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Nondisjunction rates of mouse specific chromosomes involved in heterozygous Rb rearrangements measured by chromosome painting of spermatocytes II. I. The effects of the number of trivalents

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Abstract. Dual-colour FISH painting with alternative fluorescent chromosome-specific probes allowed us to distinguish chromosomes 1, 4, 6 and 14. The purpose was to check whether nondisjunction rates of specific chromosomes involved in heterozygous Robertsonian fusions are independent of the number of trivalents, or an epistatic effect among Rb chromosomes takes place affecting nondisjunction rates. Probes were used on DAPI-stained metaphases of spermatocytes II of laboratory strains of mice with reconstructed karyotypes heterozygous for one, two, three or four Robertsonian metacentrics in an all-acrocentric background. The existence of such epistatic interactions was not verified.

A large number of races of *Mus musculus domesticus* is characterised by the fixation of different numbers and/or combinations of Rb chromosomes. They have been described since 1970 (Gropp et al., 1970; Winking and Gropp, 1976) up to now (Castiglia and Capanna, 1999, 2000; Britton-Davidian et al., 2000). These races, wherever they share a common habitat subjected hence to the same exogenous selective forces, have been maintaining their karyotypic differentiation. If these stable karyotypic races are differentiated by the fixation of multiple, different Rb translocations, they show low levels of gene flow (e.g. Castiglia et al., 2002). On the other hand, the genetic similarity observed between races diverging by only one or a few Rb translocations suggests the persistence of gene flow or a recent divergence in these cases (Britton-Davidian et al., 1989).

It has been suggested that these Robertsonian rearrangements may trigger cladogenetic processes due to their underdominance (White, 1978). The assessment of the real heterozygote disadvantage of the hybrids, generated by inter-crosses between such chromosomal populations, is critical to understanding the role of Robertsonian translocations in speciation (see Searle, 1993). Reduced fertility, consequent to abnormal segregation in meiosis I, represents a key-factor causing the reduction of hybrid fitness. Several laboratory and field studies have addressed this issue (Cattanach and Moseley, 1973; Ford and Evans, 1973; Winking and Gropp, 1976; Said et al., 1993; Baulch et al., 1996; Hauffe and Searle, 1998; Rizzoni and Spirito, 1998; Castiglia and Capanna, 2000; Wallace et al., 2002).

The intrinsic paradox of the underdominant rearrangements makes it difficult to evaluate their cladogenetic role: if the heterozygote disadvantage is weak, the probability of fixation for newly arisen rearrangements is high and isolation efficiency is low; the reverse occurs if such a disadvantage is strong.

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Several factors have been suggested which might help underdominant rearrangements to play a significant role in speciation, such as meiotic drive, genetic drift, inbreeding and selective advantage for the "new" rearrangement in a homozygous condition (Barton, 1979, 1983; Lande, 1979, 1984; Hedrick, 1981; Walsh, 1982; Barton and Bengtsson, 1986). Recently "unequal centromere number rule" (Pardo-Manuel de Villena and Sapienza, 2001) has been proposed to explain meiotic drive of heterozygous Robertsonian metacentrics in oocytes I of the mouse: metacentrics and their two homologous acrocentrics could have a different probability to migrate towards the egg or the polar body side of the meiotic I spindle because of their different numbers of centromeres. Preferential cosegregation to the same pole of chromosomes with the same shape (metacentrics with metacentrics, acrocentrics with acrocentrics) during meiosis I has been detected by analyzing spermatocytes II of the mouse (Rizzoni and Spirito, 1999; Scascitelli et al., 2003). This non-random segregation helps underdominant rearrangements to persist in small populations (Scascitelli et al., 2003).

Epistatic interactions among several Rb heterozygous rearrangements have been suggested to affect the structure of the hybrid zones between mouse chromosomal races with many Rb metacentric chromosomes and races with the standard all-acrocentric karyotype, and the amount of gene flow between them (see Searle, 1993). Such epistatic effect should consist in an increasing frequency of nondisjunction for each trivalent as the number of trivalents increases. On this basis euploid gamete formation and fertility should decrease in a more-than-multiplicative ratio as the number of trivalents increases.

In the present paper the behaviour is described of two chromosomes (4 and 6) involved, as chromosome arms, in the same Rb fusion (Rb[4.6]2Bnr) or that of two chromosomes (1 and 14) involved, as chromosome arms, in two different Rb fusions (Rb[1.3]1Bnr; Rb[9.14]6Bnr). We studied the spermatocytes II of single, double, triple or quadruple heterozygous mice, using dual-colour FISH painting with alternative fluorescent chromosome-specific probes. Our aim was to check whether the nondisjunction rate of specific arms of specific trivalents is affected by the presence and the number of other trivalents (epistatic interaction for nondisjunction among Rb heterozygous rearrangements).

