Chromosoma (2009) 118:291–301
DOI 10.1007/s00412-008-0197-x

RESEARCH ARTICLE

Quantitative analysis of NOR expression in a B chromosome of the grasshopper *Eyprepocnemis plorans*

María Teruel · Josefa Cabrero · Francisco Perfectti · Juan Pedro M. Camacho

Received: 10 October 2008 / Revised: 6 November 2008 / Accepted: 12 November 2008 / Published online: 2 December 2008

Abstract The B_{24} chromosome in the Torrox population of the grasshopper *Eyprepocnemis plorans* is recurrently attached to a nucleolus in diplotene cells, indicating the activity of its distally located ribosomal DNA (rDNA). The frequency of males expressing the B chromosome nucleolus organizer region (B-NOR) almost doubled in 4 years. The likelihood of expressing the B-NOR increased with the B number and, in males expressing it, about 20% of their cells showed a nucleolus attached to the B. When active, the B-NOR contributed more than 25% of total cell nucleolar area (NA). Within males expressing the B-NOR, total cell NA did not differ between cells showing the active or inactive B-NOR, suggesting that total cell NA is tightly regulated in this species. However, this parameter tended to increase in this population from 1999 to 2004, in parallel to the neutralization process which is taking place in this population. Finally, an analysis of A chromosome NOR interdependence for activity revealed a positive correlation among autosomes but a negative correlation between autosomes and the X chromosome, the manifestation of which depends on B-NOR activity. These results are discussed in the context of the nucleolus as a sensor of the stress caused by parasitic B chromosomes.

Introduction

The major class of ribosomal RNA (rRNA) genes (28S, 18S and 5.8S) are located at the chromosomal loci known as nucleolus organizer regions (NORs), which are responsible for nucleolus formation and ribosome synthesis. A large part of the cell's energy is devoted to ribosome production (Warner 1999), a tightly regulated process requiring the coordinated activity of all three eukaryotic RNA polymerases: Pol I for rRNA, Pol II for ribosomal protein genes, and Pol III for 5S rRNA (Rudra and Warner 2004). The transcription of rDNA is catalyzed by RNA polymerase I in a highly controlled process (Sollner-Webb and Tower 1986), with Pol I activity regulation playing a critical role (Laferte et al. 2006). This rDNA transcription by RNA polymerase I initiates nucleolus formation (Karpen et al. 1988; Nogi et al. 1991; Santoro 2005) and ribosome production (Grummt 2003).

The synthesis of rRNA can be regulated by varying the number of active genes and/or transcription rate per gene (Grummt 2003). Only about half of rRNA genes are active at a given moment, suggesting that eukaryotes control the effective dosage of these genes (Lawrence and Pikaard 2004) through chromatin-mediated epigenetic modifications, such as histone acetylation and methylation or DNA methylation (Lewis and Pikaard 2001), although it is not clear what physiological signals dictate the proper rRNA gene dosage per cell (Lawrence and Pikaard 2004). Recent results in *Arabidopsis thaliana* have suggested that NORs, rather than individual rRNA genes, are the units of regulation (Lewis et al. 2004).

In mammals, a chromatin-remodeling complex (NoRC) has been discovered, which controls rDNA transcription and size and number of nucleoli (Li et al. 2005). It works by recruiting DNA-methylating and histone-modifying activities to the rDNA promoter, thus determining a repressive higher-order chromatin structure (Santoro et al. 2002; Zhou et al. 2002). Recently, it has been shown that RNA transcribed from the intergenic spacer (IGS) also

Communicated by I. Grummt.

M. Teruel · J. Cabrero · F. Perfectti · J. P. M. Camacho (✉)
Departamento de Genética, Universidad de Granada,
18071 Granada, Spain
e-mail: jpmcamac@ugr.es

plays a role in rDNA silencing through the NoRC complex (Grummt 2007).

The mechanisms regulating the activation of ribosomal chromatin are less understood than those responsible for repression (Huang et al. 2006). Chromosome context appears to be important for NOR activity, as deduced from changes in the on/off activity status following chromosome rearrangements moving NORs to new locations (for a review, see Pikaard 2000).

The nucleolus is a dynamic cell compartment involved in the control of numerous cellular functions, with an amalgamation of many protein–protein and protein–nucleic acid interactions which are constantly changing (Hiscox 2007). Nucleolus is a sensor of stress caused by a variety of factors as, for instance, nutrient starvation (Langerstedt 1949), environmental contaminants (Hudson and Ciborowski 1996), or viruses (Hiscox 2007). In general, nucleoli are smaller under stressful conditions (see Olson 2004). Likewise, conditions causing stress and harming cellular metabolism (e.g., starvation, toxic lesion, cancer, and viral infection) down-regulate rDNA transcription and impair ribosome and protein synthesis (Grummt 2003). Nutrient starvation leads to significant nucleolar size reduction in both yeast and mammalian cells (Tsang et al. 2003). In yeast, this morphological change is accompanied by the release of RNA Pol I from the nucleolus, deacetylation of the histone H4, and the inhibition of rDNA transcription (Tsang et al. 2003). Therefore, there seems to be a chromatin-mediated mechanism modulating the nucleolar structure, RNA Pol I location, and RNA gene expression in response to nutrient availability (Tsang et al. 2003).

The former results clearly indicate that nucleolus size reflects the activity of rRNA genes, as was first shown by Caspersson (1950). Additional evidence comes from the observation that: (1) nucleolus size correlates with cell-division activity, being larger in stages with high protein demand (Mosgoeller 2004); (2) that nucleolus hypertrophy coincides with an increase in ribosome synthesis (Nakamoto et al. 2001); and (3) that increases in rDNA transcription result in an overall greater volume of the nucleolus relative to the nucleus (Caperta et al. 2007).

Silver staining distinctly labels RNA Pol I transcription machinery, including B23, nucleolin, UBF, and RNA Pol I subunits (Roussel et al. 1992; Roussel and Hernandez-Verdun 1994; Roussel et al. 1996). It has been shown that the degree of silver staining of the nucleoli and the rate of RNA synthesis are strongly correlated (see references in Pebusque and Seite 1981). Recently, Caperta et al. (2007) have shown that rRNA gene transcription, silver staining, and NOR decondensation are interrelated in rye. Therefore, silver staining provides a simple and dependable technique to measure nucleolus size as an indirect measure of rRNA gene transcription.

B chromosomes are supernumerary (accessory) chromosomes found in some individuals of about 15% of eukaryote species, which frequently show drive (i.e., higher transmission rate than standard (A) chromosomes) and decrease the fitness of individuals carrying them, thus behaving as genome parasites (for recent reviews, see Camacho 2004, 2005; Jones et al. 2008). B chromosomes in many species carry rRNA genes (Camacho 2005), in most cases having been detected by silver staining thus being active, but only in the plant *Crepis capillaris* have transcripts derived from the B chromosome rDNA hitherto been detected (Leach et al. 2005).

