficient (SMR of a 1 g lizard) and b is the mass exponent (slope of a double logarithmic plot of SMR and M). The relationship for mass-specific metabolic rate (e.g., ml O_2 g^{-1} h^{-1}) is $SMR/M = aM^{b-1}$.

Andrews and Pough (1985) report the mean mass exponent (b) of the multiple regression equation for 107 species of squamates as 0.80 (±SE b = 0.012). They also report no difference in the mass exponent ($P < 0.05$) between Varanidae (the family with the highest mean SMR) and Boidae (the family with the lowest SMR).