

INTRASPECIFIC VARIATION IN CHROMOSOMAL NUCLEOLUS ORGANIZER REGIONS IN *NOTROPIS CHRYSOCEPHALUS* (PISCES: CYPRINIDAE)

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Over the past few years, we have been studying the chromosomal locations of the nucleolus organizer regions (NORs) in North American cyprinid fishes and using the data to infer hypotheses of species or species-groups relationships (Gold and Amemiya, 1986; Amemiya and Gold, 1988; Gold et al., 1988). Thus far, we have surveyed chromosomal NOR phenotypes of nearly 50 cyprinid species (Amemiya and Gold, 1988; Gold et al., 1988; J. R. Gold, pers. obser.), the majority of which belong to the extremely speciose genus *Notropis*. The NORs themselves are the chromosomal sites for the 18S and 28S ribosomal RNA genes (Ritossa and Spiegelman, 1965; Wallace and Birnstiel, 1966) and hence allow the identification of unique, individual chromosomes within cyprinid complements. Our studies have generally shown that species-specific chromosomal NOR phenotypes occur in North American cyprinids and the interspecific NOR differences are taxonomically and systematically informative. Examples of this and how chromosomal NOR data are used to infer hypotheses of phylogenetic relationships may be found in Amemiya and Gold (1988).

The use of chromosomal NORs (or any character) as a means to infer phylogenetic relationships depends in large part on the stability of the character(s) and the degree to which they vary within a taxon. In the case of chromosomal NORs, the differences between cyprinid species typically involve the chromosomal position of a NOR (e.g., terminal versus interstitial), the absolute number of chromosomal NORs per genome (generally between one and three pairs of NOR-bearing chromosomes, although most cyprinids have only a single pair), and the type of chromosome on which a NOR is located (e.g., metacentric versus submetacentric as well as considerations of the relative size of NOR-bearing chromosomes in the complement). Intraspecific variations in cyprinid chromosomal NORs have been observed in roughly 10% of all specimens examined regardless of species but are of a qualitatively different

nature than the NOR variants found among species. The intraspecific variants include either differences in NOR size between chromosomally homologous NOR sites or differences in apparent transcriptional activity of a given NOR site. More complete details regarding these intraspecific variants may be found in Gold (1984) and Gold and Amemiya (1986). In no instance has either of these two types of intraspecific NOR variants been found to differentiate cyprinid species.

We have found six instances from over 1,000 examined specimens where the types of NOR variants generally found among cyprinid species have apparently occurred within a species. These include a presumed inversion involving the NOR in *Pimephales vigilax*, a significant increase in the length of the long arm of one of the NOR-bearing chromosomes in *Nocomis asper* and *Nocomis leptocephalus*, a decrease in the length of the short arm and a presumed inversion of one of the NOR-bearing chromosomes in an unidentified specimen of *Hybognathus*, a presumed translocation of the NOR in *Notropis lutrensis*, and an addition of a NOR chromosome in *Notropis potteri* (Gold, 1984; Amemiya, 1987; J. D. Jenkin and J. R. Gold, pers. obser.). All of these 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. With the exception of *N. asper* (where two aberrant individuals were found), the variants were detected in single individuals from populations where all other individuals examined had the chromosomal NOR phenotypes characteristic of each species. The fact that the types of NOR differences generally found between species do occur within species is important since interspecific variants would be expected to arise intraspecifically. The fact that they occur so rarely (i.e., seven individuals total from more than 1,000 individuals examined to date) indicates the stability of interspecies differences in NOR as phylogenetic characters.

In this note, we report an intraspecific chro-

mosomal NOR polymorphism in the striped shiner, *Notropis chrysocephalus*, which is of the type generally found between species and which appears to be fixed in a population from the Blue River in Oklahoma. Although a number of interpretations are possible, we favor the hypothesis that the polymorphism became fixed via a founder-type or bottleneck event.

Specimens of *N. chrysocephalus* were collected by seine from five localities (including the Blue River) and include representatives of the two nominal subspecies of *N. chrysocephalus*. The subspecies (collection localities) were as follows: *N. c. isolepis* (Dugdemona River, La Salle Parish, Louisiana; Black Creek, Lamar Co., Mississippi; and Cornhouse Creek, Randolph Co., Alabama); and *N. c. chrysocephalus* (Spring River, Sharp Co., Arkansas). The population in the Blue River (Johnston Co., Oklahoma) is considered to be *N. c. isolepis* (Gilbert, 1964; Lee et al., 1980). All specimens karyotyped will ultimately be deposited in the Texas Cooperative Wildlife Collection (TCWC) at Texas A&M University.