The grasshopper *Eyprepocnemis plorans* harbors B chromosomes passing through parasitic and neutralized stages as a consequence of their arms race with the host (A) chromosomes (Camacho et al. 1997). The most parasitic B was found in the Torrox (Málaga, Spain) population, where the B_{24} variant was transmitted to 70% of progeny through females, and it significantly decreased egg fertility (Zurita et al. 1998). Another sign of the parasitic nature of B_{24} was a higher increase in cell chiasma frequency than other variants showing signs of neutralization (Camacho et al. 2002).

E. plorans carries rRNA genes in the paracentromeric region of almost all chromosomes, although the different clusters may be classified into large (in chromosomes 9, 10, 11, and X) and small (in most remaining A chromosomes), with the largest rDNA cluster being distally located on B chromosomes (López-León et al. 1994; Cabrero et al. 2003). The large clusters on A chromosomes correspond to the so-called primary NORs, which are the only active NORs detected in Spanish populations, and the small clusters contain secondary NORs which are usually inactive in Spanish populations (López-León et al. 1995b), but are sometimes active in Moroccan populations (Bakkali et al. 2001). The rRNA genes located on the B chromosomes are usually inactive, with the exception of a male from the Salobreña (Granada, Spain) population carrying a B_2 chromosome fused to the longest autosome (Cabrero et al. 1987), and the B_{24} chromosome in Torrox which has recently been observed recurrently expressing its NOR (Teruel et al. 2007).

The frequency of activity of the primary NORs appears to be influenced by B chromosome presence in some populations. For instance, in Salobreña, B-carrying males showed a higher number of active NORs per cell than 0B males (Cabrero et al. 1987). In Jete (Granada, Spain), however, this difference was not observed (López-León et al. 1995b). In two Moroccan populations, the situation was similar to that of Salobreña, with B-carrying males showing a significantly higher number of active NORs per cell (Bakkali et al. 2001).

However, all these analyses referred to the active/inactive status of the different NORs without consideration

of the size of the nucleoli attached to these active NORs. In rye, Morais-Cecilio et al. (2000) reported that nucleolar activity, measured as the size of the silver-stained Ag-NORs in metaphase chromosomes, was significantly lower in B-carrying plants. Recently, we measured nucleolus size at several meiotic stages in the grasshopper *E. plorans* and found that it was lower in Torrox than in Salobreña or Smir populations, which were exposed to a lower B-chromosome load than in Torrox (Teruel et al. 2007). In addition, we observed the recurrent activity of the B chromosome NOR in a Torrox population sample collected at 1999, as indicated by the attachment of nucleolus material to the B chromosomes in diplotene cells (Teruel et al. 2007). In the present paper, we have scored the number of nucleoli and measured nucleolus size in diplotene cells from males collected in 2003 and 2004 from the Torrox population, in order to investigate whether the frequency of B chromosomes expressing their NOR and the size of the produced nucleolus is changing over time and how this B chromosome activity influences A-chromosome NOR activity.

Materials and methods

Males of the grasshopper *E. plorans* were collected at Torrox (Málaga, Spain) in 2003 and 2004. The 2003 sample was representative of B-chromosome frequency in the field since males were collected at random. The 2004 sample, however, was chosen among more than 100 males collected in the field to include about 12 males in each of the 0B, 1B, 2B, and 3B classes, in order to get a balanced design for statistical analyses. Testes were fixed in freshly prepared 3:1 ethanol–acetic acid and stored at 4°C. Squash preparations were silver impregnated following Rufas et al. (1982) for visualizing nucleoli. In addition, a faint Giemsa staining was performed to differentially staining nucleoli (dark brown to black) and chromatin (blue-green). Twenty diplotene cells per male were photographed with a digital camera (Olympus DP70) coupled to an Olympus microscope.

Two nucleolar parameters were analyzed, i.e., the number of nucleoli (NN; attached to each chromosome bivalent or univalent per cell) and nucleolus area (NA). The latter was measured, in arbitrary units (au), with the help of a digital pen tablet and the ImageJ program (Rasband 1997), and then converted to squared microns after calculating magnification scale. As shown previously (Teruel et al. 2007), the measurement error of the nucleolus area by the former method was low (1.53%), and squashing produced only negligible effects on measuring the area of the nucleolus.

In addition, in each diplotene cell, we scored the total number of chiasmata and determined whether B chromosomes were forming bivalents in males with two or more Bs. The variables used for statistical analyses were: the number of chiasmata per cell, the number of nucleoli per cell (NN), nucleolus area per cell (NA), and nucleolus area attached to chromosome 9 (NA_9), chromosome 10 (NA_{10}), chromosome 11 (NA_{11}), X chromosome (NA_X), and B chromosomes (NA_B).

In males where B chromosomes showed NOR activity, it was found consistently in only part of the cells analyzed, and therefore, we calculated the different variables separately for those cells showing and those not showing B-NOR activity . For each male, we calculated the mean for each variable, and the resulting data were used for intraindividual comparisons (between cells with active or inactive B chromosomes, and between cells with B bivalents or B univalents) as well as interindividual comparisons (with respect to collection year or B chromosome number). Statistical analyses were performed by means of contingency χ^2, Student's t test, analysis of variance (ANOVA), multivariate analysis of variance (MANOVA), and analysis of covariance (ANCOVA).

Results

Frequency of males showing NOR activity in B chromosomes across years

Twelve out of 22 B-carrying males (55%) collected in 2003, and 19 out of 36 B-carrying males (53%) analyzed in 2004 (Table 1), showed NOR activity in some of their B chromosomes (Fig. 1). There was no significant difference between these consecutive years (χ^2=0.2, df=1, P=0.90), but the frequency of B-carrying males showing B-NOR activity was greater than the 31% observed in a sample collected 4 years before in this same population (Teruel et al. 2007).

Within B-carrying males showing B-NOR activity, the mean proportion of cells showing B-NOR activity was 0.236 (SE=0.063) in 2003 and 0.195 (SE=0.046) in 2004, the difference lacking significance (Student t=0.53, df=29, P=0.60). These figures were very similar to the 0.213 (SE=0.043) observed in four males collected in 1999 (Teruel et al. 2007). Table 1 also shows that the chance of a specimen bearing a B chromosome having an active NOR grows with B number (about 40% for 1B, 50% for 2B and 66% for 3B), but the proportion of active cells within males showing B-NOR activity was larger in males with 1 and 3 Bs, especially in 2003.

Number of nucleoli per cell (NN)

The NN in diplotene cells (3.54±0.18 in 2003 and 3.57±0.10 in 2004) did not vary between years (t=0.14, df=74, P=0.89)

Table 1 Number of *E. plorans* males analyzed from the Torrox population in 2003 and 2004

Year	Number of Bs	Males with		Total	Percent males with B-NOR activity	Percent cells showing B-NOR activity
		B-NOR inactive	B-NOR active			
2003	0	–	–	6	–	–
	1	6	3	9	33.33	22.54
	2	3	5	8	62.50	10.00
	3	1	2	3	66.67	60.00
	4	0	2	2	100.00	22.50
	Total	10	12	28	42.86	23.55
2004	0	–	–	12	–	–
	1	7	6	13	46.15	14.17
	2	6	5	11	45.45	13.11
	3	4	8	12	66.67	27.50
	Total	17	19	48	39.58	19.50

and was similar to that found in 1999 (3.34±0.10; F=0.57, df=2, 88, P=0.57). In 31 males from 2003 and 2004, where the B-NOR was active, those diplotene cells where the B-NOR was active showed a significantly higher NN (4.08±0.28) than those lacking B-NOR activity (3.51±0.0.12; t=2.29, df=34, P=0.028).

