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CHROMOSOMAL NOR KARYOTYPES AND GENOME SIZES IN *DIONDA* (OSTEICHTHYES: CYPRINIDAE) FROM TEXAS AND NEW MEXICO

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ABSTRACT—Chromosome numbers, chromosomal nucleolar organizer region (NOR) phenotypes, and genome sizes (DNA contents) were documented for all nominal members of the North American cyprinid genus *Dionda* inhabiting Texas and New Mexico. All examined taxa, including four members of the *Dionda episcopa* complex (*D. episcopa, D. argentosa, D. serena,* and an undescribed species inhabiting the Colorado and Guadalupe river drainages in Texas) and *D. diaboli,* possessed 2n = 50 chromosomes, with a single pair of NOR-bearing chromosomes. In each, the chromosomal NOR was located terminally on the short arm of a small-sized acrocentric chromosome. All of the NOR chromosomes appeared to be homologous in trypsin G-banding pattern. Mean genome sizes ranged from 1.94 picograms (pg) of DNA in *D. serena* from the Nueces River to 2.09 pg of DNA in *D. argentosa* from Baker's Crossing in the Devils River. Divergence in genome size among nearly all of the samples of *Dionda* was less than that maximally observed, on average, between individuals within populations of most North American fishes. Collectively, the chromosomal NOR and genome size data suggest that the taxonomic status of *Dionda* inhabiting Texas and New Mexico may warrant further investigation.

Recently, Mayden et al. (1992) used a phylogenetic analysis of allozyme allele-frequency data to assess evolutionary relationships among samples of *Dionda* from the southwestern United States and Mexico. Mayden et al. (1992) proposed that there were five species of *Dionda* inhabiting the southwestern United States. These included *D. episcopa* from the Pecos River and its tributaries in Texas and New Mexico and four species from drainages in Texas: *D. argentosa* and *D. diaboli* from the Devils River, *D. serena* from the Nueces, Frio, and Sabinal rivers, and an undescribed species (*D.* sp. cf. *episcopa*) from the Colorado and Guadalupe river systems.

In this paper, we document the chromosome numbers, chromosomal nucleolar organizer region (NOR) phenotypes, and genome sizes (DNA contents) of several samples of *Dionda* from the southwestern United States. Previous studies in our laboratory have documented extensive differences in chromosomal NOR phenotypes among North American cyprinid fishes, many of which are useful both in identifying (or distinguishing) individual species and in the inference of phylogenetic relationships (Amemiya and Gold, 1988, 1990*a*, 1990*b*; Powers and Gold, 1992; Amemiya et al., 1992). Genome sizes also have proven useful in identifying individual species in that relatively large and significant differences in genome sizes have often been found between cyprinid species thought to be closely related (Gold et al., 1990*a*; Gold et al., 1992).

MATERIALS AND METHODS—Specimens were collected by seine from natural populations. The taxa and collection localities (catalogue numbers for voucher specimens deposited in the Texas Cooperative Wildlife Collection, TCWC, at Texas A&M University) were as follows: *D. episcopa,* Bitter Creek near Bitter Lake National Wildlife Refuge, Pecos River drainage, Chaves Co., New Mexico (TCWC-6882.01); Cottonwood Creek on private land, Pecos River drainage, Eddy Co., New Mexico (TCWC-6883.01); *D. argentosa,* Devils River at Baker's Crossing, Val Verde Co., Texas (TCWC-6879.01); Finegan Springs at the Devils River State Natural Area, Val Verde Co., Texas (TCWC-6877.01); *D. diaboli,* San Felipe Creek at Del Rio, Val Verde Co., Texas (TCWC-6878.02); *D. serena,* Nueces River at Barksdale, Real Co., Texas

TABLE 1—Summary of silver-stained material examined (includes individuals from the Pecos River, *Dionda episcopa,* and the Nueces River, *Dionda serena,* examined by Amemiya, 1987). All specimens had one pair of NOR chromosomes of the B phenotype (NOR situated on the short arm of a small acrocentric chromosome).

