Results and discussion

The NOR chromosome phenotypes of the six species were known from previous studies (Gold 1984, unpubl., Amemiya and Gold 1988): *C. lepida*, *C. lutrensis*, and *C. venusta* each possess a single pair of NOR chromosomes of the *C′* phenotype defined as a NOR terminal on the short arm of a submetacentric chromosome which is the largest chromosome in the complement; *N. amabilis* and *N. shumardi* each possess a single pair of NOR chromosomes of the *F′* phenotype defined as a NOR terminal on the short arm of a subtelo-/acrocentric chromosome which is the largest chromosome in the complement; and *O. emiliae* possesses a single pair of NOR chromosomes of the *E′* phenotype defined as a NOR subterminal (interstital) on the short arm of a submetacentric chromosome which is the largest chromosome in the complement. Based on the similarities in size and the presence of an unusually large, long-arm C-band, Gold and Amemiya (1986) and Amemiya and Gold (1988) suggested that the *C′* NOR chromosomes of the *Cyprinella* species were homologous and that the *E′* NOR chromosome in *O. emiliae* differed from the *C′* NOR chromosome by a small paracentric inversion in the short arm. G-banded preparations of the NOR chromosomes of the six species are shown in Figure 1. The NOR chromosomes of all six species possess at least six dark G-bands on the long arm of each chromosome, indicating (i) homology of the long arms among the six species, and (ii) chromosomal rearrangements among the six species have all involved the short arm.

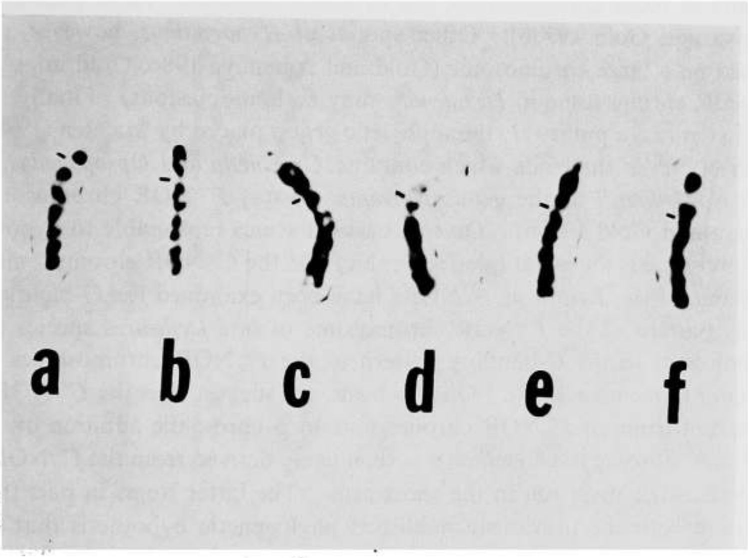

Fig. 1. Trypsin G-banded NOR chromosomes of: a) *Notropis amabilis*; b) *Notropis shumardi*; c) *Cyprinella lepida*; d) *Cyprinella lutrensis*; e) *Cyprinella venusta*; and f) *Opsopoeodus emiliae*. Bars indicate positions of centromeres.

Phylogenetic considerations

Mayden (1989, pers. comm.) and Coburn and Cavender (1991) proposed hypotheses of species relationships for the majority of eastern North American cyprinids based primarily on morphological (including osteological) characters. While salient differences in the placement of several species or species-groups occur between the two studies, both are in agreement that the majority of "*Notropis*"-like shiners (which includes the six species examined in this

technological difficulties remain in terms of resolving trypsin-induced G-bands on all chromosomes of a given metaphase, completely G-banded metaphases have been obtained (Fig. 2), indicating that G-bands exist on all chromosomes within cyprinid complements. Taken together, these results suggest that G-bands do occur on chromosomes of fish species with homogeneous DNA base compositions.

Alternatively, we have been unable to resolve serial (G- or R-) bands on cyprinid chromosomes using DNA binding fluorochromes. Both quinacrine and DAPI, fluorochromes which are known to resolve (presumably AT-rich) G-bands in higher vertebrates (Benn and Perle 1986, Bickmore and Sumner 1989), yield fairly uniform fluorescence on cyprinid chromosomes except for quenched regions corresponding to the chromosomal NORs and to a number of centromeres. Both of the latter are presumed to be enriched in GC base pairs, and in the case of the centromeric regions, to be comprised largely of highly repetitive, satellite type DNA sequences (John and Miklos 1979).