Nucleolar area per cell

To analyze temporal changes in nucleolar area (NA), we also used the 1999 sample analyzed previously (Teruel et al. 2007), in order to get a longer temporal scope. An ANCOVA with the number of B chromosomes (0-3) as a fixed factor, year as continuous variable, and total cell NA as a dependent variable, indicated that all B-carrying types of male showed nonsignificant lower NA values than the 0B males (F=1.09, df=3, 83, P=0.36), but there was a significant increase in NA across years (F=21.24, df=1, 83, P=0.000014; Fig. 2).

Nucleolar area yielded by B chromosomes

The mean nucleolar area attached to B chromosomes (NA_B) was 6.31 μm^2 (SE=0.88) in 12 males collected in 2003, and 7.97 μm^2 (SE=1.08) in 19 males from 2004, but the difference was not significant (t=1.08, df=29, P=0.288). These figures were slightly higher than the 5.63 μm^2 (SE=0.78) found in 1999 (Teruel et al. 2007), suggesting a tendency to increase for NA_B, but the result was not significant (one-way ANOVA: F=0.96, df=2, 32, P=0.39).

The relative contribution of B chromosomes to total NA was 0.275 (SE=0.041) in 2003 and 0.264 (SE=0.041) in 2004, the difference lacking significance (t=0.18, df=29, P=0.86), these figures being remarkably similar to those observed in 1999 by Teruel et al. (2007; 0.283, SE=0.030; F=0.03, df=2, 32, P=0.97). This indicates that, when the B chromosome NOR is active, it contributes more than 25% of total NA in the cell.

B pairing

To investigate whether B-NOR activity was influenced by meiotic pairing between B chromosomes, we scored the frequency of B univalents and bivalents in a total of 379 diplotene cells from individuals with two B chromosomes from both years (Table 2). The frequency of B bivalent formation in 2004 (64.38%) was significantly higher than that in 2003 (43.13%) (χ^2=16.91, df=1, P<0.0001), but the frequency of cells showing NOR activity in the B chromosome (6.25% in 2003 and 5.94% in 2004) did not differ between the 2 years (χ^2=0.02, df=1, P=0.899). No association was found between B-NOR activity, and the formation of B-bivalents either in 2003 (χ^2=2.33, df=1, P=0.127) or in 2004 (χ^2=0.14, df=1, P=0.707).

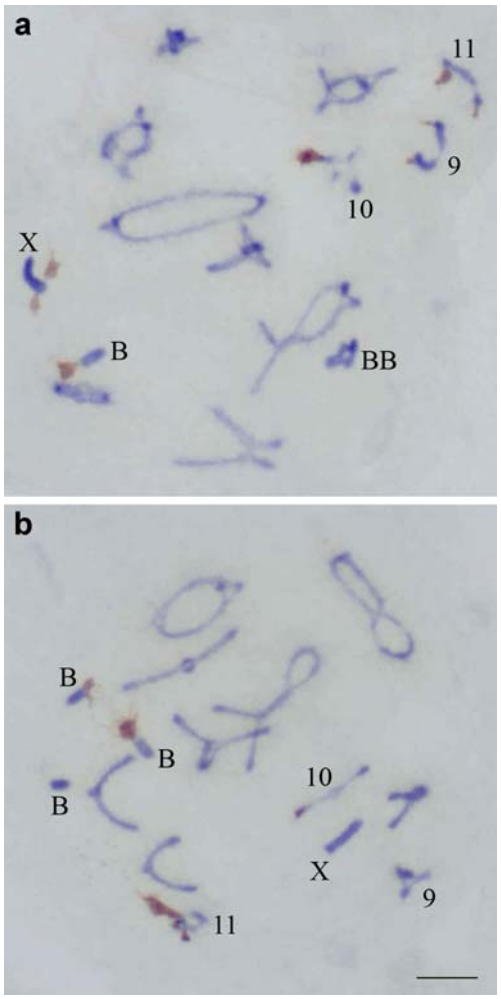

Fig. 1 Diplotene cells submitted to silver impregnation and Giemsa staining, showing chromosome material in *blue* and nucleoli in *brown* color. Note the presence of NOR-activity in one (**a**) or two (**b**) B chromosomes. *Bar*=5 μm

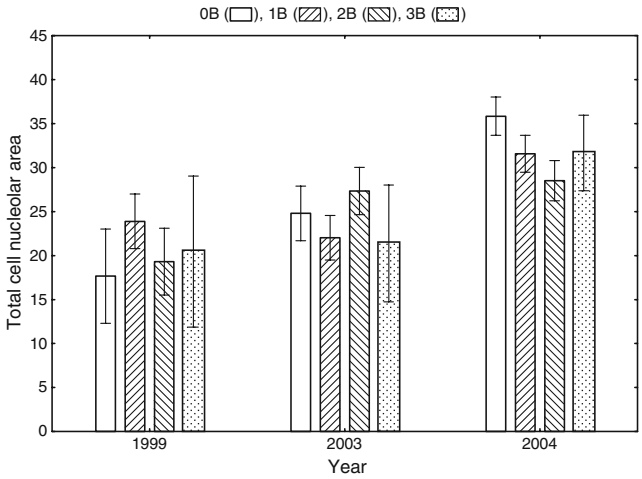

Fig. 2 Temporal variation of total cell nucleolar area (in μm^2) in diplotene cells of males with different numbers of B chromosomes. *Bars* indicate ±1 SD

In diplotene cells from males with two or more Bs, the size of the nucleoli attached to B-univalents (5.78 μm^2, SE=0.74) and B-bivalents (6.55 μm^2, SE=1.93) did not differ significantly in 2003 (t=0.44, df=41, P=0.66) or 2004 (9.78±1.18 when univalent and 7.37±1.04 when bivalent; t=1.42, df=55, P=0.16) or both years together (8.01±0.77 for B-univalents and 7.04±0.98 for B-bivalents; t=0.77, df=98, P=0.44). This suggests that the activity of the B-NOR is not influenced by pairing between B chromosomes.

Changes in NA yielded by A chromosomes, associated with B-NOR activity

To analyze the influence of B-NOR activity on the activity of NORs on the A chromosomes (9-11 and X), we firstly compared the total cell NA between cells where the B-NOR was active and cells where it was inactive, in 12 males from 2003 and 19 males from 2004 showing B-NOR activity. In 2003, total cell NA did not differ significantly (t=0.71, df=11, P=0.49) between cells where the B-NOR was inactive (mean=23.41 μm^2, SE=1.67) and those where it was active (mean=26.35 μm^2, SE=4.96). The 2004 sample yielded the same result: 30.89 μm^2 (SE=2.18) in cells where the B-NOR was inactive and 33.64 μm^2 (SE=2.45) in cells where it was active (t=1.41, df=18, P=0.18). This suggests that the total cell NA is tightly regulated.

