Brief Reports

High incidence of NOR associations in metaphases of *Lateolabrax latus* (Perciforms, Pisces)

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Previously we found nucleolus organizer regions (NORs) stained by a silver method in two species of serranid fishes, Lateolabrax japonicus and Lateolabrax latus (Tatewaki and Kitada, 1994). L. japonicus had 2n=48 with all acrocentrics, while L. latus had 2n=48and comprised 46 acrocentric and 2 subtelocentric chromosomes (Kitada et al., 1981). Silver-stained NORs (Ag-NORs) located at secondary constrictions in long arms of one median-sized acrocentric pair in L. japonicus, and at the satellite or stalk regions of the short arms of the subtelocentric chromosomes in L. latus (Tatewaki and Kitada, 1994). These findings demonstrated different location and size of Ag-NORs between these two species and apparent heteromorphism of Ag-NORs in L. japonicus. During the course of cytogenetic studies of these two species, we found association of chromosomes with Ag NORs and its different frequency of the association between the two species. We therefore made a quantitative assessment of the chromosome association between the two species.

Fifteen or more specimens of each sex of male and female in *L. japonicus* and one of each sex of *L. latus* were used in this study. Cultivation was initiated from their muscle tissues in Eagle's MEM with 10% fetal calf serum. Subcultivation was also carried out when cells were grown enough. Air dried chromosome preparations were made from serially passaged subcultures (from primary cultures to 13th passages) by our routine method. The method used by Howell and Black (1980) was employed for Ag-NORs.

As shown in Fig. 1, we found association of chromosomes with NORs in both metaphases of *L. japonicus* and *L. latus*. This association was confirmed by silver staining (Fig. 1B), and proposed as NOR associations in the present report. The frequency of NOR associations in these two species was estimated from cultured cells, as shown in Table 1. In *L. latus* the incidence of the NOR association ranged from 19.1% to 34.4% (average : 26.8%) in female and 19.1% to 27.3% (average : 24.7%) in male metaphases, respectively. Although variation in the association frequency was found in each sampling, no remarkable sex difference was observed (average in both sexes:25.9%). In *L. japonicus* the incidence of the NOR association was 3.1% in average lower than that found in *L. latus* and no sex difference in female (average : 3.2%) and male (average : 3.0%), respectively (Table 1). These results apparently indicated a significant difference in the NOR associations between *L. japonicus* and *L. latus* (0.5>X²).

Differences in the location and the size of Ag-NORs were also reported in some other fish species (Takai, 1985; Ueno and Ojima, 1991), as shown in the present species. Further noticeable is that Ag-NORs located at the secondary constriction appeared to be more heteromorphic in intra- and interspecies than Ag-NORs located at satellite or stalk region (Tatewaki and Kitada, 1994). However, NOR association was higher in chromosomes with Ag-NORs in the satellite regions like in L. latus. The high frequency of NOR-associations found in the present study may be reflected in the localization of NORs in satellite regions rather than variation of silver staining intensity, since satellite or nucleolar association was commonly found in human acrocentric chromosomes with satellites, irrespective of NOR variability in each acrocentric. As shown in Table 1. no remarkable age difference was found in the NOR association in cultured cells, indicating that serial passages in vitro were little or no interference for NOR associations.

It seemed that the nucleolar association of human acrocentric chromosomes provides the cytogenetic explanation for Robertsonian translocations and is more

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76

NOR association in serranid fishes



Figure 1. Partial metaphases of *L. japonicus* (A) and *L. latus* (B). A: Giemsa-stained, B: Silver-stained. Each arrowhead indicates NOR association.

species	Sex	Passage	Nos of mitosis cells	Nos of NOR associations	Percentage of NOR associations	Average		
						Female & male	Tota	.1
L. latus	female	2nd	166	51	30.7	26.8	25.9 –	
		3rd	150	29	19.3			
		4th	230	52	22.6			
		10th	183	63	34.4			
	male	primary	150	41	27.3	24.7		#
		4th	152	42	27.6			
		8th	89	17	19.1			
L. japonicus	female	primary	155	5	3.2	3.2	3.1	
		18th	157	5	3.1			
	male	2nd	110	4	3.7	3.0		
		13th	264	6	2.3			

Table 1. Percentage of NOR associations in cultured cells of L. latus and Q. japonicus

0.001 > p

prone to nondisjunction. The high frequency in the NOR-association between acrocentrics as found in the present findings may be characteristics probably acquired prior to Robertsonian fusion. Although we have not yet found any Robertsonian rearrangements

between Ag-NOR-bearing chromosomes in the present species, even in their cultured cells. Although more detailed analyses using such as banding and molecular cytogenetic methods are required for chromosome rearrangements in these species, the present findings could be attributed to its unique characteristics in their karyotype evolution.

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