Chromosome preparations were made either directly from kidney tissue of colchicine-injected specimens (following Gold, 1984) or from fibroblast cultures (following Amemiya et al., 1984). NOR-banding was accomplished by silver-staining using the procedure of Howell and Black (1980) as modified by Gold and Ellison (1983). Photomicrographs of silver-stained metaphases were taken on Kodak Technical Pan 2415 film (ASA 25-40) developed in Diafine (Acufine).

Summary data of the NOR-banded material are given in Table 1; representative metaphases are shown in Fig. 1. All individuals examined possessed $2n = 50$ chromosomes as previously reported for the species (Gold et al., 1980). All individuals sampled except those from the Blue River possessed two pairs of NOR-bearing chromosomes (Table 1); one pair exhibited a *C* NOR phenotype, whereas the other exhibited a *D* NOR phenotype. All individuals from the Blue River had only a single pair of NOR-bearing chromosomes of the *C* NOR phenotype.

There are at least two interpretations of these data. The first is that the single pair of NOR-bearing chromosomes in the Blue River population could represent a fixed intraspecific polymorphism within *N. chrysocephalus*. This would suggest that the "normal" NOR condition for *N. chrysocephalus* is to have two pairs of NOR-bearing chromosomes, which seems reasonable given

TABLE 1—Summary of NOR-banded material examined for *Notropis chrysocephalus*.

Sample	No. of specimens examined	No. of meta-phases examined	No. of pairs of NOR chromosomes	NOR chromosome phenotypes ¹
<i>N. c. isolepis</i>				
Blue River	13	82	1	C
Dugdemona River	10	115	2	C, D
Black Creek	1	17	2	C, D
Cornhouse Creek	3	18	2	C, D
<i>N. c. chrysocephalus</i>				
Spring River	7	137	2	C, D

¹ NOR chromosome phenotypes: C, terminal on the short arm of a large-sized submetacentric; D, terminal on the short arm of a medium-sized submetacentric. Justifications for assignment of NOR chromosome phenotypes are in Gold and Amemiya (1986).

that *N. c. chrysocephalus* and *N. c. isolepis* sampled from other localities possess both *C* and *D* NOR chromosomes. Moreover, several North American cyprinids possess more than one pair of NOR-bearing chromosomes (Amemiya, 1987; Amemiya and Gold, 1988), including other members of the *Notropis cornutus* species-group (P. K. Zoch and J. R. Gold, pers. obser.) to which *N. chrysocephalus* presumably belongs (Buth, 1979). Accordingly, the situation in the Blue River could then be explained either as a founder event where a few *N. chrysocephalus* individuals possessing only a *C* NOR chromosome pair originally colonized the Blue River or as a bottleneck event where only a few *N. chrysocephalus* individuals (possessing only a *C* NOR chromosome pair) survived a population-size reduction and then gave rise to the present-day population. Both alternatives are predicated on founder-type events or genetic drift (Mettler and Gregg, 1969). A third alternative might be directional selection against individuals carrying *D* NOR chromosomes, although this seems highly unlikely given that all other members of the *N. cornutus* species-group possess two pairs of NOR chromosomes and that it would be difficult to envision how a decrease in the number of ribosomal RNA genes would be selectively beneficial. From a phylogenetic perspective, the first interpretation would appear to suggest that two