To analyze the effect of the activity of the B-NOR on the activity of each of the other NORs in the A chromosomes, we compared NA associated with each A chromosome (NA_9, NA_{10}, NA_{11}, and NA_X) between cells where the B-NOR was inactive and cells showing activity in the B-NOR. As Table 3 shows, NA_9 and NA_{10} tended to decrease when the B-NOR was active, and this was apparent in both years. Student's t test for dependent samples, however, showed that this decrease had borderline significance for NA_9 in 2003 and significance for NA_{10} in 2004, although these differences vanished with the sequential Bonferroni correction.

Temporal changes in NA produced from NORs in the A chromosomes

A MANOVA with NA_9, NA_{10}, NA_{11}, and NA_X as dependent variables, and year (1999, 2003, and 2004) as grouping variable, showed significant differences among years (Wilks test: F=3.32, df=8, 168, P=0.0015). Univariate tests showed significant tendencies to temporal increase in NA of chromosomes 9 (F=4.76, df=2,87, P=0.0109), 10 (F=8.12, df=2,87, P=0.00059) and 11 (F=5.29, df=2,87, P=0.0068), but not X (F=1.55, df=2,87, P=0.218; Fig. 3).

B chromosome effects on chiasma frequency

A comparison of chiasma frequency in the same diplotene cells where NA was analyzed showed no significant differences between the 2003 (mean=14.68, SE=0.18) and 2004 (mean=15.04, SE=0.15) samples (Student's t=1.47, df=74, P=0.146). Significant effect of B chromosome number on mean cell chiasma frequency was found in both years (2003: F=3.48, df=3, 24, P=0.031; 2004: F=3.84, df=3, 44, P=0.016), but there was an odd–even pattern in 2003 and a dose-effect in 2004 (Fig. 4). Mean cell chiasma frequency in A chromosomes was not correlated with total NA in A chromosomes (2003: r=0.156, P=0.427; 2004: r=0.157, P=0.286). The net increase in chiasma frequency of 1B males with respect to 0B males was about 0.5 in both study years (see Fig. 4).

Interdependence among A and B chromosome NORs

To analyze whether NORs located in each of the four A chromosomes (9, 10, 11, and X) and that in the B

Table 2 B-NOR activity and B-bivalent formation in 2B males

Year	Meiotic B configuration	B inactive	B active	Total cells	Percent active
2003	B univalents	83	8	91	8.79
	B bivalent	67	2	69	2.90
	Total cells	150	10	160	6.25
	Percent bivalents	44.67	20.00	43.13	
2004	B univalents	74	4	78	5.13
	B bivalent	132	9	141	6.38
	Total cells	206	13	219	5.94
	Percent bivalents	64.08	69.23	64.38	

Table 3 Comparison of NA associated with each A chromosome carrying a NOR (9, 10, 11, X) between cells where the B-NOR was active or inactive, in 12 males from 2003 and 19 males from 2004 where the B-NOR showed activity

Year	Item	B-NOR	Mean	SE	*t*	*df*	*P*
2003	NA_9	Inactive	4.17	0.79			
		Active	2.80	0.63	2.15	11	0.055
	NA_{10}	Inactive	7.91	1.03			
		Active	6.11	1.63	1.39	11	0.191
	NA_{11}	Inactive	6.04	0.97			
		Active	7.22	3.62	−0.37	11	0.719
	NA_X	Inactive	4.02	1.05			
		Active	3.00	1.02	1.27	11	0.232
	NA_B	Active	6.31	0.88			
2004	NA_9	Inactive	5.46	0.76			
		Active	4.02	1.23	1.50	18	0.152
	NA_{10}	Inactive	7.99	0.86			
		Active	5.14	1.37	2.19	18	0.042
	NA_{11}	Inactive	8.83	1.28			
		Active	8.80	2.09	0.02	18	0.986
	NA_X	Inactive	6.57	0.94			
		Active	5.99	1.36	0.62	18	0.541
	NA_B	Active	7.97	1.08			

NA associated with the B chromosome (NA_B) is also included
SE Standard error, *t* Student test for dependent samples

chromosome showed any kind of interaction regarding the size of the nucleolus with which they were associated, we performed a series of correlation analyses in the diplotene cells of 0B males and B-carrying males. The results showed that, in the absence of B chromosomes, the NORs on A chromosomes behaved independently, i.e., the size of the NA for any of them was independent of the NA in the remaining A chromosomes (see the first set of analyses in Table 4).

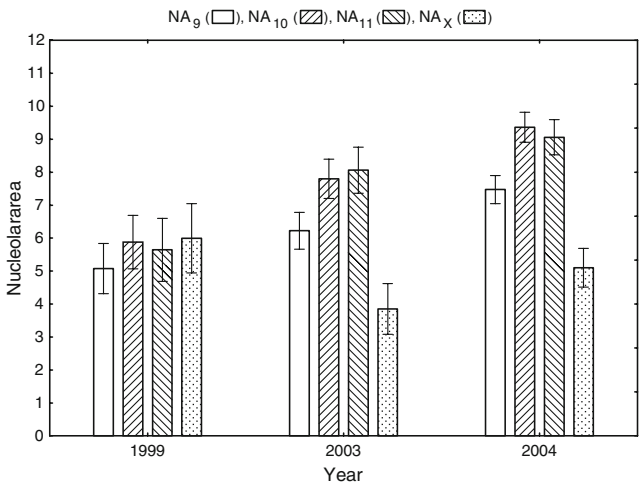

Fig. 3 Temporal variation of nucleolar area (in μm^2) attached to A (9, 10, 11, X) chromosomes. *Bars* indicate ±1 SD

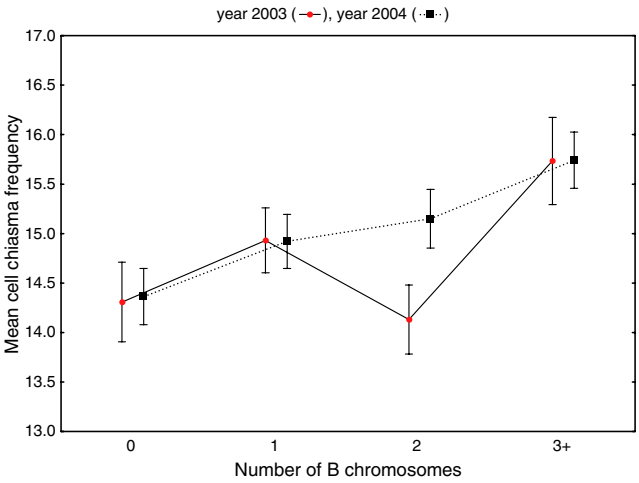

Fig. 4 Mean cell chiasma frequency in diplotene cells of males with different numbers of B chromosomes in 2003 and 2004 in the Torrox population. *Bars* indicate ±1 SD

In the presence of B chromosomes, however, this independence was lost in some cases, i.e., NA_{10} and NA_{11} showed strong positive correlation in 2003, as well as NA_9 and NA_{10} in 2004 (see the second set of analyses in Table 4). Since there were two types of B-carrying males, i.e., those carrying Bs being always inactive and those carrying Bs with B-NOR activity in part of the analyzed cells, we performed additional analyses in order to test the effect of B chromosome activity. When all B chromosomes in a male were inactive, no interdependence was found among A-chromosome NORs (see the third set of analyses in Table 4), as noted in the absence of Bs. However, in males where B-NOR activity was observed, the result of these correlation analyses depended on whether it had been performed on cells where the B was inactive or active. In the first case, highly significant correlations were observed, especially in 2004, and they were again positive among autosomes and negative between X and autosomes (see the fourth set of analyses in Table 4). In cells where the B-NOR was active, however, no significant correlation was detected among the size of the nucleoli attached to the different A-chromosome NORs, that of the B-NOR also being independent (see the fifth set of analyses in Table 4).

