Cytogenetics of bisexual/unisexual species of *Poecilia*. IV. Sex chromosomes, sex chromatin composition and Ag-NOR polymorphisms in *Poecilia latipinna*: a population from Mexico

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Cytogenetic analysis using C-banding, silver staining and fluorescent staining was carried out on a population sample of *Poecilia latipinna* derived from Tampico, Mexico, to verify the presence of sex chromosomes in individuals from the southern areas of this species range and to investigate the extent of C-band and Ag-NOR polymorphisms. Females were found to have W heteromorphic chromosomes, with large amounts of heterochromatin-rich in AT nucleotide sequences. C-banding corresponded to the pattern proposed as typical for the genus. Specimens share one of the Ag-NOR locations previously described in populations from the U.S.A. and show additional ones as well.

Keywords: fish, NOR polymorphism, *Poecilia latipinna*, Poeciliidae, sex chromatin, sex chromosomes.

Introduction

Previous observations on three populations of *Poecilia latipinna* from northern areas of the species range (Texas and Florida, U.S.A.) have shown the existence of sex chromosomes of the ZW (♀♀)/ZZ (♂♂) type (Sola *et al.*, 1990). Sex determining mechanisms in the genus *Poecilia* are variable. For example, both male and female heterogametic species, with different degrees of sex chromosome differentiation, coexist with species having no obvious sex chromosomes (for review see Sola *et al.*, 1992a,b); moreover, in other poeciliid species polymorphic sex-determining mechanisms exist (Kallman, 1984). These facts led us to analyse a fourth population sample of *P. latipinna* from Tampico, Mexico, a region that is near the southern limit of this species range (Miller, 1983). We wanted to verify the presence of sex chromosomes in specimens from widely disparate regions. Analysis of a Tampico population of *P. latipinna* is also of interest because in this area these fish are sympatric with the

unisexual species, *P. formosa*, and the sexual species, *P. mexicana*. Together, these three species constitute a breeding complex in which triploid unisexuals are produced (Balsano *et al.*, 1989). Note that in the latter species chromosomes similar to the W chromosome of *P. latipinna* have not been found (Sola *et al.*, 1992a, b).

In the three U.S.A. populations of *P. latipinna* previously analysed (Sola *et al.*, 1990), the W chromosome is characterized by a large amount of heterochromatin. Our aim in the present manuscript is to report on our analysis of sex chromatin using AT- and GC-specific fluorochromes. In addition, chromosomes from the three U.S.A. populations show polymorphic C-bands and a high level of Ag-NOR variability. We wanted to see if this variability persists in other populations of *P. latipinna* as well.

Materials and methods

The specimens analysed were laboratory-reared in Rome but were derivatives from specimens collected in the Laguna de la Puerta, Altamira, near Tampico, Mexico, in June 1988. Somatic metaphases were prepared from spleen, gill and cephalic kidney cells (Hitotsumachi *et al.*, 1969). NORs were silver (Ag) stained by

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the method of Howell & Black (1980) and C-banding was done according to Sumner (1972). Fluorescent staining with DAPI, quinacrine and chromomycin A₃ (CMA₃) was carried out as described in Sola *et al.* (1992b) on slides made from the Tampico specimens and on slides made from the three U.S.A. populations (Sola *et al.*, 1990).

Results

Cytogenetic observations from five specimens of *P. latipinna* collected near Tampico are summarized in Table 1.

Giemsa-stained metaphases from female specimens show a slightly heteromorphic chromosome, that for its size can be considered a homologue of pair number 2 (Fig. 1a). After C-banding, metaphases from male (Fig. 1b) or female (Fig. 1c) specimens are clearly distinguishable because of the large amount of heterochromatin on the W chromosome (Fig. 1c and 2c). In both male and female specimens, one pair of small chromosomes shows telomeric heterochromatin (Fig. 1b and c). In specimens from all populations, hetero-

chromatic regions of the W chromosome appear brightly fluorescent following DAPI-staining both in previously C-banded (Fig. 2a and d) and untreated (Fig. 2b and e) metaphases. The telomeric heterochromatin of the small size chromosomes, on the other end, appears to be bright only in previously C-banded metaphases (Fig. 2a). Quinacrine and DAPI on untreated slides produce a similar pattern: uniform staining of euchromatin and heterochromatin in the chromosome complement with the exception of the terminal region of the W chromosome (Fig. 2b and e). An AT-rich nature of the W chromosome heterochromatin is also suggested by CMA₃-staining because these regions show less fluorescence than the remainder of the chromosome (Fig. 2f).

Individual specimens show one to two, one to four and one to five Ag-NORs per cell (Fig. 3a). Similar numbers of nucleoli were observed on Ag-stained nuclei (Table 1). Five nucleoli were not observed in animal UR121, the only specimen with five Ag-NORs, possibly because of the highest number of NOR associations shown by this specimen, 22 out of the total 38 NOR associations observed. NORs are located on

Table 1 Summary of cytogenetic observations

Animal	Sex	Metaphases examined						Total number of Ag-NOR chromosomes	Ag-NORs location			Ag-stained nuclei	Ag-stained nucleoli			
		Total	C-bands	DAPI	Q	CMA ₃	Ag		A	B	C		1	2	3	4
UR61	F	147	27	30	—	12	35	51	51	0	0	69	45	21	3	0
UR85	F	152	13	20	10	32	42	147	79	0	68	26	3	17	5	1
UR116	F	212	33	34	25	34	107	360	199	0	161	85	43	31	5	6
UR121	F	96	33	16	—	24	45	168	85	73	10	72	10	35	12	15
UR145	M	63	21	21	—	18	30	57	0	29	28	77	32	45	0	0
Total		670	127	121	35	120	259	822	414	102	267	405	170	183	30	22

Q = quinacrine.