Materials and methods

Animals

Heterozygous specimens with different karyotypes were obtained as follows:

F1 heterozygous males for one Rb metacentric chromosome (Rb[4.6]2Bnr) were obtained mating all-acrocentric C57Bl/B6 females (Harlan, Italy) with Rb2Bnr/Ei males, homozygous for the Rb metacentric chromosome (The Jackson Laboratory, Bar Harbor, Maine, USA).

F1 heterozygous males for two Rb metacentric chromosomes (Rb[1.3]1Bnr, Rb[9.14]6Bnr) were obtained mating all-acrocentric C57Bl/B6 females (Harlan) with Rb16Bnr/Ei males, homozygous for the two Rb metacentric chromosomes (The Jackson Laboratory).

F1 heterozygous males for three Rb metacentric chromosomes (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr) were obtained mating all-acrocentric C57Bl/B6 females (Harlan) with Rb126Bnr/Ei males, homozygous for the three Rb metacentric chromosomes (The Jackson Laboratory).

F1 heterozygous males for four Rb metacentric chromosomes (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr, Rb[16.17]7Bnr) were obtained mating Rb7Bnr homozygous females carriers of the metacentric chromosome Rb[16.17]7Bnr (The Jackson Laboratory) with Rb126Bnr/Ei males, homozygous for the three Rb metacentric chromosomes (The Jackson Laboratory).

Spermatocyte II preparation, hybridisation procedure and analysis

Air-dried metaphases of spermatocytes II (Evans et al., 1964), collected from specimens with the different karyotypes, were hybridised with specific probes for different chromosomes.

Heterozygous males for one Rb metacentric (Rb[4.6]2Bnr), three Rb metacentrics (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr) and four Rb metacentrics (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr, Rb[16.17]7Bnr) were studied with dual-colour FISH for chromosomes 4 and 6; heterozygous males for two Rb metacentrics (Rb[1.3]1Bnr, Rb[9.14]6Bnr), three Rb metacentrics (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr) and four Rb metacentrics (Rb[1.3]1Bnr, Rb[4.6]2Bnr, 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 probes specific for chromosomes 4 and 6 or, alternatively, 1 and 14, conjugated with FITC and CY-3, respectively (Cambio, Cambridge UK). Besides dual-colour FISH, one-colour FISH for chromosome 1 was applied to preparations from double and triple Rb heterozygotes and one-colour FISH for chromosome 6 applied to preparations from single and triple Rb heterozygotes, to check whether double hybridization affected estimation of nondisjunction rates. One-colour FISH was performed using CY-3 conjugated DNA probes specific for chromosome 1 or, alternatively, chromosome 6 (Appligene, Oncor).

One-colour FISH protocol

According to the company standard protocol, slides were pretreated in 2× SSC, 0.1% Tween20 at 37°C for 30 min, dehydrated in 70, 80 and 95% ethanol for 2 min each, denatured in 70% formamide, 2× SSC at 65°C for 2 min and then dehydrated again using the serial ice-cold ethanol. Meanwhile pre-warmed probes (for 5 min at 37°C) were denatured for 5 min at 72°C and immediately placed on ice. Hybridisation was achieved overnight at 37°C in a moist chamber. The slides were washed in 0.5× SSC for 2 min at 72°C, and in 1× PBD for 2 min at room temperature.

Dual-colour FISH protocol

After dehydration by serial ethanol washing (70, 90, 100%) and air drying, slides were denatured for 2 min at 65°C in 70% formamide, 2× SSC, and immediately dehydrated using serial ice-cold ethanol. Meanwhile prewarmed probes were mixed and denatured for 10 min at 65°C and then preannealed for 60 min at 37°C. Hybridisation was achieved overnight at 37°C in a moist chamber. The slides were washed twice for 5 min at 45°C in 50% formamide, 2× SSC, and twice in 0.1× SSC.

After the FISH procedure spreads were counterstained with DAPI in antifade (0.2 μg/ml). Metaphases were observed with single propidium iodide, FITC and DAPI filters using a Leitz Diaplan microscope. The images collected by a Leica DC250 monochromatic digital camera were elaborated with a Q-fluoro Leica software.

Nondisjunction rate evaluation

Results of chromosomes 1 and 6 with one-colour- and dual-colour FISH were highly comparable (see Results); therefore nondisjunction rates for these chromosomes were calculated on the basis of data obtained by both techniques.