Discussion

B chromosomes in many species have been shown to harbor rRNA genes (for a review, see Camacho 2005). In most cases, these genes have been detected by silver impregnation, which reveals only active rRNA genes, but the growing use of the fluorescent in situ hybridization (FISH) is expected to uncover more cases of B chromo-

Table 4 Correlation analysis on NA associated with each A chromosome NOR in diplotene cells

Type of male	Type of cells	NA associated with chromosome		Correlation (2003)					Correlation (2004)				
				r	*t*	P	Pb	N	*r*	*t*	P	Pb	N
0B	Lacking Bs	9	10	0.139	0.281	0.7930	6		0.349	1.178	0.2662		12
		9	11	0.603	1.511	0.2054			0.016	0.051	0.9600		
		9	X	−0.325	−0.687	0.5297			−0.034	−0.108	0.9162		
		10	11	0.650	1.709	0.1627			0.302	1.001	0.3404		
		10	X	−0.548	−1.312	0.2599			−0.291	−0.962	0.3585		
		11	X	−0.060	−0.119	0.9108			−0.056	−0.179	0.8616		
+B	B active + B inactive	9	10	0.372	1.790	0.0886	22		0.579	4.144	0.0002	**0.0013**	36
		9	11	0.405	1.983	0.0612			0.282	1.714	0.0957		
		9	X	−0.151	−0.684	0.5020			−0.291	−1.774	0.0851		
		10	11	0.705	4.441	0.0003	**0.0015**		0.204	1.217	0.2321		
		10	X	−0.482	−2.461	0.0231	0.0923		−0.331	−2.043	0.0488	0.2442	
		11	X	−0.519	−2.718	0.0132	0.0662		−0.304	−1.860	0.0715		
B inactive	B inactive	9	10	−0.249	−0.726	0.4883		10	0.586	2.804	0.0133	0.0801	17
		9	11	0.105	0.300	0.7720			0.122	0.478	0.6397		
		9	X	0.135	0.385	0.7104			−0.448	−1.938	0.0717		
		10	11	0.585	2.040	0.0757			0.043	0.168	0.8691		
		10	X	−0.478	−1.539	0.1623			−0.384	−1.610	0.1283		
		11	X	−0.509	−1.673	0.1330			−0.462	−2.015	0.0622		
B active	B inactive	9	10	0.090	0.287	0.7797		12	0.561	2.798	0.0124	**0.0371**	19
		9	11	0.156	0.500	0.6280			0.533	2.594	0.0189	**0.0378**	
		9	X	−0.254	−0.830	0.4260			−0.231	−0.978	0.3418		
		10	11	0.842	4.938	0.0006	**0.0035**		0.737	4.493	0.0003	**0.0019**	
		10	X	−0.649	−2.697	0.0224	0.1121		−0.598	−3.073	0.0069	**0.0276**	
		11	X	−0.550	−2.085	0.0637			−0.602	−3.110	0.0064	**0.0318**	
B active	B active	9	10	0.390	1.337	0.2107		12	−0.024	−0.100	0.9215		19
		9	11	0.611	2.439	0.0349	0.2791		0.074	0.306	0.7636		
		9	X	−0.194	−0.627	0.5448			−0.243	−1.032	0.3164		
		9	B	0.170	0.545	0.5977			−0.013	−0.053	0.9583		
		10	11	0.612	2.444	0.0346	0.3114		0.390	1.748	0.0985		
		10	X	−0.632	−2.577	0.0276	0.2755		−0.398	−1.789	0.0915		
		10	B	0.195	0.628	0.5441			−0.310	−1.346	0.1961		
		11	X	−0.333	−1.116	0.2906			−0.453	−2.097	0.0512		
		11	B	0.276	0.908	0.3854			−0.489	−2.310	0.0337	0.3370	
		X	B	−0.157	−0.502	0.6265			0.244	1.038	0.3138		

Correlations being significant after applying the sequential Bonferroni method (Pb) are noted in bold type letter

somes carrying inactive rRNA genes (see also Cabrero and Camacho 2008).

In the grasshopper *E. plorans*, most A chromosomes carry rDNA (López-León et al. 1994), although the most active NORs are those located in chromosomes 9-11 and X (López-León et al. 1995b). In addition, the great majority of B chromosomes hitherto analyzed by FISH have been shown to carry rDNA in amounts higher in eastern (Daghestan, Armenia, Turkey, and Greece) than western (Spain and Morocco) populations (López-León et al. 2008). However, rDNA in *E. plorans* B chromosomes is inactive in most cases, with the exception of a male carrying the B_2 chromosome fused to the longest autosome (Cabrero et al. 1987), suggesting the importance of chromosome context for NOR activity (see Pikaard 2000), and the recurrent activity of the B_{24} NOR in the Torrox population (Teruel et al. 2007). Our present analysis indicates that the frequency of *E. plorans* males showing B-NOR activity increased from 31% to 53% between 1999 (Teruel et al. 2007) and 2003–2004 (this paper).

The total number of nucleoli per diplotene cell remained roughly constant during these years (3.3–3.5), whereas the nucleolus area (NA) per cell augmented 31% from 2003 to 2004. Therefore, in parallel to the increase in the frequency of B chromosomes contributing rRNA to the cell through B-NOR activity, there was an increase in NA that was attributable mainly to larger nucleoli attached to autosomes 9, 10, and 11 (see Fig. 3). The number of nucleoli per cell within males showing B-NOR activity, however, was significantly higher in cells with B-NOR activity than in

those with inactivity. In the case of activity, the relative contribution of B chromosomes to total cell NA was about 27%, a figure remaining rather constant from 1999 to 2004.

Repression of ribosome synthesis can come from stress of many kinds (see Rudra and Warner 2004), and this is manifested by a decrease in nucleolus size (see Olson 2004). B chromosomes may cause stress that is manifested by a decrease in some fitness-related traits. One of these is frequently fertility, which decreases with B-chromosome number. For instance, in *E. plorans*, B chromosomes and mite ectoparasites decrease egg fertility (Muñoz et al. 1998). In fact, the B chromosome variant showing the highest drive (B_{24} in the Torrox population in 1992) in this species was also the one producing the largest decrease in egg fertility (Zurita et al. 1998). In a previous work, we found that the Torrox population showed lower total NA per cell than two other populations (Salobreña in Spain and Smir in Morocco) carrying less parasitic B chromosomes, thus suggesting that parasitic Bs are associated with smaller nucleoli (Teruel et al. 2007).