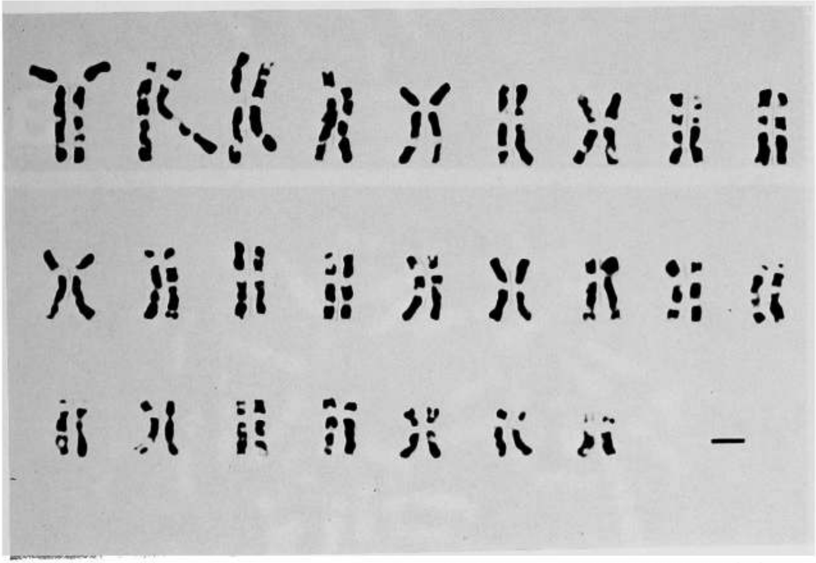

Fig. 2. Trypsin G-banded karyogram from the cyprinid fish *Plagopterus argentissimus*. Specimens were obtained from the Dexter National Fish Hatchery in Dexter, New Mexico. Bar equals 5 μm.

The finding that cyprinid chromosomes possess trypsin-induced G-bands which cannot be differentiated with quinacrine or DAPI in part supports the hypothesis of Holmquist *et al.* (1982) that the organization of eukaryotic chromosomes into compartments preceded the evolution of AT- and GC-rich G- and R-band regions. Their hypothesis related to temporal clusters of units of DNA replication (replicons) which presumably represented the "ancient basic pattern" which then evolved into the differentially AT- and GC-base pair rich G- and R-bands. Since both replication bands (using BrdU substitution) and trypsin-induced G-bands are present on cyprinid chromosomes (Gold *et al.* 1990, this paper), the apparent absence of fluorochrome-resolved serial bands may suggest that the evolution of at least trypsin-induced G-bands also preceded the evolution of differential AT-/GC-richness. This suggestion should be considered tentative, however, given the frequent difficulty in producing quinacrine- or DAPI-resolved G-bands on the chromosomes of certain mammals known to have heterogeneous DNA base compositions (J. W. Bickham, pers. comm.).