Taxon	Number of specimens examined	Number of metaphases examined
Dionda episcopa	12	133
Dionda argentosa	6	78
Dionda serena	9	141
Dionda sp.[1]	6	72
Dionda diaboli	9	194

[1] Undescribed species of *Dionda* from the Colorado and Guadalupe river drainages in Texas.

(TCWC-6880.01); Frio River near Concan, Uvalde Co., Texas (TCWC-6881.01); and *Dionda* sp., Bailey Creek near Junction, Colorado River drainage, Kimble Co., Texas (no vouchers); headsprings of the South Concho River near Cristoval, Colorado River drainage, Tom Green Co., Texas (TCWC-6898.01).

Specimens used in chromosomal analyses were taken live to the laboratory and maintained in well-aerated aquaria until sacrificed. Whole blood for genome-size determinations was taken either in the field or from live specimens taken to the laboratory. Cryopreservation of whole blood samples follow procedures outlined in Gold et al. (1991).

Metaphase chromosomes were prepared either directly from solid tissues or from cultured fibroblasts using procedures outlined in Gold et al. (1990*b*). Silver-staining (AgNOR-banding) followed the one-step method of Howell and Black (1980). G-banding (using trypsin) of NOR chromosomes followed methods described by Gold et al. (1990*b*) and Gold and Li (1991). Bright-field photomicroscopy followed Gold and Amemiya (1986).

Genome size determinations of individual fish were made from erythrocyte nuclei as described in Gold et al. (1991). Erythrocytes from both chicken and common carp were used as internal standards. Homogeneity of sample-mean DNA values was tested using one-way analysis of variance, and mean separation was accomplished using Duncan's multiple range test. All statistical analyses were conducted with SAS (SAS Institute, Inc., 1985) programs.

RESULTS—Summary data of the NOR-stained material are presented in Table 1 and include data from Amemiya (1987). All specimens possessed 2n = 50 chromosomes, as do most North American cyprinids (Gold et al., 1980; Amemiya et al., 1992). The chromosome numbers of *D. diaboli* and of *Dionda* from the Devils, Frio, and Colorado river drainages are reported here for the first time.

Metaphases from the five species of *Dionda* are shown in Fig. 1. All five possessed a single pair of NOR-bearing chromosomes, with the NOR situated terminally on the short arm of a small acrocentric chromosome (NOR phenotype B, following the terminology of Gold and Amemiya, 1986, and Amemiya and Gold, 1988). Trypsin G-banding patterns (Fig. 2) indicated that the B NOR chromosomes in all five taxa are homologous.

Mean genome size values ranged from 1.94 picograms (pg) of DNA in *D. serena* from the Nueces River to 2.09 pg in *D. argentosa* from Baker's Crossing in the Devils River (Table 2). Previously, Gold et al. (1990*a*), using scanning microdensitometry, reported a mean DNA value of 2.13 pg from a sample of five individuals of *Dionda* taken from Baker's Crossing in 1984. The genome-size values of these samples of *Dionda* are the smallest yet found among the nearly 70 species of North American Cyprinidae thus far examined for genome size (Gold et al., 1990*a*; Gold et al., 1992; J. R. Gold, pers. obser.).

Heterogeneity testing of mean genome sizes (Table 2) revealed three homogeneous groups of samples: 1) *D. episcopa* and *D. argentosa* (mean sample DNA values ranging from 2.05 to 2.09 pg of DNA); 2) *Dionda* sp., *D. serena* from the Frio River, and *D. diaboli* (mean sample DNA values ranging from 1.96 to 2.01 pg of DNA); 3) *D. serena* from the Nueces River (mean DNA value of 1.94 pg). The percent difference in genome size between groups was 3.24 (group 1 versus group 2), 6.7 (group 1 versus group 3), and 3.35 (group 2 versus group 3). Gold et al. (1990*a*) estimated that the average maximum percent difference in genome size between individuals within populations of North American cyprinid species was 4.86 ± 0.31%. Thus, excepting the comparison of *D. serena* from the Nueces River (group 3) versus group 1 (*D. episcopa* and *D. argentosa*), genome-size divergence among the significantly different test groups is less than that maximally observed, on average, between individuals within populations of cyprinid species.