A second symptom of the stress caused by B chromosomes is the increase in chiasma frequency. Our present results have shown that mean chiasma frequency was about 15 per diplotene cell in both years analyzed, a figure very close to that observed in the Torrox population in samples from 1992, 1997, and 1998 (Camacho et al. 2002). The significant increase in chiasma frequency observed in B-carrying males from both 2003 and 2004, with respect to 0B males, suggests that the B_{24} chromosome is still being perceived as a parasitic element in the Torrox population. However, the net chiasma increase with regard to 0B males was 1.4 in the samples from the 1990s (see Camacho et al. 2002) but only 0.5 in 2003 and 2004 (present results), a figure resembling that found in partially neutralized Bs in Moroccan populations (Camacho et al. 2002). This suggests a decline in the degree of parasitism of B_{24} in Torrox during recent years.

Another indication for B-chromosome neutralization in the Torrox population comes from the significant fall in the transmission rate of the B_{24} chromosome through females from 1992 (0.7) to 1998 (near 0.5) (Perfectti et al. 2004). Therefore, the B_{24} chromosome in Torrox appears to be currently less parasitic (lower chiasma increase per B), in coincidence with its absence of drive, and is causing less stress since NA has increased during the last years.

Ribosome biogenesis is the main energy consumer within cells since, in yeast, rDNA transcription by RNA Pol I represents about 60% of the total transcription, and ribonucleoprotein mRNA production represents about 50% of all transcription initiation events by RNA Pol II (Warner 1999). In mammals, nearly 50% of the transcriptional activity is dedicated to the synthesis of 45S rRNA, even though only about 50% of rRNA genes are transcribed (Moss and Stefanovsky 2002). This highlights the need for a tight regulation of ribosome production (Rudra and Warner 2004). It is thus logical that the presence of extra (B) chromosomes composed of about 100×10^6 bp (as it is the case in *E. plorans*, Cabrero et al., unpublished), with a high proportion of rDNA (López-León et al. 2008), may distort rRNA gene-activity regulation, especially if B-rDNA genes are capable of contributing to rRNA production, as what occurs in the Torrox population. In this context, future research needs to be focused on the molecular analysis of rDNA transcripts from A and B chromosomes and their quantization in relation with B-NOR activity in Torrox and other populations.

Ribosomal chromatin can exist in three states, an inactive state similar to heterochromatin, a transcriptionally competent, but inactive, state, and a transcriptionally productive state (Huang et al. 2006). Pol I transcription factors are associated with transcriptionally silent mitotic NORs (Weisenberger and Scheer 1995; Roussel et al. 1996). Mitotic silencing is controlled primarily by post-translational modification of the transcription machinery (see Huang et al. 2006). Inactive NORs can thus sequester part of the transcription machinery and cause stress to cells. We suggest that the B chromosome NOR can also exist in three different states: silent, competent or active. In most populations hitherto analyzed, the B-NOR was silent, whereas in Torrox, from 1999 onwards, there were also some B chromosomes showing competent and active NORs, the two latter states occurring, for the same B, in different cells within the same male. The coexistence of competent and active NORs in the same chromosome is also a normal property of A chromosomes (9–11 and X) since all of them are active in only a proportion of diplotene cells per male, and the total NA yielded by them is of the same order of magnitude as that in the B_{24} chromosome (see Table 3). As shown in Table 1, the likelihood of showing a B chromosome with an active NOR grows with B number. The NOR neo-activity of B_{24} in Torrox could be explained by two hypotheses: (1) the appearance of a new B-variant escaping silencing, and/or (2) a mutation in an A chromosome gene regulating NOR activity (or B chromosome silencing in general).

It is not known how nucleolus size is regulated, but a possible molecule transmitting information from the surrounding environment to the nucleolus is nucleostemin (Tsai and McKay 2005; Tuma 2005). Intraindividual comparisons in *E. plorans* males showing B-NOR activity indicated the absence of significant difference in total NA between cells showing the B-NOR to be active and those showing it to be inactive. This suggests that total cell NA is tightly regulated to reach the demanded levels, irrespectively of how many chromosomes are contributing rRNA, most likely by means of a feedback mechanism balancing the number of active rRNA genes with the transcription rate

per gene to achieve homeostasis (Lawrence et al. 2004). In agreement with this statement, Grummt (2007) suggested the existence of an "integrative cooperativity and multiple interlocking feedback mechanisms between DNA methyltransferases, histone-modifying enzymes and nucleosome-remodeling activities that mediate the stable commitment of specific rDNA clusters to a particular state of activity." Our present analyses have shown that B-NOR activity is associated with decreases in the activity of the NORs in chromosomes 9 and 10 (about 30% decrease, averaging 2003 and 2004 data), so that the total cell NA did not significantly vary after B-NOR activation. As Table 3 shows, B chromosomes yielded NA amounts comparable to, and in some cases larger than, those in the A chromosomes. This suggests that, once activated, the behavior of the B-NOR is comparable to those in A chromosomes in terms of nucleolus size.

Preuss and Pikaard (2007) have remarked that "there is still much that we do not know and much to be learned about the chromosomal control of NORs and rRNA genes," and have suggested a large-scale mechanism for NOR-activity regulation dictated by unlinked loci. The identification of a gene in *Arabidopsis thaliana*, by QTL analysis, being unlinked to the NORs in this species but influencing their relative expression level (Lewis et al. 2004) is one of the few proofs for this assertion. Evidence on this subject remains elusive because of the difficulty in identifying rRNA transcripts from the different chromosomes within the same genome, although this limitation is being overcome in recent studies of nucleolar dominance in allopolyploids (for instance, see Earley et al. 2006). Silver staining of nucleoli attached to chromosome bivalents at diplotene cells can also help in this task. This technique has revealed, for instance, the activation of the NOR in the B_2 chromosome in *E. plorans* after its fusion with the longest autosome, which was explained by the possible existence of a NOR-regulating gene in this autosome (Cabrero et al. 1987), a finding consistent with changes in nucleolar dominance associated with chromosome rearrangements (for a review, see Lewis and Pikaard 2001).

