SHORTER CONTRIBUTIONS: ICHTHYOLOGY

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CYTOSYSTEMATIC EVIDENCE THAT THE GENUS *RICHARDSONIUS* BELONGS IN THE WESTERN CLADE OF PHOXININ CYPRINIDS.—The cyprinid genus *Richardsonius* is currently comprised of two species: the redside shiner (*R. balteatus*), distributed mostly west of the Rocky Mountains from Nass River, British Columbia, to the Bonneville Basin in northern Utah (Wallace, 1980), and the Lahontan redside (*R. egregius*), distributed in the Lahontan basin and related waters of Nevada and California (Allen-Grimes, 1980). Several authors (Bailey, 1951; Uyeno, 1961; Buhan, 1969) have hypothesized that *Richardsonius* is closely allied to the cyprinid genera *Clinostomus*, *Gila*, and *Ptychocheilus*. The distribution of *Richardsonius* and the hypothesis that *Richardsonius* is allied to *Gila* and *Ptychocheilus* suggest that *Richardsonius* may have phyletic affinities with cyprinid genera endemic to western North America.

Based on morphological evidence, Cavender and Coburn (1992) hypothesized that all Nearctic cyprinids except monotypic *Notemigonus* belonged to a clade they termed phoxinins. Coburn and Cavender (1992) divided phoxinins into three clades: western, chub, and shiner, with shiners forming the sister group to a western-chub clade. The western clade included all cyprinid genera in western North America except *Richardsonius* and monotypic *Oregonichthys*. Coburn and Cavender placed *Richardsonius* in the shiner clade and hypothesized that a *Richardsonius-Clinostomus* clade was sister to an *Oregonichthys*-notropin clade. Notropins (sensu Coburn and Cavender) included nearly all endemic cyprinid genera in eastern North America.

Jenkin et al. (1992) documented chromosomal nucleolus organizer region (NOR) phenotypes of several cyprinids, including *Richardsonius*, from western North America. Both species of *Richardsonius* were found to possess multiple NOR chromosomes, including two pair of NOR phenotype *A* (NOR terminal on the short arm of a medium-sized acrocentric chromosome). At least two pair of *A* NOR chromosomes were found in 15 of 19 other cyprinid species endemic to western North America, prompting the suggestions that (1) an *AA* NOR chromosome state might represent a chromosomal synapomorphy for the western clade, and (2) *Richardsonius* might properly belong in the western clade. The latter suggestion was based on the (untested) hypothesis that *A* NOR chromosomes in *Richardsonius* were homologous to *A* NOR chromosomes in other members of the western clade.

The purpose of this study was to determine whether the *A* NOR chromosomes are informative phylogenetically relative to the placement of *Richardsonius* in phoxinins. NOR chromosomes of both species of *Richardsonius* were examined for trypsin G-band patterns and compared to those documented by Gold and Li (1994) for the western-clade genera *Lavinia*, *Mylopharodon*, and *Ptychocheilus*. Trypsin G-banding patterns of *A* NOR chromosomes found in *Notemigonus crysoleucas* and *Scardinius erythrophthalmus* (two leuciscin cyprinids) were also examined to provide an outgroup perspective to NOR chromosome states in phoxinins. Genome sizes of specimens of both species of *Richardsonius* also were documented.

Methods.—Specimens of *Richardsonius* examined in this study were obtained by seine from natural populations. Collection localities and voucher material are given in Jenkin et al. (1992). Specimens of *N. crysoleucas* and *S. erythrophthalmus* were obtained from Anderson Minnow Farms in Lonoke, Arkansas. Seeding of fibroblast cultures and preparation of metaphase chromosomes followed procedures in Gold et al. (1990). Trypsin G-banding employed methods in Gold et al. (1990) and Gold and Li (1991). G-banding of NOR chromosomes was carried out on at least three individuals of each species.

Whole blood for genome size determinations was cryopreserved following procedures outlined in Gold et al. (1991). Genome size determinations of five individuals of each species were made using flow cytometry of erythrocyte nuclei. Erythrocytes from both chicken and common carp were used as internal standards.