DISCUSSION—Both the chromosome and genome-size data revealed little evidence of differ-

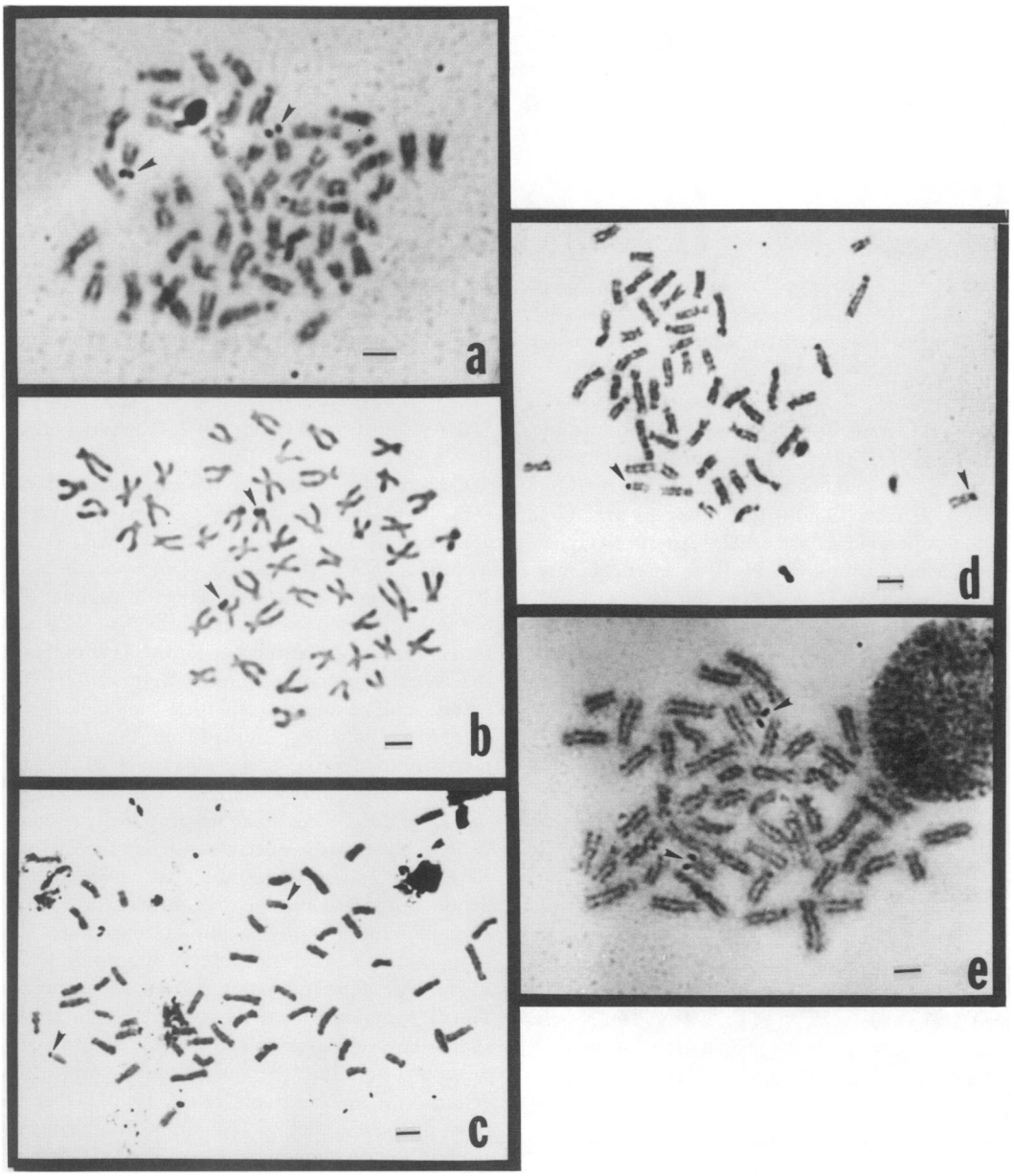

FIG. 1—Silver-stained metaphases of *Dionda argentosa* (a), *Dionda episcopa* (b), *Dionda serena* (c), *Dionda* sp. cf. *episcopa* (d), and *Dionda diaboli* (e). Chromosomal NORs are indicated by arrowheads. Bars are the equivalent of 5 μm.

entiation among the five species of *Dionda* (sensu Mayden et al., 1992) from the southwestern United States. These results are unusual compared with previous studies of closely related North American cyprinids. For example, four different NOR phenotypes occur among six species examined from the genus *Lythrurus,* two different NOR phenotypes occur among the four extant species of the genus *Pimephales,* five different NOR phenotypes occur among six species

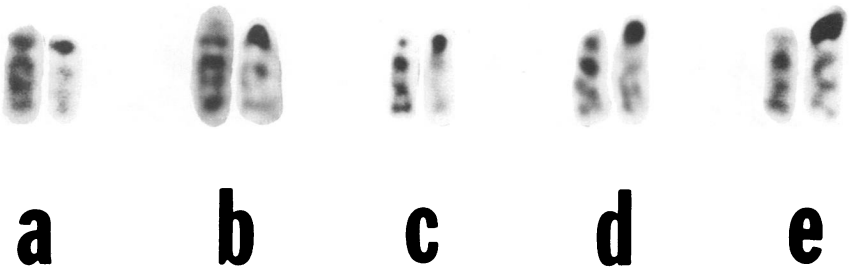

FIG. 2—Trypsin G-banded NOR chromosomes from *Dionda argentosa* (a), *Dionda episcopa* (b), *Dionda serena* (c), *Dionda* sp. cf. *episcopa* (d), and *Dionda diaboli* (e). For each pair, the chromosome on the left is from a G-banded metaphase; the chromosome on the right is from sequential silver-staining (to identify NOR chromosomes) from the same metaphase.