Since increases in rDNA transcription result in an overall greater nucleolus size (Caperta et al. 2007), NA can be considered the phenotypic expression of rRNA genes. Our present analysis of NA yielded by each chromosome NOR, in diplotene cells, thus represents a very easy approach to evaluate relative expression level of rRNA genes on different chromosomes in the same genome. In *E. plorans*, our results have revealed noteworthy variation in NA, reflecting interdependence among NORs for expression, associated with B-chromosome NOR activity. B presence is not enough to change A-NOR relationships, since they behaved with similar independence in 0B individuals and B-carrying individuals where no B-NOR activity was detected (i.e., carrying a silent B-NOR). The situation changed in individuals showing B-NOR activity, although in this case, the cells where the A-chromosome NORs behaved independently were those showing B-NOR activity (i.e., carrying an active B-NOR), whereas strong A-NOR correlations appeared in cells where the B-NOR was not active (but it was competent). A possible explanation for these results is that competent B-NORs attract part of the cell transcriptional machinery to the detriment of A-chromosome NORs which have to compete for it, whereas an active B-NOR contributes to total cell rRNA production so that the amount demanded is reached with no competition of A-chromosome NORs. Silent B-NORs, however, would not promote competition among A chromosomes for transcriptional machinery.

As with A-chromosome NORs, B-NOR silencing might be related to epigenetic modifications of B chromosomes, i.e., DNA methylation and/or histone modifications. For instance, a decrease in the level of DNA methylation was observed in the distal third of the B_2 chromosome in *E. plorans* (where the B contains rDNA) when it was fused to the longest autosome, in parallel with the activation of the B-NOR (López-León et al. 1991). However, in the male showing this fusion, the NOR of the fused B showed low DNA-methylation levels irrespective of whether it was yielding a nucleolus or not (López-León et al. 1991), suggesting that it was in a competent state in all cells. Later studies indicated that DNA methylation did not appear to be the only cause for the usual inactivity of the B-NOR (López-León et al. 1995a). Recently, we have reported that B chromosomes in *E. plorans* show marked hypoacetylation for lysine 9 in the H3 histone (Cabrero et al. 2007), suggesting that histone modifications may also be involved in B-NOR silencing. Therefore, competent B-NORs in Torrox unveil A-chromosome NOR interdependence that is cooperative among autosomes (positive correlations) and antagonistic between autosomes and the X chromosome (negative correlations), these being the same kind of relationships as previously reported in the Jete population (see López-León et al. 1995b).

It has been suggested that a stochastic factor influences which rDNA loci are silenced (Matyasek et al. 2007). The independent expression pattern of the NORs in chromosomes 9–11 and X in 0B males could be a manifestation of such a random silencing. However, when a B chromosome is contributing to rRNA production, the rules governing NOR expression are broken and significant changes occur.

Acknowledgements This study was supported by FEDER funds and grants from the Spanish Ministerio de Ciencia e Innovación (CGL2006-06307) and Plan Andaluz de Investigación (CVI-1664). MT was supported by a fellowship (FPI) from the Spanish Ministerio de Ciencia e Innovación. We also thank David Nesbitt for English corrections.

References

Bakkali M, Cabrero J, López-León MD, Perfectti F, Camacho JPM (2001) Population differences in the expression of nucleolus organizer regions in the grasshopper *Eyprepocnemis plorans*. Protoplasma 217:185–190

Cabrero J, Camacho JPM (2008) Location and expression of ribosomal RNA genes in grasshoppers: abundance of silent and cryptic loci. Chromosome Res 16:595–607

Cabrero J, Alché JD, Camacho JPM (1987) Effects of B chromosomes on the activity of Nucleolar Organizer Regions in the grasshopper *Eyprepocnemis plorans*. Activation of a latent Nucleolar Organizer Region on a B chromosome fused to an autosome. Genome 29:116–121

Cabrero J, Perfectti F, Gómez R, Camacho JPM, López-León MD (2003) Population variation in the A chromosome distribution of satellite DNA and ribosomal DNA in the grasshopper *Eyprepocnemis plorans*. Chromosome Res 11:375–381

Cabrero J, Teruel M, Carmona FD, Jiménez R, Camacho JPM (2007) Histone H3 lysine 9 acetylation pattern suggests that X and B chromosomes are silenced during entire male meiosis in a grasshopper. Cytogenet Genome Res 119:135–142

Camacho JPM (2004) B chromosomes in the eukaryote genome. Karger, Basel

Camacho JPM (2005) B chromosomes. In: Gregory TR (ed) The evolution of the genome. Elsevier, San Diego, pp 223–286

Camacho JPM, Shaw MW, López-León MD, Pardo MC, Cabrero J (1997) Population dynamics of a selfish B chromosome neutralized by the standard genome in the grasshopper *Eyprepocnemis plorans*. Amer Nat 149:1030–1050

Camacho JPM, Bakkali M, Corral JM, Cabrero J, López-León MD, Aranda I, Martín-Alganza A, Perfectti F (2002) Host recombination is dependent on the degree of parasitism. Proc Roy Soc Lond B 269:2173–2177

Caperta AD, Neves N, Viegas W, Pikaard CS, Preuss S (2007) Relationships between transcription, silver staining, and chromatin organization of nucleolar organizers in *Secale cereale*. Protoplasma 232:55–59

Caspersson T (1950) Cell growth and cell function, a cytochemical study. WW Norton, New York

Earley K, Lawrence RJ, Pontes O, Reuther R, Enciso AJ, Silva M, Neves N, Gross M, Viegas W, Pikaard CS (2006) Erasure of histone acetylation by *Arabidopsis* HDA6 mediates large-scale gene silencing in nucleolar dominance. Genes Dev 20:1283–1293

Grummt I (2003) Life on a planet of its own: regulation of RNA polymerase I transcription in the nucleolus. Genes Dev 17:1691–1702

Grummt I (2007) Different epigenetic layers engage in complex crosstalk to define the epigenetic state of mammalian rRNA genes. Hum Mol Genet 16 Spec No 1:R21–R27

Hiscox JA (2007) RNA viruses: hijacking the dynamic nucleolus. Nat Rev Microbiol 5:119–127

Huang S, Rothblum LI, Chen D (2006) Ribosomal chromatin organization. Biochem Cell Biol 84:444–449

Hudson LA, Ciborowski JJH (1996) Teratogenic and genotoxic responses of larval *Chironomus salinarius* group (Diptera: Chironimidae) to contaminated sediment. Environ Toxicol Chem 15:1375–1381

Jones RN, Viegas W, Houben A (2008) A century of B chromosomes in plants: So what? Annals of Botany 101:767–775

Karpen GH, Schaefer JE, Laird CD (1988) A *Drosophila* rRNA gene located in euchromatin is active in transcription and nucleolus formation. Genes Dev 2:1745–1763

Laferte A, Favry E, Sentenac A, Riva M, Carles C, Chedin S (2006) The transcriptional activity of RNA polymerase I is a key determinant for the level of all ribosome components. Genes Dev 20:2030–2040

Langerstedt S (1949) Cytological studies on the protein metabolism of the liver in the rat. Acta Anat Suppl 9:1–140

Lawrence RJ, Pikaard CS (2004) Chromatin turn ons and turn offs of ribosomal RNA genes. Cell Cycle 3:880–883

Lawrence RJ, Earley K, Pontes O, Silva M, Chen ZJ, Neves N, Viegas W, Pikaard CS (2004) A concerted DNA methylation/histone methylation switch regulates rRNA gene dosage control and nucleolar dominance. Mol Cell 13:599–609

Leach C, Houben A, Field B, Pistrick K, Demidov D, Timmis J (2005) Molecular evidence for transcription of genes on a B chromosome in *Crepis capillaris*. Genetics 171:269–278