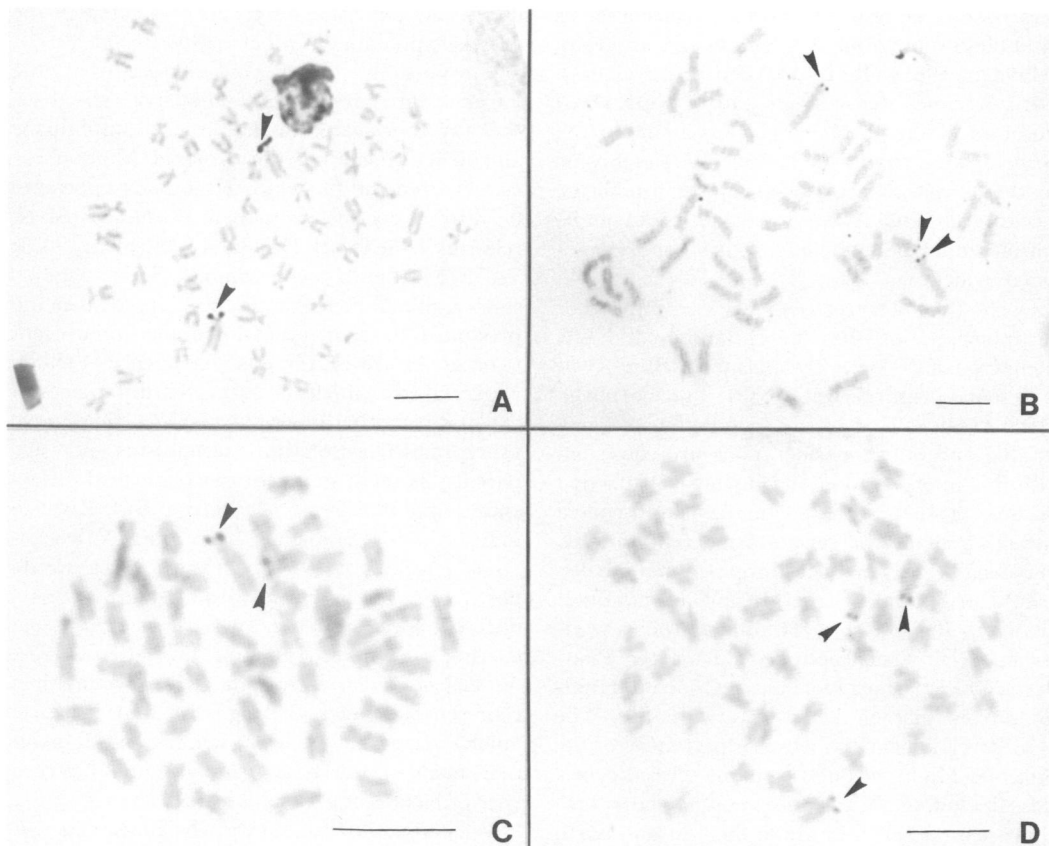


FIG. 1—Silver-stained metaphases of (A) *Notropis chrysocephalus isolepis* from Blue River, Oklahoma, (B) *N. c. isolepis* from Dugdeмона River, Louisiana, (C) *N. c. isolepis* from Black Creek, Mississippi, and (D) *N. c. chrysocephalus* from Spring River, Arkansas. Chromosomal NORs are indicated by arrowheads. Individuals in B, C, and D have activity heteromorphisms where only one of a homologous pair of NOR-bearing chromosomes are identified by silver-staining (cf. Gold and Amemiya, 1986). Bar scale equals 5 μ m.

pairs of NOR chromosomes (of the C and D phenotypes) are the plesiomorphic NOR condition for the *N. cornutus* species-group and that the loss of the D NOR pair in Blue River *N. chrysocephalus* represents an apomorphic condition. The loss of the D NOR pair would then represent an autapomorphy and the Blue River *N. chrysocephalus* would merit consideration as a separate species.

The second interpretation is that a single NOR chromosome pair of the C phenotype as found in Blue River *N. chrysocephalus* represents a plesiomorphic NOR condition. The two *N. chrysocephalus* subspecies presumably belong to the *N. cornutus* species-group (of the *Notropis* subgenus *Luxilus*) which also includes *N. cornutus* and *Notropis albeolus* (Buth, 1979). Both *N. cornutus* and

N. albeolus possess C and D NOR chromosomes which by relative size and centromere position criteria appear to be homologous to the C and D NOR chromosomes found in both subspecies of *N. chrysocephalus* (P. K. Zoch and J. R. Gold, pers. obser.). Gold and Amemiya (1986) and Amemiya and Gold (1988), however, have hypothesized that a single pair of NOR chromosomes is the plesiomorphic NOR condition for most of North American Cyprinidae including the genus *Notropis*. Moreover, a single pair of NOR chromosomes also occurs in all surveyed species of the *Notropis* subgenus *Cyprinella*, the genus *Pimephales*, *Notropis emiliae*, and the *Notropis zonatus* species-group of the subgenus *Luxilus* (Amemiya, 1987; Amemiya and Gold, 1988; Gold et al., 1988; P. K. Zoch and J. R. Gold,

pers. obser.), all of which would be considered as valid outgroups to the *N. cornutus* species-group (Mayden, 1985a; R. L. Mayden, pers. comm.). If it is assumed that a single pair of NOR chromosomes of the *C* phenotype (as found in *N. chrysocephalus* from Blue River) is plesiomorphic for the *N. cornutus* species-group, then both the species-group and *N. chrysocephalus* could not be considered as monophyletic, at least on chromosomal evidence.

