NUCLEOLAR ACTIVITY AND DISTRIBUTION OF RIBOSOMAL GENES IN *PHYLLOTIS* RODENT SPECIES AND THEIR LABORATORY HYBRIDS

ACTIVIDAD NUCLEOLAR Y DISTRIBUCIÓN DE GENES RIBOSOMALES EN ESPECIES DE ROEDORES DEL GÉNERO *PHYLLOTIS* Y EN SUS HÍBRIDOS DE LABORATORIO

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This work is dedicated to the memory of the late Professor Dr. Oliver Pearson, who was a leader in the areas of zoology and evolutionary biology. Dr. Pearson made significant contributions to the knowledge of South American mammals. He died in March 2003.

ABSTRACT

The expression of nucleolar organizer regions (NORs), distinguished through silver staining procedures, and the distribution of ribosomal genes, detected by fluorescent *in situ* hybridization with a ribosomal DNA probe, were studied in 3 *Phyllotis* rodent species and their laboratory generated hybrids. In the hybrids between the more genetically distant species, the NORs of one parental genome were preferentially expressed. This result differs from the codominant rDNA expression previously detected in hybrids between the 2 more genetically similar of the 3 species, and suggests that nucleolar dominance is related to the compatibility of parental genomes. The mean area of each fluorescent signal was significantly larger in *P. magister*, which has 3 nucleolar chromosome pairs, than in *P. darwini* and *P. xanthopygus*, which have 4 nucleolar chromosome pairs each. Nevertheless, the total area of rDNA signals per cell was similar in the karyotypes of all 3 species, strongly suggesting that rDNA genes have been redistributed in these genomes rather than gained or lost during evolutionary divergence.

Key words: nucleolar dominance, interspecific hybrids, NOR activity, FISH signal areas

RESUMEN

La expresión de las regiones organizadoras del nucléolo (NORes), revelada mediante procedimientos de tinción con plata, y la localización cromosómica de los genes ribosomales, detectada por hibridación *in situ* con una sonda para ADNr, se estudió en 3 especies de roedores del género *Phyllotis* y en sus híbridos de laboratorio. Los híbridos entre las 2 especies genéticamente más distantes expresaron preferentemente los NORes de uno de los genomas parentales. Este resultado difiere de la expresión codominante del ADNr previamente encontrada en los híbridos derivados del par de especies genéticamente más cercanas y sugiere que la dominancia nucleolar está relacionada con la compatibilidad de los genomas parentales. El área promedio de cada señal fluorescente fue significativamente mayor en *P. magister*, con 3 pares de cromosomas nucleolares, que en *P. darwini* y *P. xanthopygus*, cuyos cariotipos tienen 4 pares de cromosomas nucleolares cada uno. Sin embargo, el área total de señales de ADNr por célula fue similar en los cariotipos de las 3 especies, sugiriendo fuertemente que durante la divergencia evolutiva de estas formas, los genes ribosomales se redistribuyeron entre los genomas, no produciéndose ni ganancias ni pérdidas significativas de los mismos.

Palabras claves: dominancia nucleolar, híbridos interespecíficos, actividad NOR, áreas de señal FISH

INTRODUCTION

The chromosomal distribution and position of nucleolar organizer regions (NORs) generally are detected by silver staining procedures (Goodpasture and Bloom, 1975; Sánchez-Rufas et al., 1982). Positive Ag reaction of chromosomal NORs (AgNORs) is believed to reflect the transcriptional activity of ribosomal genes during the preceding interphase of the cell cycle (Howell, 1977; Hubbel, 1985; Jiménez et al., 1988), while *in situ* hybridization with rDNA probes makes it possible to detect all the chromosomal regions containing ribosomal genes in a karyotype. Thus, the use of both procedures is required to distinguish between active and inactive rRNA gene clusters in a particular genome (Shubert and Künzel, 1990; Pendás et al., 1993; Mellink et al., 1994).

