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Nondisjunction rates of mouse chromosomes involved in heterozygous Rb rearrangements measured by chromosome painting of spermatocytes. II. The effects of trivalent combinations and genetic background

M. Scascitelli[a] B. Gustavino[a] F. Pacchierotti[b] F. Spirito[c] M. Rizzoni[a]

[a]Department of Biology, University of Rome 'Tor Vergata';
[b]Section of Toxicology and Biomedical Sciences, ENEA CR Casaccia;
[c]Department of Genetics and Molecular Biology, 'C. Darwin', University of Rome 'La Sapienza', Rome (Italy)

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Abstract. Chromosome specific nondisjunction rates were quantified by dual-colour FISH in spermatocytes II of Robertsonian heterozygous mice with different trivalent combinations or, alternatively, with different genetic backgrounds. We found that such factors do not influence the proneness to nondisjunction of specific chromosomes.

Closely related species often differ for underdominant chromosomal rearrangements (pericentric inversion, translocation, centric fusion and fission, for a review see Searle, 1993). A direct role of such a karyotypic differentiation has been proposed to trigger the cladogenetic process (White, 1968) in different related taxa, i.e., several invertebrates and certain mammalian orders such as insectivores, rodents, primates (White, 1978). A recent hypothesis suggests that the separation between humans and apes might also be due to differences in chromosomal structure (Navarro and Barton, 2003; Rieseberg and Livingstone, 2003).

In the house mouse (*Mus musculus domesticus*) the standard diploid karyotype consists of 40 acrocentric chromosomes, but many natural populations in Western Europe (for a review see Winking and Gropp, 1976; Capanna et al., 1977; Nachman and Searle, 1995) and northern Africa (Said et al., 1993) show different karyotypes. The variation is caused by the fixation of several metacentrics – the so-called Robertsonian centric fusion – which arose from the joining of two acrocentric chromosomes.

The difference in chromosome number and structure and partially in DNA sequence loss (around the pericentromeric region during the process of Robertsonian centric fusion (Garagna et al., 2001) between all-acrocentric and Robertsonian populations could impair the fertility of hybrids, heterozygous for these rearrangements. Reduction of the gene flow via hybrid sterility would represent an essential step in the process of speciation (White, 1978) and many investigations have been focused on its evaluation (Cattanach and Moseley, 1973; Said et al., 1993; Castiglia and Capanna, 2000), as well as on checking possible factors involved in the process, that could explain the high fixation frequency of Rb fusions, the stability of wild karyotypic races and the possible gene flow reduction.

It has been demonstrated (Scascitelli et al., 2004) that the chromosome-specific nondisjunction rate for chromosomes 1, 4, 6 and 14, analyzed by dual-color FISH, was not influenced by the trivalent number (i.e. absence of epistatic interactions).

In the present paper, FISH analysis of spermatocytes II allowed us to compare chromosome-specific nondisjunction rates between males with different karyotypes or genetic backgrounds. In particular, we evaluated the nondisjunction rate for chromosomes 1 and 14 (involved respectively in the centric

Request reprints from Moira Scascitelli
Department of Biology, University of Rome 'Tor Vergata'
via della Ricerca Scientifica snc, 00133, Rome (Italy)
telephone: +39-06-72594812; fax: +39-06-2023500
e-mail: m.scascitelli@inwind.it

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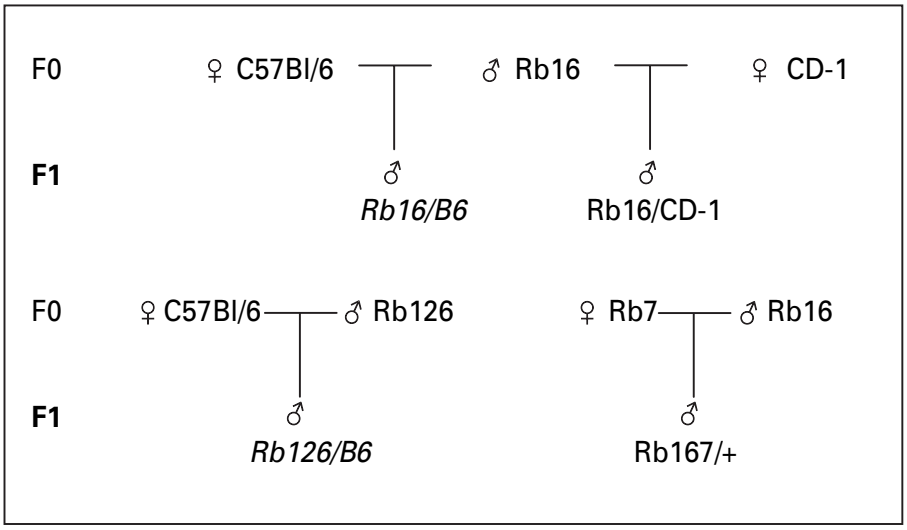