A = Short arms of chromosomes 4; B = Short arms of chromosomes 14; C = Short arms of chromosomes 20.

Fig. 1 (a) Partial Giemsa-stained karyotypes of *P. latipinna* females showing chromosome pair number 1 and the heteromorphic pair 2; (b-c) C-banded metaphase from a male (b) and female (c) specimen. The arrowhead indicates the W chromosome. Arrows indicate chromosomes with telomeric heterochromatin.

Fig. 2 (a–b) DAPI staining of previously C-banded (a) or untreated (b) metaphases from female specimens. Arrowheads indicate the W chromosome. Arrows in (a) indicate chromosomes with telomeric heterochromatin, DAPI-negative in the untreated metaphase (b). (c–f) Selected examples of the W chromosome after C-banding (c), after sequential C-banding and DAPI staining (d), after quinacrine staining (e) and after CMA₃ staining (f). In (c) (d) and (f) the sequential staining of the same W chromosome is shown for all rows except the first one.

Fig. 3 (a) Distribution of Ag-stained NOR-bearing chromosomes per specimen; (b–c) Ag-stained metaphase plates. Arrows indicate Ag-NORs.

the short arms of several chromosomes (Fig. 3b and c), which for their size were numbered as chromosomes 4, 14 and 20 (Fig. 4a). The Ag-NOR chromosomal loci found in all populations of *P. latipinna* surveyed to date, are represented as small circles on the idiograms shown in Fig. 4. Chromosomal loci that were found to have an Ag-positive NOR in at least one cell from every specimen belonging to a population are indicated as solid circles, while those loci showing Ag-positive NORs in cells from only some specimens of a population are indicated as open circles. To minimize the arbitrariness of classification, chromosomes of similar size showing NORs on the same location were considered as homologues.

Discussion

Observations presented here on the Tampico population of *P. latipinna*, coupled with our previous observa-

tions in this species (Sola *et al.*, 1990), indicate that the ZW/ZZ chromosomal mechanism of sex determination is common within individuals sampled from a broad geographical range. W chromosome differentiation in this species has been found to be related to the accumulation of AT-rich repetitive DNA. A similar accumulation of AT-rich DNA has been described for the W chromosome of *P. sphenops* var. *melanistica* (Haaf & Schmid, 1984). Thus species in the genus *Poecilia*, which underwent sex chromosome differentiation toward female heterogamety, seem to share a similar history with respect to cytological differentiation, or a common ancestral species. Contrary to this latter hypothesis, *P. mexicana*, considered part of the *P. sphenops* species complex (Miller, 1983), does not show morphologically differentiated sex chromosomes (Sola *et al.*, 1992b). Moreover, it remains peculiar that congeneric species do not share the same type of sex chromosome heterogamety. For example, males of *P.*

Fig. 4 Idiograms to detect Ag-staining patterns in all populations of *P. latipinna* surveyed to date. (a) Tampico population; (b) Gator Creek, Florida population; (c) Brownsville, Texas population; (d) San Marcos, Texas population. Solid circles represent shared Ag-NOR locations, open circles represent variable Ag-NOR locations. See text for further explanation. Refer to Sola *et al.* (1990) for details of the Florida and Texas populations.

reticulata appear to show XY heterogamety (Nanda *et al.* 1990, 1992).

Specimens of *P. latipinna* sampled from Tampico show one pair of small chromosomes with telomeric heterochromatin. Thus their C-banding pattern is similar to that shown by Florida specimens (Sola *et al.*, 1990), by melanistic *P. sphenops* (Haaf & Schmid, 1984) and by *P. m. mexicana* (Sola *et al.*, 1992b). This pattern has been proposed to be typical for the genus (Sola *et al.*, 1990). However, the telomeric bands of this chromosome pair in *P. latipinna* do not seem to be AT-rich as reported for *P. sphenops* (Haaf & Schmid, 1984). We find these bands to be fluorescent only after DAPI staining of C-banded metaphases, and not after DAPI or quinacrine staining of untreated slides. The former condition non-specifically enhances contrast between euchromatin and heterochromatin (Sola *et al.*, 1992b).

Numbers of NORs per cell in the Tampico specimens of *P. latipinna* were comparable to those observed in the population sampled from Florida. However, NORs on the short arms of chromosome 1, common to the Florida population, or telomeric

NORs, common to the Texas populations, were not observed in the specimens derived from the Tampico population. Additional NOR locations were found on short arms of chromosome pairs number 4 and 20, even if both homologues of the latter pair were never simultaneously Ag-stained. The data on Tampico specimens suggest that intraspecific polymorphism of Ag-NORs is more widespread than previously suspected (Sola *et al.*, 1990). Studies with CMA₃-staining, which should yield information on structural NORs, are presently underway. However, data discussed above already indicate that while some NOR loci appear to be differentially distributed among widely disparate populations, variable distributions of NORs among specimens from the same population are also possible.

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