Interchromosomal, intercellular, and inter-individual variability in the number and size of AgNOR bands and *in situ* hybridization signals have been reported in most species that have been analyzed (Sánchez et al., 1989; Zurita et al., 1997, 1998, 1999). It is generally accepted that, while variability among AgNOR bands mainly depends on specific metabolic cell demands (Sánchez et al., 1989; Berríos et al., 1992), differences among *in situ* hybridization signals reflect variations in the number of ribosomal genes present in each particular NOR (Wachtler et al., 1986; Suzuki et al., 1990; Leitch and Heslop-Harrison, 1992; Mellink et al., 1994). Moreover, in some mammal species a direct relationship has been established between the number of ribosomal genes in a particular NOR and its level of expression (Zurita et al., 1998, 1999).

Interspecific hybrids have often shown the existence of nucleolar dominance, that is, the preferential expression of ribosomal genes from one of the 2 parental species (Honjo and Reeder, 1973). First described in plants (Navashin, 1928, 1934), nucleolar dominance is now known in insects, amphibians, and mammals (Reeder, 1985; Pikaard

and Chen, 1998). The mechanisms that, in the cells of the hybrids, discriminate between parental sets of rRNA genes and initially establish nucleolar dominance remain obscure. Nevertheless, the subsequent enforcement of dominance through successive mitoses is an epigenetic phenomenon, under the influence of both DNA methylation and histone deacetylation (Chen and Pikaard, 1997).

We have previously obtained laboratory hybrids between 3 species of the Andean rodent genus *Phyllotis* (Rodentia, Muridae): *P. darwini*, *P. xanthopygus*, and *P. magister* (Walker et al., 1984, 1999). Each of these species has small and multiple AgNORs, located in the telomeric regions of 3 or 4 chromosomal pairs (Walker et al., 1998, 1999; Spotorno et al., 2001). We have also previously shown that in the cells of the hybrids between the sister species *P. darwini* and *P. magister*, both parental NOR sets are expressed with similar frequencies (Walker et al., 1999).

In the present paper we will demonstrate that, to the contrary, nucleolar dominance occurs in the cells of the hybrids between the phylogenetically more distant species *P. darwini* and *P. xanthopygus*. This and previous results suggest that in phyllotine hybrids, nucleolar dominance or codominance would be established at a genomic level, according to the distance and compatibility between the progenitor genomes. The use of a procedure to directly measure every fluorescent rDNA *in situ* hybridization signal area, has allowed us to assess the amount and evolution of ribosomal genes in the genomes of these 3 species.

MATERIALS AND METHODS

Specimens. Specimens of the 3 *Phyllotis* species were trapped alive in Chilean territory or were the direct laboratory descendants of wild-caught individuals. Skulls and skins of all the animals studied were prepared as voucher specimens and were deposited in the collection of the Laboratorio de Citogenética, Facultad de Medicina, Universidad de Chile (LCM).

Taxa, original localities, number and sex of the animals examined (with collection numbers between brackets) were as follows: *P. darwini* (4 males and 2 females): IV Región: Las Tacas, 1 female (LCM 667); Aucó, 1 male (LCM 755); Talinay, 1 male (LCM 1008); Pichidangui, 1 male (LCM 1850) and 1 female (LCM 1848); Fray Jorge, 1 male (LCM 1955). *P. magister*: II Región: Ojo Opache, mouth of the Loa River, 4 males (LCM 1693, 1728, 1795, 2034) and 3 females (LCM 1691, 1741, 2054). *P. xanthopygus* (2 males and 3 females): II Región: San Pedro de Atacama, 1 female (LCM 996); IV Región: El Indio, Río Elqui, 1 male (LCM 1696); Región Metropolitana: Baños Morales, 1 female (LCM 587); Farellones, 1 male (LCM 1798) and 1 female (LCM 1913).

Interspecific hybrids were obtained from laboratory crosses between the parental species. The crosses, that always included the reciprocal ones, the parental species (male x female), the number and sex of the examined hybrids (with collection numbers between brackets) were: *darwini* x *xanthopygus*, 2 males (LCM 624, 937) and 2 females (LCM 764, 998); *xanthopygus* x *darwini*, 1 male (LCM 758); *darwini* x *magister*, 1 female (LCM 1964); *magister* x *darwini*, 1 male (LCM 1740).