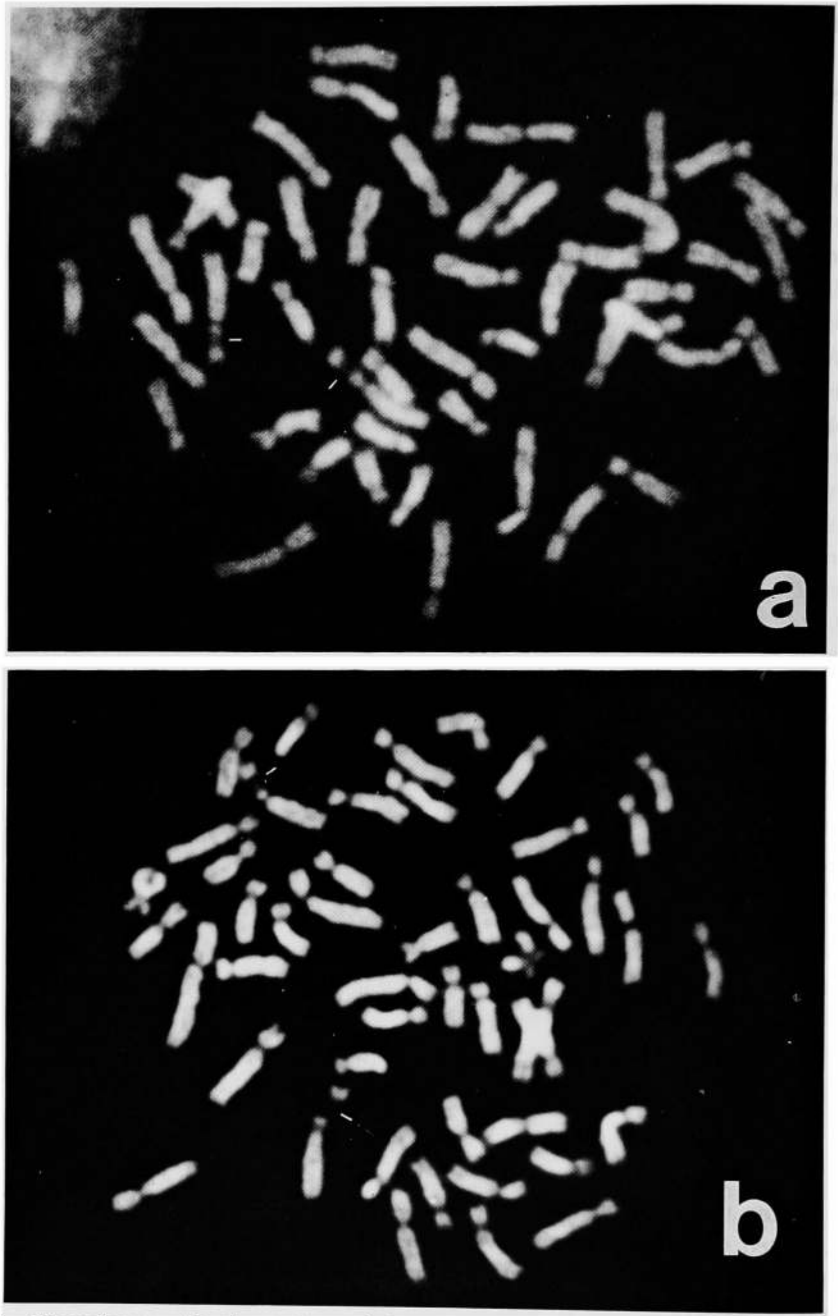

Fig. 3. Fluorochrome-stained metaphases of *Opsopoeodus emiliae*: a) quinacrine dihydrochloride; and b) DAPI. Bars indicate chromosomal NORs.

Chromosomal Polymorphism

In several of our early studies (Gold *et al.* 1978, 1979), the apparent conservatism of gross chromosomal evolution (viz., differences in chromosome and chromosome arm numbers) in North American cyprinids was considered noteworthy in view of the relatively rapid rate of organismal speciation in the group and the correlations found in other organisms between chromosomal evolution and organismal evolution. We noted, however, that the possibility of cryptic chromosomal rearrangement (undetectable in gross or standard karyotypes) represented a major caveat. NOR-banding of cyprinid complements has provided evidence of several chromosomal differences between species (Amemiya and Gold 1990a, Amemiya *et al.* 1991), and, in a few instances, evidence of chromosomal polymorphism within species (Gold and Zoch 1990). In Figure 4, a G-banded metaphase from an individual of *C. lutrensis* clearly shows two chromosomal polymorphisms. One of these, involving a large chromosome pair, appears to be an extensive chromatic addition; whereas the other, involving a small chromosome pair, appears to be a pericentric inversion. Further study of cyprinids using G-banding may well document other previously cryptic chromosomal rearrangements both within and among species and force a reconsideration of the hypothesis that cyprinids are chromosomally conservative.

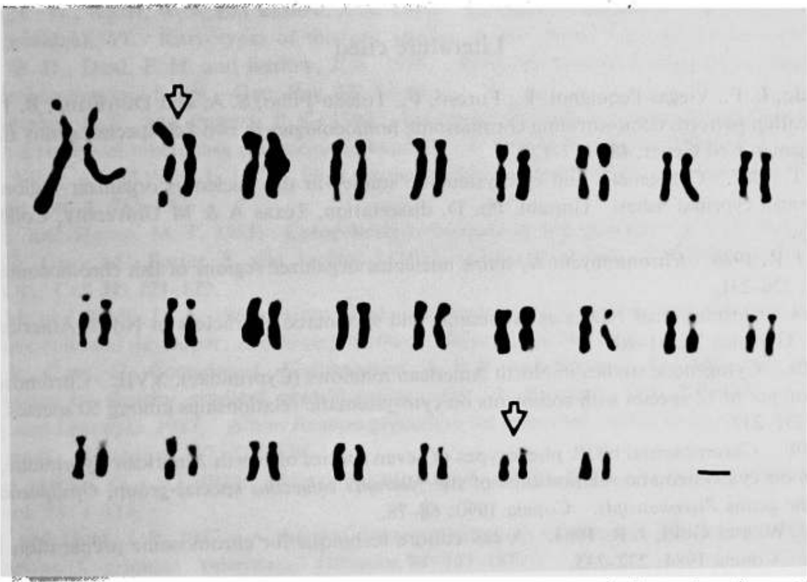

Fig. 4. Trypsin G-banded karyogram from an individual of *Cyprinella lutrensis*. Arrows indicate putative heteromorphic chromosomes. The second largest pair of chromosomes appears heteromorphic in the length of the short arm. The second smallest pair of chromosomes appears heteromorphic for a pericentric inversion.

Summary

Trypsin G-banding of chromosomes from North American cyprinid fishes was used to address phylogenetic problems within the group and to demonstrate the occurrence of G-bands which are not differentially rich in AT DNA base pairs. G-band homology of the long arm of the *F′*, *C′*, and *E′* NOR chromosomes found among six North American cyprinid species, in concert with a hypothesis of species relationships based on morphology, suggests that a NOR situated terminally on the largest chromosome in the complement may represent the plesiomorphic NOR character state within the large "*Notropis*"-like shiner assemblage. Outgroup comparison suggests that this chromosome may also represent a synapomorphy for the same lineage. Evidence exists which suggests that the *F′* NOR is ancestral and that the *C′* and *E′* NORs are derived. The occurrence in cyprinids of trypsin-induced G-bands which are not differentially rich in AT-/GC-DNA base pairs may indicate that the evolution of trypsin-induced G-bands preceded the evolution of differential AT-/GC-richness. The use of trypsin G-banding in cyprinids is expected to permit the identification of previously cryptic chro-