Of the two interpretations, we currently favor the interpretation that the chromosomal NOR phenotype of *N. chrysocephalus* from Blue River represents an intraspecific NOR polymorphism which became fixed in that population. Biogeographic and other considerations are consistent with this interpretation and further suggest that fixation of the NOR polymorphism occurred through a chance or genetic drift event. Briefly, Mayden (1985b, 1987) has proposed that the disjunct distributions of several cyprinid and other species in the Ouachita Highlands reflect a vicariant event which occurred during the Pleistocene and separated biota of the Ouachita Highlands from that of the Ozark Highlands. The Blue River is near the western periphery of the Ouachita Highlands and contains several cyprinids (including *N. chrysocephalus*) whose presumed closest relatives are to the east and north in the Ozark Highlands. Such a vicariant event could have isolated *N. chrysocephalus* in the Blue River and permitted fixation of a NOR polymorphism through a small population, chance event. Evidence in further support of an isolation hypothesis is that unlike most other cyprinid fauna of the Ouachita Highlands, *N. chrysocephalus* appears, at least at present, to be restricted in the area to the Blue River and Little River drainages (W. J. Matthews, pers. comm.).

The second interpretation will require further study of *N. chrysocephalus* from Blue River using more traditional morphological and genetic characters. This interpretation, however, seems unlikely in that, even if *N. chrysocephalus* from Blue River represent a distinct species, it almost certainly would be expected to be most closely related phylogenetically to *N. chrysocephalus* from elsewhere than the latter would be to other members of the *N. cornutus* species-group. Moreover, the second interpretation might appear to suggest that *N. chrysocephalus* (and perhaps the *N. cornutus* species-group) originated in the Ouachita Highlands, whereas most of the zoogeographic evi-

dence suggests that the group originated in the Mississippi Valley (Gilbert, 1964).

The possibility that *N. chrysocephalus* from Blue River could represent a distinct species is theoretically conceivable in that several amphibians and fishes (including the cyprinid *Notropis rubellus*) from the Ouachita Highlands appear to be distinct species from their presumed closest relatives in the Ozark Highlands (Mayden, 1985b; W. J. Matthews, pers. comm.). This suggests, simply, that sufficient time has elapsed since the presumed vicariant event for a speciation event to occur. However, there is no evidence of which we are aware which indicates that differences in the number of chromosomal NORs could serve as reproductive-isolating mechanisms, nor apparently is there sufficient morphological differentiation in *N. chrysocephalus* from Blue River to indicate that it is distinct from other *N. chrysocephalus* (Gilbert, 1964). On these bases, we do not view *N. chrysocephalus* from Blue River as a different species relative to *N. chrysocephalus* occurring elsewhere. Assuming the latter is true, *N. chrysocephalus* from Blue River would then represent to our knowledge the first instance, among vertebrates at least, where a NOR polymorphism of the type usually found between species has become fixed within a species.

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CLUTCH SIZE AND PARASITISM OF THE TEXAS SPOTTED WHIPTAIL,
CNEMIDOPHORUS GULARIS (SAURIA: TEIIDAE),
FROM SOUTH-CENTRAL TEXAS

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The Texas spotted whiptail, *Cnemidophorus gularis*, is a teiid lizard found in the prairies, grasslands, and rocky hillsides of Texas, southern Oklahoma, eastern New Mexico, and northern Mexico. It occupies a variety of habitats ranging from relatively undisturbed to completely altered (Walker et al., 1986). Although common throughout its well-documented distribution (Webb, 1975; Raun and Gehlbach, 1976; Dixon, 1987), relatively few reports have documented

any aspect of its natural history. Food habits have been reported previously by Scudday and Dixon (1973) and Best and Polechla (1983). Walker et al. (1987) provided an anecdotal account of mating behavior between two individuals from southern Texas. Few reports of reproduction and clutch size (Ballinger and Schrank, 1972; Trauth, 1987) and only one account of parasitic infestation (Whitworth and Wangberg, 1985) appears in the literature. Here, we present additional infor-