Cytogenetic Analyses.   Chromosome preparations were made from bone marrow cells using the conventional *in vivo* colchicine, hypotonic method, preceded by yeast injection to improve the mitotic index (Lee and Elder, 1980). Active NORs were

detected by the silver staining procedure described by Sánchez-Rufas et al. (1982). To unambiguously identify the chromosomes carrying NORs, we used a sequential AgNOR G-banding technique (NOR-G banding), developed in our laboratory. The number and chromosomal distribution of active NORs were then recorded in the cells of each parental species and compared with those found in the cells of the hybrids.

Fluorescent *in situ* hybridization (FISH) was performed using a chicken rDNA probe formed by the terminal 3′ sequences of the 18S gene. The probe was labeled with biotin by nick translation, using the Bionick Labelling System (Gibco BRL) and according to the instructions of the manufacturer. FISH and detection of the hybridization signals were performed following the protocols previously described by Iturra et al. (1998, 2001). Metaphases were counterstained with propidium iodide-antifade solution (Oncor). Chromosome signals were detected using a Nikon fluorescence microscope equipped with the appropriate filter and photographed with Kodak Ektachrome 400 color film. Images from FISH were then scanned and the probe signal areas were measured in pixels, using a commercial software package (IPLab, v2.420, Scanalytics). Chromosomes having fluorescent signals in each karyotype were identified according to their previously described sizes and morphologies (Walker et al., 1979, 1984). The mean values obtained for the total FISH signal areas in each of the 3 karyotypes were compared using the Student *t*-test for independent variables.

RESULTS

Phyllotis darwini showed 4 chromosomal pairs carrying active NORs (Nos. 3, 7, 9, 12; Fig. 1A) and *P. magister* had a maximum of 3 chromosomal pairs bearing NORs (Nos. 3, 9, 12). These observations on the number and chromosomal distribution of telomeric AgNOR bands confirmed those previously described for both species (Walker et al., 1999). In the cells of *P. magister* x *P. darwini* hybrids, both parental NOR sets exhibited similar frequencies of activity, as it was previously reported (Walker et al., 1999), indicating that ribosomal genes of the 2 species were codominantly expressed.

Also according to previous descriptions (Spotorno et al., 2001), *P. xanthopygus* showed telomeric NORs located in 4 chromosomal pairs (Nos. 3, 6, 12, 16; Fig. 1B). The abundant pericentromeric heterochromatin present in all the *P. xanthopygus* chromosomes (Walker et al., 1984, 1991) was also stained with silver nitrate, producing Ag^+ centromeric bands (Fig. 1B); these bands were absent in *P. darwini* chromosomes (Fig. 1A), where there are smaller quantities of heterochromatin (Walker et al., 1984, 1991).

In the NOR-banded metaphases of *P. darwini* x *P. xanthopygus* hybrids, a minimum of 2 and a maximum of 6 chromosomes carrying active NORs were detected. As expected, the Ag-banded karyotype of the hybrids displayed 2 distinct kinds of chromosomes: half of them had pericentromeric Ag^+ bands, as in *P. xanthopygus*, whereas in the other half, such Ag^+ bands were lacking, as in *P. darwini* (Fig. 1C–E), making it possible to distinguish the parental origin of NOR chromosomes. One of the 2 metaphases of the same hybrid female appearing in Fig. 1 showed 4 active NORs, half of them being located in *P. darwini* chromosomes (Fig. 1C, E), and the other, had 5 active NORs, 3 of which were located in *P. darwini* chromosomes (Fig. 1D).