examined from the subgenus *Notropis,* and four different NOR phenotypes occur among the nine species of the genus *Luxilus* (Amemiya and Gold, 1990*b*; Li and Gold, 1991; Powers and Gold, 1992; Amemiya et al., 1992). Alternatively, the absence of chromosomal NOR differences among these samples of *Dionda* does not necessarily falsify the hypothesis of Mayden et al. (1992), because there are instances where apparently valid cyprinid species possess homologous chromosomal NOR character states (Amemiya and Gold, 1990*b*; Powers and Gold, 1992; Amemiya et al., 1992).

TABLE 2—Summary genome-size data (in picograms of DNA per nucleus). Sample size equals 5 for all samples except for *Dionda diaboli* where $n = 10$.

Sample	Mean[1] ± SE	Range
Dionda episcopa		
Bitter Creek	2.08 ± 0.02[a,b]	2.01–2.14
Cottonwood Creek	2.06 ± 0.01[a,b,c]	2.04–2.08
Dionda argentosa		
Baker's Crossing	2.09 ± 0.01[a]	2.05–2.11
Finegan Springs	2.05 ± 0.01[b,c]	2.03–2.08
Dionda sp.[2]		
South Concho River	2.01 ± 0.01[d]	1.99–2.04
Bailey Creek	2.01 ± 0.01[d]	1.99–2.02
Dionda serena		
Frio River	2.01 ± 0.01[d]	2.00–2.01
Nueces River	1.94 ± 0.01[e]	1.91–1.99
Dionda diaboli		
San Felipe Creek	1.99 ± 0.01[d]	1.96–2.03

[1] Mean DNA values of samples with the same letter (Duncan's test grouping) are not significantly different at $\alpha = 0.05$.