Lewis MS, Pikaard CS (2001) Restricted chromosomal silencing in nucleolar dominance. Proc Natl Acad Sci USA 98:14536–14540

Lewis MS, Cheverud JM, Pikaard CS (2004) Evidence for nucleolus organizer regions as the units of regulation in nucleolar dominance in *Arabidopsis thaliana* interecotype hybrids. Genetics 167:931–939

Li J, Santoro R, Koberna K, Grummt I (2005) The chromatin remodelling complex NoRC controls replication timing of rRNA genes. EMBO J 24:120–127

López-León MD, Cabrero J, Camacho JPM (1991) A nucleolus Organizer Region in a B chromosome inactivated by DNA methylation. Chromosoma 100:134–138

López-León MD, Cabrero J, Camacho JPM (1995a) Changes in DNA methylation during development in the B chromosome NOR of the grasshopper *Eyprepocnemis plorans*. Heredity 74:296–302

López-León MD, Cabrero J, Camacho JPM (1995b) Changes in NOR activity pattern in the presence of supernumerary heterochromatin in the grasshopper *Eyprepocnemis plorans*. Genome 38:68–74

López-León MD, Neves N, Schwarzacher T, Heslop-Harrison JS, Hewitt GM, Camacho JPM (1994) Possible origin of a B chromosome deduced from its DNA composition using double FISH technique. Chromosome Res 2:87–92

López-León MD, Cabrero J, Dzyubenko VV, Bugrov AG, Karamysheva TV, Rubtsov NB, Camacho JPM (2008) Differences in ribosomal DNA distribution on A and B chromosomes between eastern and western populations of the grasshopper *Eyprepocnemis plorans plorans*. Cytogenet Genome Res 121:260–265

Matyasek R, Tate JA, Lim YK, Srubarova H, Koh J, Leitch AR, Soltis DE, Soltis PS, Kovarik A (2007) Concerted evolution of rDNA in recently formed *Tragopogon* allotetraploids is typically associated with an inverse correlation between gene copy number and expression. Genetics 176:2509–2519

Morais-Cecilio L, Delgado M, Jones R, Viegas W (2000) Modification of wheat rDNA loci by rye B chromosomes: a chromatin organization model. Chromosome Res 8:341–351

Mosgoeller W (2004) Nucleolar ultrastructure in vertebrates. In: Olson MOJ (ed) The Nucleolus. Kluwer, New York, pp 10–20

Moss T, Stefanovsky VY (2002) At the center of eukaryotic life. Cell 109:545–548

Muñoz E, Perfectti F, Martín-Alganza A, Camacho JPM (1998) Parallel effects of a B chromosome and a mite that decrease female fitness in the grasshopper *Eyprepocnemis plorans*. Proc Roy Soc Lond B 265:1903–1909

Nakamoto K, Ito A, Watabe K, Koma Y, Asada H, Yoshikawa K, Shinomura Y, Matsuzawa Y, Nojima H, Kitamura Y (2001) Increased expression of a nucleolar Nop5/Sik family member in metastatic melanoma cells: evidence for its role in nucleolar sizing and function. Am J Pathol 159:1363–1374

Nogi Y, Yano R, Nomura M (1991) Synthesis of large rRNAs by RNA polymerase II in mutants of *Saccharomyces cerevisiae* defective in RNA polymerase I. Proc Natl Acad Sci USA 88:3962–3966

Olson MOJ (2004) Sensing cellular stress: another new function for the nucleolus? Sci STKE 2004:e10

Pebusque MJ, Seite R (1981) Electron microscopic studies of silver-strained proteins in nucleolar organizer regions: location in nucleoli of rat sympathetic neurons during light and dark periods. J Cell Sci 51:85–94

Perfectti F, Corral JM, Mesa JA, Cabrero J, Bakkali M, López-León MD, Camacho JPM (2004) Rapid suppression of drive for a parasitic B chromosome. Cytogenet Genome Res 106:338–343

Pikaard CS (2000) The epigenetics of nucleolar dominance. Trends Genet 16:495–500

Preuss S, Pikaard CS (2007) rRNA gene silencing and nucleolar dominance: insights into a chromosome-scale epigenetic on/off switch. Biochim Biophys Acta 1769:383–392

Rasband WS (1997) ImageJ, volume http://rsb.info.nih,gov/ij. Bethesda, Maryland, USA: U. S. National Institutes of Health

Roussel P, Andre C, Comai L, Hernandez-Verdun D (1996) The rDNA transcription machinery is assembled during mitosis in active NORs and absent in inactive NORs. J Cell Biol 133:235–246

Roussel P, Belenguer P, Amalric F, Hernandez-Verdun D (1992) Nucleolin is an Ag-NOR protein; this property is determined by its amino-terminal domain independently of its phosphorylation state. Exp Cell Res 203:259–269

Roussel P, Hernandez-Verdun D (1994) Identification of Ag-NOR proteins, markers of proliferation related to ribosomal gene activity. Exp Cell Res 214:465–472

Rudra D, Warner JR (2004) What better measure than ribosome synthesis? Genes Dev 18:2431–2436

Rufas JS, Iturra P, de Souza W, Esponda P (1982) Simple silver staining procedure for the localization of nucleolus and nucleolar organizer under light and electron microscopy. Arch Biol 93:267–274

Santoro R (2005) The silence of the ribosomal RNA genes. Cell Mol Life Sci 62:2067–2079

Santoro R, Li J, Grummt I (2002) The nucleolar remodelling complex NoRC mediates heterochromatin formation and silencing of ribosomal gene transcription. Nat Genet 32:393–396

Sollner-Webb B, Tower J (1986) Transcription of cloned eukaryotic ribosomal RNA genes. Annu Rev Genet 55:801–830

Teruel M, Cabrero J, Perfectti F, Camacho JPM (2007) Nucleolus size variation during meiosis and NOR activity of a B chromosome in the grasshopper *Eyprepocnemis plorans*. Chromosome Res 15:755–765

Tsai RY, McKay RD (2005) A multistep, GTP-driven mechanism controlling the dynamic cycling of nucleostemin. J Cell Biol 168:179–184

Tsang CK, Bertram PG, Ai W, Drenan R, Zheng XF (2003) Chromatin-mediated regulation of nucleolar structure and RNA Pol I localization by TOR. EMBO J 22:6045–6056

Tuma RS (2005) A GTP signal to the nucleolus. J Cell Biol 168:172

Warner JR (1999) The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 24:437–440

Weisenberger D, Scheer U (1995) A possible mechanism for the inhibition of ribosomal RNA gene transcription during mitosis. J Cell Biol 129:561–575

Zhou Y, Santoro R, Grummt I (2002) The chromatin remodelling complex NoRC targets HDAC1 to the ribosomal gene promoter and represses RNA polymerase I transcription. EMBO J 21:4632–4640

Zurita S, Cabrero J, López-León MD, Camacho JPM (1998) Polymorphism regeneration for a neutralized selfish B chromosome. Evolution 52:274–277