NOR expression variability, previously registered in *P. darwini* and *P. magister* (Walker et al., 1999), was also detected in *P. xanthopygus*. The average number and
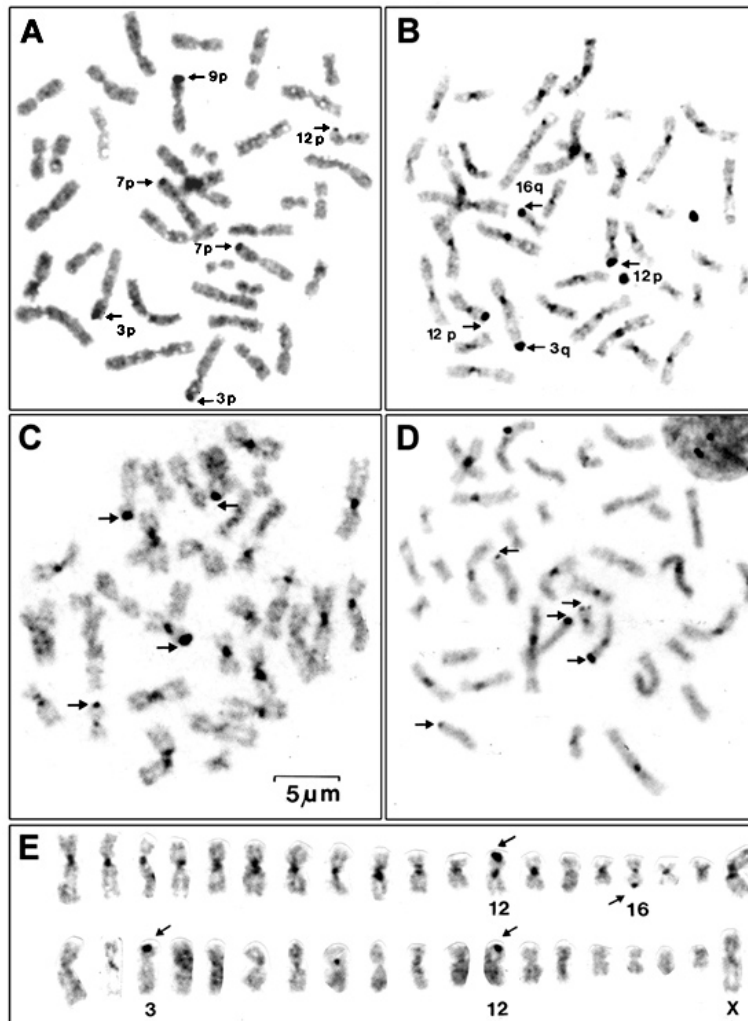
Figure 1. Multiple telomeric AgNORs (arrows) in NOR-G banded metaphases of *Phyllotis darwini*, *P. xanthopygus*, and their hybrids. (A) *P. darwini* male. (B) *P. xanthopygus* female (note also the Ag+ pericentromeric bands in all the chromosomes; see text). The NORs of chromosome 6 were not Ag positively stained in this metaphase. (C and D) *P. darwini* x *P. xanthopygus* hybrid female. (E) Same as (C), but parental chromosomal sets were ordered by size and NOR-G bands, and separated according to the presence or absence of centromeric Ag+ bands (upper row, *P. xanthopygus* origin; lower row, *P. darwini* origin).

standard deviation of active NORs per cell recorded in *P. darwini*, *P. magister* and *P. xanthopygus* were 3.58 ± 0.77 (N = 81), 3.02 ± 0.77 (N = 57), and 3.46 ± 1.58 (N = 51), respectively.

Three distinct patterns of NOR activity, each with characteristic but different

Table 1. Number of cells having both (+/+), only one (+/-) or no (-/-) active NORs in each of the nucleolar chromosome pairs of *P. darwini* (A) and *P. xanthopygus* (B).