Fig. 1. Mating scheme: F0 represents the parental generation: C57Bl/6 and CD-1 are two different strains of all-acrocentric homozygous females; Rb7, Rb16 and Rb126 are animals homozygous for one (Rb[16.17]7Bnr), two (Rb [1.3]1Bnr, Rb[9.14]6Bnr), or three (Rb[1.3] 1Bnr, Rb [4.6]2Bnr, Rb[9.14]6Bnr) metacentric chromosomes. F1 represents the heterozygous generation: double heterozygotes – shortened as Rb16/B6 and Rb16/CD-1 – share their male parent, therefore have the same Rb chromosomes (Rb[1.3]1Bnr, Rb[9.14]6Bnr), but have a different genetic background with regard to the maternal all-acrocentric strain. Triple heterozygotes, shortened as Rb126/B6 and Rb167/+, have a different combination of Rb fusion: both of them have the chromosomes Rb[1.3]1Bnr, and Rb[9.14]6Bnr), whilst the third one is respectively Rb[4.6]2Bnr or Rb[16.17]7Bnr. Males analysed in the past work are written in italics.

fusion Rb[1.3]1Bnr and Rb[9.14]6Bnr) in double and triple heterozygotes (Rb16/CD-1 and Rb167/+). We also used previous data (Scascitelli et al., 2004) from two other groups of double and triple heterozygotes (Rb16/B6 and Rb126/B6) in order to make the following comparisons: a) between the two groups of Rb double heterozygotes (Rb16/B6 and Rb16/CD-1) that share the same Rb heterozygous chromosomes but differ for the maternal genetic background (C57Bl/6 or CD-1, respectively); we aimed at checking whether the genetic background affects chromosome-specific nondisjunction rates; b) between two groups of Rb triple heterozygotes (Rb126/B6 and Rb167/+) that share two Rb chromosomes (Rb[1.3]1Bnr and Rb[9.14] 6Bnr) whilst the third one is, alternatively, either Rb[4.6] 2Bnr or Rb[16.17]7Bnr. We checked a possible influence of the third Rb chromosome on the nondisjunction rate of specific chromosomes involved in the first two Rb rearrangements.

Materials and methods

Animals

All the heterozygotes analyzed in the present study were obtained from laboratory crosses between mice homozygous for acrocentric or Robertsonian chromosomes. These crosses are represented in Fig. 1 that also includes crosses used in the previous work (Scascitelli et al., 2004).

Robertsonian homozygotes were imported from The Jackson Laboratory (Bar Harbor, Maine, USA). All-acrocentric homozygous C57Bl/6 and CD-1 females were purchased from Harlan (Italy).

Spermatocyte II preparation, hybridisation procedure and analysis

Air-dried spermatocyte II metaphases (Evans et al., 1964) were obtained from heterozygotes with different karyotypes/genetic backgrounds.

Rb16/CD-1 males, heterozygous for two Rb metacentrics (Rb[1.3]1Bnr, Rb[9.14]6Bnr) with a CD-1 maternal background and Rb167/+, heterozygous for the three Rb metacentrics Rb[1.3]1Bnr, Rb[9.14]6Bnr, Rb[16.17]7Bnr, were studied with dual-colour FISH for chromosomes 1 and 14. Dual-colour FISH was performed using a mix of two probes specific for chromosomes 1 and 14, directly conjugated with CY-3 and FITC respectively (Cambio, Cambridge UK). See Scascitelli et al. (2004) for the protocol of the dual-color technique.