[2] Undescribed species of *Dionda* from the Colorado and Guadalupe river drainages in Texas.

Genome sizes of the samples examined fell into three homogeneous groups: 1) *D. episcopa* and *D. argentosa*; 2) *D. serena* from the Frio River, *D. diaboli,* and *Dionda* sp.; 3) *D. serena* from the Nueces River. The distinctiveness in genome size of the sample of *D. serena* from the Nueces River is of interest since Hubbs and Brown (1956) found that *Dionda* from the Nueces River differed in external morphology from other members of the *D. episcopa* complex sampled from the Colorado, Guadalupe, Devils, and Pecos river drainages. Girard (1856) also apparently noticed some of the same differences since he proposed the name *Dionda texensis* for specimens from the Nueces River. Nonetheless, the observed divergence in genome size among the five species of *Dionda* examined in this study is, with one exception, less than that typically encountered among individuals within geographic populations or samples of North American cyprinid species. On average, individuals within populations of cyprinid species differ maximally in genome size by close to 5% (Gold et al., 1990*a*; Gold et al., 1992); whereas most of the samples of *Dionda* examined in this study differ in genome size by a little >3%. The one exception was the sample of *D. serena* from the Nueces River which differed in genome size by >6% from the samples of *D. episcopa* and *D. argentosa.*

Other than the initial descriptions of Girard (1856), the evidence that five species of *Dionda* occur in the southwestern United States rests on the description of *D. diaboli* by Hubbs and Brown (1956) and on the allozyme data of Mayden et al. (1992). Hubbs and Brown (1956) diagnosed *D. diaboli* on the basis of several morphological

characters, the most prominent of which was heavy pigmentation on the dorsal scale pockets. In their single sample of *D. diaboli*, Mayden et al. (1992) found a fixed, unique allele at the Est-1 locus and a unique allele (occurring at a frequency of 0.20) at the Pgm-A locus. For the remaining species of *Dionda* from Texas and New Mexico, Mayden et al. (1992) did find a few locally fixed allelic diffferences or unique alleles (relative to all *Dionda* examined), but in only one instance was the same diagnostic allele (fixed or otherwise) found among all samples of any of the putative taxa. The exception was an allele at the Est-2 locus in *D. argentosa* which occurred at a frequency of 0.20 in a sample from Baker's Crossing in the Devils River and at a frequency of 0.40 in a sample from San Felipe Creek. In short, the only "diagnostic" alleles in the sense of having been found in all samples of any single species of *Dionda* from the southwestern United States were those at Est-1 and Pgm-A in the single sample of *D. diaboli* and at Est-2 in the two samples of *D. argentosa*. Of these, only the allele at Est-1 in *D. diaboli* was completely diagnostic relative to all *Dionda* examined.

Based on the data obtained to date, we believe that the taxonomic status of *Dionda* in the various river drainages of the southwestern United States merits further investigation. A strong case can be made for the continued recognition of *D. diaboli* as a species. Both *D. diaboli* and one other member of the *D. episcopa* complex (*D. argentosa*) coexist sympatrically in the Devils River, and are well-differentiated morphologically (Hubbs and Brown, 1956), allozymically (Mayden et al., 1992), and in genome size. This strongly suggests that the two are isolated reproductively from one another, since unrestricted gene flow between them would be expected to homogenize both allele frequencies and genome sizes. In addition, the two species exhibit some degree of ecological segregation in the Devils River (Hubbs and Garrett, 1990; Garrett et al., 1992). Studies employing restriction site variation in mitochondrial DNA relative to further examining genetic differentiation among the various "forms" of *Dionda* inhabiting Texas and New Mexico are now in progress in our laboratory.

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