(A) P. darwini

Specimens LCM number* (sex)	Number of cells	chr 3			chr 7			chr 9			chr 12		
		+/+	+/-	-/-	+/+	+/-	-/-	+/+	+/-	-/-	+/+	+/-	-/-
1850 (m)	17	9	8	0	1	16	0	0	5	12	1	11	5
755 (m)	13	3	9	1	2	9	2	1	6	6	1	9	3
667 (f)	12	4	8	0	4	8	0	2	6	4	0	9	3
Total	42	16	**25**	1	7	**33**	2	3	17	**22**	2	**29**	11

(B) P. xanthopygus

Specimens LCM number* (sex)	Number of cells	chr 3			chr 6			chr 12			chr 16		
		+/+	+/-	-/-	+/+	+/-	-/-	+/+	+/-	-/-	+/+	+/-	-/-
1798 (m)	14	1	9	4	0	4	10	0	5	9	1	7	6
587 (f)	14	2	11	1	2	8	3	2	3	9	2	11	1
1913 (f)	17	4	10	3	10	7	0	7	7	3	2	10	5
Total	45	7	**30**	8	13	**19**	13	9	15	**21**	5	**28**	12

*The specimen numbers are those of the mammal laboratory collection (LCM); m = male, f = female. Bold characters indicate the most frequent pattern of NOR activity (+/+, +/- or -/-) detected for each chromosomal pair.

frequencies, were detected for each of the 4 *P. darwini* and *P. xanthopygus* nucleolar chromosomes (Table 1): active NORs were present in both homologous chromosomes (+/+), in only one of them (+/–) or in none of them (–/–). In these 2 species, as in *P. magister* (Walker et al., 1999), the most frequent condition was that in which only one member of each nucleolar chromosome pair was active (+/–, Table 1). The exceptions were one pair of NOR chromosomes in each species karyotype, which showed absence of activity in both homologous members as the most frequent condition (*P. darwini* No. 9 and *P. xanthopygus* No. 12, Table 1), and thus revealing the low expression level of the ribosomal genes located herein.

The analysis of 51 metaphases from 4 *P. darwini* x *P. xanthopygus* hybrids (male *P. darwini* x female *P. xanthopygus*, LCM 764, 937, 998, and male *P. xanthopygus* x female *P. darwini*, LCM 758, Table 2) indicated that the 4 *P. darwini* NOR chromosomes showed, in general, a higher frequency of expression than the *P. xanthopygus* NOR chromosomes (Table 2), independently of the *P. darwini* progenitor sex. The small *P. xanthopyygus* nucleolar chromosome No.16 was the only exception to this rule, since its ribosomal genes were expressed in a relatively high proportion of the examined cells (51%, Table 2). On the other hand, a total of 161 active NORs were detected in the cells of these hybrids, 104 in *P. darwini* chromosomes and only 57 in *P. xanthopygus* ones (Table 2).

Representative results obtained after *in situ* hybridization with the rDNA probe are shown in Fig. 2. Four to 6 telomeric fluorescent signals were recorded in the parental species and hybrid metaphases (Fig. 2A–E). The *P. darwini* x *P. xanthopygus* hybrids showed rDNA-FISH signals on the nucleolar chromosomes of both parental sets. Since constitutive heterochromatin fluoresces strongly with the propidium iodide counterstain and the karyotypes of the parental species differ substantially in the amount of pericentromeric heterochromatin (Walker et al., 1984), we could

Table 2. Number and frequency of cells showing active NORs in each of the parental nucleolar chromosomes of the *P. darwini* x *P. xanthopygus* hybrids.

Hybrid specimens LCM number* (sex)	Number of cells	Nucleolar active chromosomes of the two parental genomes							
		P. darwini				*P. xanthopygus*			
		chr 3	chr 7	chr 9	chr 12	chr 3	chr 6	chr 12	chr 16
764 (f)	8	8	2	1	1	4	1	1	5
937 (m)	13	10	4	5	4	0	2	3	4
998 (f)	17	16	8	8	8	1	7	4	10
758 (m)	13	7	9	9	4	3	3	2	7
Total	51	41	23	23	17	8	13	10	26
%	100.0	80.4	45.1	45.1	33.3	15.7	25.5	19.6	51.0