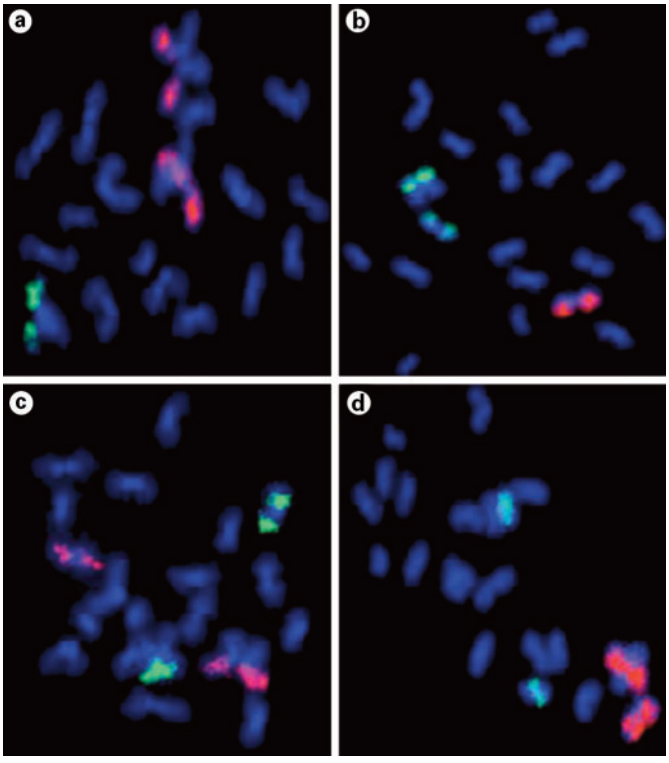

Fig. 2. Hyperhaploid spermatocyte II metaphases disomic for chromosome 1 (**a**) and 14 (**b**) from heterozygotes for two Rb metacentrics (Rb[1.3]1Bnr, Rb[9.14]6Bnr); (**c**, **d**) double disomic spermatocytes for chromosomes 1 and 14 from heterozygotes for three Rb metacentrics (Rb[1.3]1Bnr, Rb[9.14]6Bnr, Rb[16.17]7Bnr).

Nondisjunction rate evaluation

We analyzed 150 haploid/hyperhaploid (20/21–22 chromosome arms) spermatocytes for each specimen and all the hypohaploid cells (18–19 chromosome arms) found during the scoring.

MII spermatocytes were classified on the basis of the chromosome arm count and of the number of fluorescent signals for chromosomes 14 and 1.

Table 1. Spermatocytes II from double (Rb16/CD-1) and triple (Rb167/+) Rb heterozygotes, named respectively Da–Dd and Ta–Td, with 18–22 chromosome arms. The classification has been made on the basis of the number of fluorescent signals (dual-colour FISH) relative to chromosomes 14 and 1.

Heterozygous karyotype	Subject	N° chromosome arms	MII with the following No. of copies for chromosomes 14 (left)/1 (right):								
			0/0	0/1	1/0	0/2	1/1	2/0	1/2	2/1	2/2
Rb16/CD-1	Da	18		3	2		13				
		19		5	13		32				
		20					105	2	5		
		21					18		9	7	
		22					1		2		1
	Db	18	2	5	5		5				
		19		7	12		37				
		20			2	1	102	2	6	2	
		21					14		9	8	
		22							2		2
	Dc	18			2		2				
		19		5	7		23				
		20			3		107		3		
		21					13		14	8	
		22							1		1
	Dd	18	2	1			10				
		19		7	7		22				
		20					114		1		
		21				2	12	2	10	9	
		22									
Rb167/+	Ta	18	1	1	2		6				
		19		8	10	1	26				
		20		1		1	92	3	3	3	
		21					23		7	8	
		22					1		4	2	2
	Tb	18		4	4		14				
		19		6	8		40				
		20					120		3	2	
		21					13		5	3	
		22					1		1	1	1
	Tc	18			4		6				
		19		7	7		28				
		20				1	113		4		
	Td	21					13		11	5	
		22					1			1	1
	Tc	18	1	4	4		10				
		19		7	11		38				
		20		3	1		102	2	2	3	
		21					17		12	4	
		22					1		1		2

Nondisjunction rate for each of those chromosomes[1] was calculated summing up the frequencies of disomic and nullisomic MII spermatocytes (i.e. cells with two or no fluorescent spots of the same color, Fig. 2). Then the mean value was obtained for each karyotype among four specimens.

Data on nondisjunction rate of chromosomes 1 and 14 in Rb16/B6 and Rb126/B6 were taken from the previous work (Scascitelli et al., 2004).

The absolute frequency of double nondisjunction events has been evaluated on the basis of the number of dual-colour painted spermatocytes II with two signals or without signal for both the probes and with two signals for one of the probes and no signal for the other one.

[1] Since all the mice analysed were heterozygotes for Rb chromosomes, probes labelled either a specific acrocentric chromosome or its fused homologue, i.e. a single chromosome arm involved in a Robertsonian centric fusion.

Statistical analysis

Fisher's exact test was used to compare chromosome-specific nondisjunction rates in double and triple heterozygotes with different genetic backgrounds or trivalent combinations.