Results.—All specimens of *R. balteatus* and *R. egregius* possessed multiple NOR chromosomes. Two pair of NOR chromosomes in both species were of the A NOR phenotype (NOR terminal on the short arm of a medium-sized acrocentric chromosome): *R. egregius* possessed a third pair of NOR chromosomes of the *J* NOR phenotype (NOR terminal on the short arm of a small-sized submetacentric chromosome), whereas *R. balteatus* possessed two additional pair of NOR

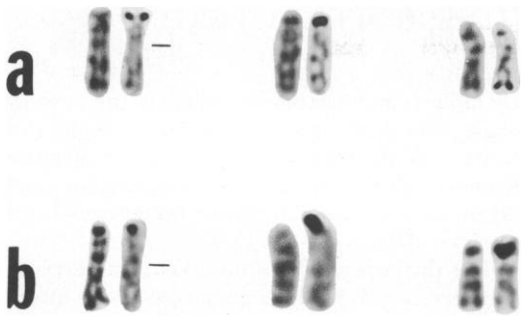

Fig. 1. Trypsin G-banded NOR chromosomes from (a) *Richardsonius balteatus* (A_1A_2L), and (b) *Richardsonius egregius* (A_1A_2J). For each pair, the chromosome on the left is from a trypsin G-banded metaphase; the chromosome on the right is from sequential silver-staining (to identify NOR chromosomes) from the same metaphase. Bar denotes large G^- (negative) band on the A_1 NOR chromosome.

Fig. 2. Trypsin G-banding patterns of A NOR chromosomes. A_1 and A_2 NOR chromosomes are from (left to right) *Richardsonius balteatus*, *Richardsonius egregius*, and *Mylopharodon conocephalus*. The A_3 NOR chromosome is from *Notemigonus crysoleucas*, and the A_4 NOR chromosome is from *Scardinius erythrophthalmus*.

chromosomes, both of the *L* NOR phenotype (NOR terminal on the long arm of a small-sized submetacentric chromosome). Silver-stained metaphases documenting these NOR phenotypes may be found in Jenkin et al. (1992).

Trypsin G-bands were obtained on all NOR chromosomes except for one pair of *L* NOR chromosomes in *R. balteatus*. Both pair of *A* NOR chromosomes in both species possessed four G^+ (dark) bands on the long arm and a single G^+ band on the short arm (Fig. 1). The A_1 NOR chromosomes could be distinguished by the presence of a large G^- (light) band near the middle of the long arm on one of the NOR chromosome pairs (Fig. 1). The large G^- band was observed on two of the four NOR chromosomes identified in all G-banded, midmetaphase preparations examined from both species. The G-banded *L* NOR chromosome in *R. balteatus* possessed three G^+ bands on the long arm and one G^+ band on the short arm, as did the *J* NOR chromosome in *R. egregius*; on both NOR chromosomes, the G^- band distal on the long arm was invariably larger than the G^- band proximal on the long arm (Fig. 1). The similarity in size, centromere position, and trypsin G-banding pattern indicates that the *L* and *J* NOR chromosomes are homologues that differ only in the position of the NOR.

Gold and Li (1994) documented trypsin G-bands on two pair of A NOR chromosomes found in five other cyprinid species (three genera) from western North America: *Lavinia exilicauda*, *Mylopharodon conocephalus*, and three species of *Ptychocheilus*. Trypsin G-banding patterns of nonhomologous *A* NOR chromosomes (designated A_1 and A_2) indicated homology (in the chromosomal sense) of A_1 and A_2 NOR chromosomes among the five species. Comparison of G-banded *A* NOR chromosomes of *Richardsonius* with those of *Mylopharodon* (Fig. 2) indicates chromosomal homology of A_1 and A_2 NOR chromosomes among all cyprinids from western North America examined to date. Also included in Figure 2 are G-banded *A* NOR chromosomes from *N. crysoleucas* and *S. erythrophthalmus*, two leuciscin cyprinids that possess only a single pair of *A* NOR chromosomes (Gold, 1984; Mayr et al., 1986). The *A* NOR chromosomes in *N. crysoleucas* (A_3) and *S. erythrophthalmus* (A_4) appear smaller in size and differ in trypsin G-band pattern from *A* NOR chromosomes found in *Richardsonius* (and other phoxinins): three G^+ bands are evident on the long arm of the NOR chromosome in *N. crysoleucas*, whereas only two G^+ bands are evident on the long arm of the NOR chromosome in *S. erythrophthalmus*.

Genome sizes of five individuals from both species of *Richardsonius* were assayed using flow cytometry. Mean genome sizes (± SE, range of genome sizes of individuals) in picograms of DNA were: *R. balteatus* (2.54 ± 0.03, 2.46–2.62) and *R. egregius* (2.70 ± 0.05, 2.61–2.85).