*The specimen numbers are those of the mammal laboratory collection (LCM); m = male, f = female.
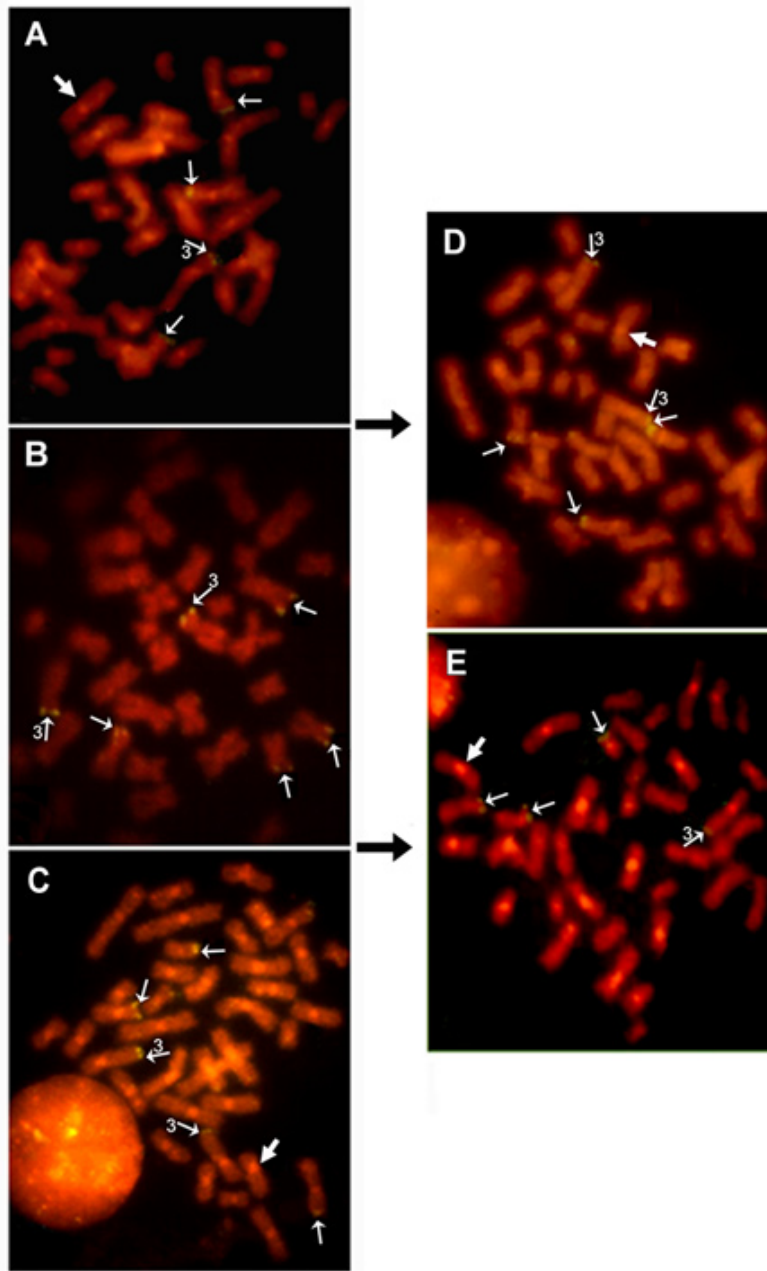
Figure 2. Fluorescence *in situ* hybridization of a18S rDNA biotinilated probe in metaphases of the 3 parental species and the 2 types of hybrids: (A) *P. magister*, (B) *P. darwini*, (C) *P. xanthopygus*, (D) *P. darwini* x *P. magister* hybrid, and (E) *P. darwini* x *P. xanthopygus* hybrid. Thin arrows indicate the location of hybridization signals (A – E) and thick arrows show the pericentromeric heterochromatin, fluorescing brilliantly with the propidium iodide counterstain (A, C, D, E).

distinguish the parental origin of the NOR-bearing chromosomes in the metaphases of the hybrids. Thus, some signals were located in *P. xanthopygus* chromosomes, with propidium iodide fluorescent centromeres, and others in *P. darwini* chromosomes, with non-fluorescent centromeres (Fig. 2E).