Results

Data about dual-color FISH on spermatocyte II metaphases of mice heterozygous for two (Rb[1.3]1Bnr, Rb[9.14]6Bnr) or three (Rb[1.3]1Bnr, Rb[9.14]6Bnr, Rb[16.17]7Bnr) Rb chromosomes are reported in Table 1. Columns named 0/2, 2/0, 2/2, 0/0 represent cells with double nondisjunction events for both chromosomes 1 and 14 (Fig. 2c, d).

Mean nondisjunction rates for double and triple heterozygotes (Rb16/CD-1 and Rb167/+) are reported in Table 2. It

Table 2. Chromosome-specific nondisjunction rates in spermatocytes II of double and triple heterozygotes (Rb16/CD-1 and Rb167/+) compared respectively to double (Rb16/B6) and triple (Rb126/B6) heterozygotes from previous data (Scascitelli et al., 2004). *P* was calculated on the basis of Fisher's exact text.

Heterozygous karyotype		Mean NDJ rate ± SE for the chromosomes:	
		1	14
Double	Rb16/B6	0.163 ± 0.013[a]	0.077 ± 0.001[a]
	Rb16/CD-1	0.158 ± 0.030	0.101 ± 0.027
		$P = 0.8$	$P = 0.2$
Triple	Rb126/B6	0.135 ± 0.024[a]	0.119 ± 0.008[a]
	Rb167/+	0.140 ± 0.031	0.104 ± 0.037
		$P = 0.8$	$P = 0.4$

[a] Data from Scascitelli et al. (2004).

also includes previous estimations (Scascitelli et al., 2004) for the karyotypes Rb16/B6 and Rb126/B6 (double and triple heterozygotes, respectively).

The comparison of the nondisjunction rate of chromosomes 1 and 14 (Table 2) between the two groups of double heterozygotes with different genetic background (Rb16/B6 and Rb16/CD-1) did not show statistically significant differences for either chromosome.

The same comparisons between the triple heterozygotes Rb126/B6 and Rb167/+, whose karyotype differs for the presence of the Rb heterozygous chromosome Rb[4.6]2Bnr or, alternatively, Rb[16.17]7Bnr, showed non-significant differences for nondisjunction rates of chromosomes 1 and 14 (Table 2).

Frequency of double nondisjunction events, observed in the present data (Table 1), was not significantly higher than that expected following the hypothesis of independent occurrence of nondisjunction events for different chromosomes, for both the karyotypes (17 vs 13.229, $P = 0.296$ for double heterozygotes; 16 vs 12.549, $P = 0.326$ for triple heterozygotes), in agreement with previous results (Scascitelli et al., 2004).

Discussion

The chromosomal speciation hypothesis is still at issue and is attracting new interest since it has been supposed to also concern the process of diversification between great apes and humans (Navarro and Barton, 2003; Rieseberg and Livingstone, 2003). It has been suggested that chromosomal rearrangements can act as barriers between populations, diverging in their chromosomal structure, via recombination reduction or underdominance of hybrids. The latter hypothesis introduces a paradox, because the probability of fixation of new rearrangements and their efficiency as barriers to the gene flow are inversely proportional, unless other factors are involved in the whole process (Barton, 1979, 1983; Lande, 1979, 1984; Hedrick, 1981; Walsh, 1982; Barton and Bengtsson, 1986; Pardo-Manuel de Villena and Sapienza, 2001; Scascitelli et al., 2003).

In the present work we tried to quantify the missegregation frequency and in particular, to throw some light upon possible factors that can influence its intensity, such as: the identity of Rb fusions, their number being the same, and the difference in the genetic background.

This paper confirms the outline of previous studies (Scascitelli et al., 2004) about possible interactions among different Rb heterozygous chromosomes on the chromosome-specific nondisjunction rate. Estimated nondisjunction rate values are independent of the karyotypic 'environment', i.e. from the number of additional Rb heterozygous metacentrics (Scascitelli et al., 2004) or the identity of additional Rb fusions and, furthermore, it is not influenced by the genetic background (present paper).

Chromosomes 1 and 14, labeled in the present work, are involved in two Rb fusions that differ not only for their acrocentric combination, but also because the first one (Rb[1.3]1Bnr) is derived by the fusion of two large acrocentrics with similar lengths, whilst the latter (Rb[9.14]6Bnr) has two quite-different sized chromosome arms. Nondisjunction rate values found in our samples were consistently higher for chromosome 1 than 14: it suggested that the decrease of the chromosome arm ratio (i.e. ratio short:long arm) in Rb biarmed chromosomes does not entail an increase of nondisjunction events, as supposed by White (1973).

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