Discussion.—Results of this study demonstrate that trypsin G-band patterns of the two pair of *A* NOR chromosomes in both species of *Richardsonius* are identical to those of the A_1 and A_2 NOR chromosomes, respectively, of *Lavinia*, *Mylopharodon*, and *Ptychocheilus*. We previously hypothesized (Gold and Li, 1994) that the A_1A_2 NOR character state represented a chromosomal synapomorphy uniting *Lavinia*, *Mylopharodon*, and *Ptychocheilus*. The presumed homology of *A* NOR chromosomes in *Richardsonius* with those of *Lavinia*, *Mylopharodon*, and *Ptychocheilus* indicate that all four genera belong to the same clade. The western cyprinid genera

Acrocheilus, *Gila* (*Temeculina*), *Orthodon*, *Plagopterus*, and *Rhinichthys* also possess at least two pair of *A* NOR chromosomes (Jenkin et al., 1992) and may belong to this clade. The *A* NOR chromosomes of these western cyprinid genera, however, have yet to be tested for homology using trypsin G-banding.

The hypothesis that the A_1A_2 NOR state represents a synapomorphy uniting *Richardsonius* with at least three other genera of the western clade (sensu Coburn and Cavender, 1992) is supported by outgroup comparison of chromosomal NOR phenotypes. Cavender and Coburn (1992) hypothesized that leuciscin cyprinids comprised the sister group to phoxinin cyprinids. Chromosomal NOR data are available for five leuciscins (Mayr et al., 1986; Rab et al., 1990; this paper): all five possess only a single pair of NOR chromosomes, and only two (*N. crysoleucas* and *S. erythrophthalmus*) possess a NOR chromosome pair of the *A* phenotype. Among other leuciscine cyprinids from the gobionin, cultrinin, and acheilognathin clades, 13 of 19 species examined possess only a single pair of NOR chromosomes, and most are not of the *A* NOR phenotype (Zhou et al., 1980; Takai and Ojima, 1986). Within phoxinins, multiple NORs have been found in both the chub (eight of 15 species) and notropin (18 of 65 species) clades (references available from JRG). However, nearly all of the chub or notropin species with multiple NORs do not possess even a single pair of *A* NOR chromosomes, and the plesiomorphic NOR state for both chub and notropin clades has been hypothesized to be a single pair of NOR chromosomes (Amemiya and Gold, 1990; Gold and Li, 1991). The A_1A_2 NOR character state, thus, appears to be derived within phoxinins and to unite *Richardsonius* with at least three other genera in the western clade.

The *L* and *J* NOR chromosomes found in the two species of *Richardsonius* appear to comprise a chromosomal synapomorphy. The two NOR chromosomes are identical in trypsin G-band pattern and differ only in the position of the NOR, i.e., terminal on the long arm in *R. balteatus* and terminal on the short arm in *R. egregius*. *L* NOR chromosomes have not been documented previously in North American cyprinids, and *J* NOR chromosomes have been found only in *Rhinichthys* (=*Tiaroga*) *cobitus* (a member of the western clade) and in three species of notropins. The *J* NOR chromosomes in the three notropin species have been hypothesized to be derived in their respective clades (Amemiya and Gold, 1990; Amemiya et al., 1992), and the *J* NOR chromosome in *R. cobitus* is smaller and possesses a different trypsin G-banding pattern from the *J* NOR chromosome in *R. egregius* (unpubl.). Because the occurrence of a NOR on the long arm of a biarmed chromosome is very rare among North American cyprinids (Amemiya et al., 1992), the *L* NOR chromosome may represent a derived condition in *Richardsonius*. This suggests that *R. balteatus* could be derived from an ancestor with an *R. egregius*-like *J* NOR phenotype.

One final point regards the difference in genome size (0.16 picograms of DNA) between the two species of *Richardsonius*. This difference is greater, on average, than that between other closely related phoxinin cyprinids thus far assayed (Gold et al., 1992). Given the hypothesis that changes in DNA quantity in North American cyprinids are small in amount (Gold et al., 1992), and that differences among species are proportional to elapsed time, the separation of the two species of *Richardsonius* may be quite old.

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SPAWNING BEHAVIOR OF *ETHEOSTOMA ZONALE* (PISCES: PERCIDAE).—Recently, greater attention has been given to the value of behavioral traits in discerning phylogenetic relationships. For example, egg-deposition behavior has been used in formulating hypotheses of phylogenetic history of the darters (Page, 1985). Actual courtship and spawning behavior may be of equal or greater value in determining these relationships. Here, I describe the courtship and spawning behavior of the banded darter, *Etheostoma zonale*. There is disagreement regarding whether *E. zonale* should be placed in the subgenus *Etheostoma*, which includes the greenside darter, *E. blennioides* (Bailey and Etnier, 1988), or included with species of the *Ulocentra* group in the subgenus *Nanostoma* (Page, 1981). *Etheostoma zonale* is known to be an egg-attaching species (Page, 1985), but little information on its spawning behavior has been reported.

Methods.—Observations of spawning banded darters were made while snorkeling in the Buf-