Significant differences exist between the mean area of fluorescent signal for *P. magister* (141.67 ± 76.61 px, *N* = 69) and those of the other 2 species, *P. darwini* (119.94 ± 62.56 px, *N* = 189, *t* =2.32, *d.f.* = 256, *p* = 0.021) and *P. xanthopygus* (116.20 ± 52.33 px, *N* = 276, *t* = 3.26, *d.f.* = 343, *p* = 0.001). Nevertheless, the total area of rDNA signal per cell was very similar in the 3 karyotypes: 545.6 ± 96.9 px; 520.1 ± 130.2 px, and 492.4 ± 102.2 px, in *P. darwini, P. magister* and *P. xanthopygus*, respectively. The small variations of these values were not significantly different ($t_{darwini-magister}$ = 0.111, *d.f.* = 17, *p* = 0.913; $t_{magister-xanthopygus}$ = 1.778, *d.f.* = 34, *p* = 0.084), despite the distinct number of nucleolar chromosomes present in each species.

DISCUSSION

The number of structural NORs per karyotype and their chromosomal distribution are thought to be species-specific cellular characters. However, NOR transcriptional activity is quite variable and, for many mammalian species, is often considered an individual characteristic (Mikelsaar et al., 1977; Mayr et al., 1987; Suzuki et al., 1990; Zurita et al., 1997) dependent mainly on specific metabolic cell demands (Sánchez et al., 1989; Berríos et al., 1992).

In the *Phyllotis* species studied here, the ribosomal genes were more dispersed in *P. darwini* and *P. xanthopygus* genomes, with 4 chromosomal pairs carrying NORs (Fig. 1), than in *P. magister* genome, with 3 NOR chromosomal pairs (Walker et al., 1999). Each species showed the expected interchromosomal, intercellular, and inter-individual NOR activity variation (Table 1; Walker et al., 1999), although all 3 species had very similar levels of total NOR activity. Thus, the indices of NOR activity, calculated as the ratio between the number of active and structural NORs per cell (Walker et al., 1999), were nearly equal for *P. darwini, P. magister* and *P. xanthopygus* (0.448, 0.503, and 0.495, respectively).

Because the most frequent NOR activity pattern found in the 3 species was that in which only one member of each nucleolar chromosome pair was active (Table 1; Walker et al., 1999), specific regulation of rDNA transcription, by either suppression or activation, likely occurs. As in other mammal species, rDNA cytosine methylation and histone deacetylation could be operating over the genomic compartment containing the ribosomal genes, thus favoring the expression of only one member of each NOR chromosomal pair in the parental species or only one parental genome in the hybrids (Flavell et al., 1988; Chen and Pikaard, 1997; Houchins et al., 1997; Pikaard, 1999).

Previously, we found that in the hybrids of the sister species *P. darwini* and *P. magister*, the ribosomal genes of both parental species were active and functioned in a codominant form. Moreover, from a reproductive point of view, these hybrids conformed to Haldane's rule, since females were fertile and males were infertile (Walker et al., 1999). Thus, codominant NOR expression, meiotic normality and gametic differentiation of, at least, female hybrids, indicate that *P. darwini* and *P. magister* genomes can function in relative harmony and compatibility when they are placed together in the nuclei of their laboratory generated hybrids.

Contrary to this observation of codominance, there appears to be nucleolar dominance for ribosomal genes in hybrids between *P. darwini* x *P. xanthopygus*, with the parental genome of *P. darwini* expressed far more frequently than that of the *P. xanthopygus* (Table 2). Moreover, if we assume *a priori* that both parental NOR sets have the same probability to be expressed in the cells of hybrids, half of the 161 active NORs (80-81) recorded in the 4 hybrid individuals studied (Table 2), should be located in *P. darwini* chromosomes and the other half in those of *P. xanthopygus*. However, 104 active NORs were found to be in *P. darwini* chromosomes and only 57 were registered in those of *P. xanthopygus* (Table 2), indicating clearly and significantly that the expression of *darwini* nucleolar organizer regions dominate over those of *P. xanthopygus* ones ($\chi^2 = 7.924$, *d.f.* = 3, *p* < 0.05).

Previous results (Walker et al., 1984) showed that both male and female *P. darwini* x *P. xanthopygus* hybrids are completely sterile, with the meiotic process arrested in their gonads. We suspect that sterility is caused by chromosomal differences and incompatibility between the parental species genomes. The nucleolar dominance in rDNA gene expression detected here would be another manifestation of the difficulty for those two genomes to function together in the nuclei of the hybrid individuals.

Taken as a whole, our data suggest that in the *Phyllotis* species analyzed here, the level of nucleolar dominance is positively correlated with the phylogenetic divergence. These results are in accordance with previous cytogenetic data, showing that there are larger differences between *P. darwini* and *P. xanthopygus* karyotypes than between those of *P. darwini* and *P. magister*, which have only small dissimilarities (Walker et al., 1979, 1984, 1991). The results also agree with data obtained from molecular analyses performed in these 3 species, since in the phylogenetic trees based on protein electrophoretic data or cytochrome *b* gene sequences, *P. xanthopygus* is always placed in a clade separate from that which groups *P. darwini* and *P. magister* (Steppan, 1998; Spotorno et al., 2001; Kuch et al., 2002).

Differences in the number and size of the hybridization signals between homologous and among non-homologous chromosomes were detected in the parental species and their hybrids (Fig. 2). Because the *in situ* hybridization procedure directly detects ribosomal DNA, these differences would be evidence of polymorphism for the number of ribosomal genes present at the different NOR sites (Wachtler et al., 1986; Pendás et al., 1993; Mellink et al., 1994; Zurita et al., 1998).

The rDNA fluorescent signal areas per chromosome were significantly different in the 3 species karyotypes, though the total fluorescent areas of the three species genomes were similar (*p* < 0.05). Thus, each relatively large fluorescent NOR site of *P. magister* (141.67 ± 76.61 px), with only 3 NOR chromosomes, apparently contains significantly larger numbers of ribosomal genes than the smaller fluorescent NOR sites of *P. darwini* (119.94 ± 62.56 px) and *P. xanthopygus* (116.20 ± 52.33 px), each with 4 NOR chromosomes. Nevertheless, the total number of ribosomal genes in each species is similar. These findings imply that the differences in the number of ribosomal genes located in the NORs of the 3 species can be explained through genomic redistribution of rDNA genes, rather than either gain or loss during evolution.

Since chromosomal evolution in *Phyllotis* has proceeded mainly through multiple and independent centric fusions from an ancestral telocentric karyotype (Pearson and Patton, 1976; Walker et al., 1979, 1984; Spotorno et al., 2001), the proposed karyotypic redistribution of rDNA genes can be confirmed comparing the G-banding patterns of NOR chromosomal arms in the 3 species metacentric karyotypes. The ribosomal

genes of the sister species *P. magister* and *P. darwini* appeared located in homologous chromosomal arms, those with the same G-banding patterns. On the contrary, none of the 4 NOR chromosomes of the more distantly related species, *P. xanthopygus*, showed G-banding homology with any of the *P. magister* or *P. darwini* NOR chromosomes or chromosomal arms (Spotorno et al., 2001), thus confirming that ribosomal genes have undergone a complete redistribution in these genomes.

According to morphological and cytogenetic analyses, *P. darwini* is the most primitive of these 3 species, and *P. xanthopygus* is the more derived taxon (Simonetti and Spotorno, 1981; Walker et al., 1991). Since 4 NOR chromosomes were detected in *P. darwini* and *P. xanthopygus,* and only 3 in *P. magister,* reduction in NOR number seems to have occurred during the divergence of *P. magister* genome. On the other hand, in the phyllotine genus *Loxodontomys,* which chromosomally evolved mainly by the occurrence of tandem fusions (Walker and Spotorno, 1992), there has also been a reduction from 4 to 3 NOR-bearing chromosomes (Walker and Spotorno, 1992; Spotorno et al., 2001).

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