Two mice were scored for each karyotype. Observations were made in regions of the slides in which signals were bright and clear, to avoid artifactual absence of probe signals. For each specimen slides were analysed until a total of 150 haploid or hyperhaploid MII spermatocytes with 21–22 chromosome arms were classified on the basis of the number of chromosome arms and of fluorescent signals; in addition, hypohaploid MII spermatocytes with 18–19 chromosome arms encountered during the above scoring were also classified. A slight excess of hypohaploid spermatocytes II was found compared to hyperhaploid ones, possibly as a consequence of artifactual chromosome loss.

The nondisjunction rate was calculated for every single painted chromosome (an acrocentric chromosome/an arm of an Rb metacentric chromosome) by adding the relative frequency of cells showing two fluorescent spots of the same colour, symptomatic of a disomy for the chromosome painted by

this probe, to that of cells showing no signal for the same probe, symptomatic of a nullisomy (Fig. 1). Dual-colour painted spermatocytes II with two signals for both the probes, with no signal for both the probes and with two signals for one probe and no signal for the other one were classified as the result of a double nondisjunction. Nondisjunction rates of specific chromosomes (an acrocentric chromosome/an arm of an Rb metacentric chromosome) were evaluated for each specimen, and then mean values were computed for each karyotype.

Estimates of epistatic interactions

The existence of epistatic interactions among Rb heterozygous rearrangements affecting the nondisjunction rate of the tested chromosomes was checked. It was assumed that such an interaction consists in a regular multiplicative change (either an increase or a decrease) of nondisjunction rate of specific chromosomes involved in heterozygous Rb rearrangements as a function of the number of trivalents in meiosis I. This multiplicative factor is denoted as Ep[1].

The relation which links the nondisjunction rates of a single, specific chromosome involved in a heterozygous Rb rearrangement when its trivalent is the only one in the meiosis I and when it is together with n – 1 other trivalents is the following:

$s_n = s \cdot Ep^{n-1}$, where s represents the nondisjunction rate when the involved trivalent is alone and sn represents the nondisjunction rate when the involved trivalent is together with other n – 1 trivalents.

A linear regression test was therefore performed between the number of trivalents and the log of nondisjunction rates of specific chromosomes involved in heterozygous Rb rearrangements. The values of the slopes of the different lines gave the estimates of Ep.

Estimation of the overall frequency of aneuploidy

A rough estimation of the overall frequency of aneuploidy was made for each karyotype, pooling data of single specimens with the same karyotype, after a check on interindividual homogeneity. The frequency of aneuploid cells was computed as follows (Rizzoni and Spirito, 1998): $(2f[n + 1] + 4f[n + 2])/(f[n] + 2f[n + 1] + 2f[n + 2])$, where f[x] represents the absolute frequency of cells with the x karyotype.

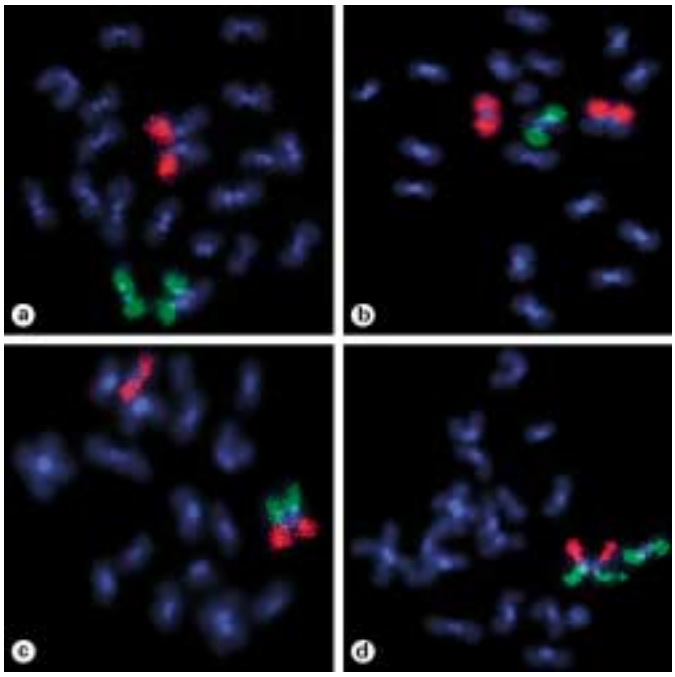

Fig. 1. Spermatocytes II disomic for specific chromosomes: (**a**) disomy for the chromosome 14, from a heterozygote for three Rb metacentrics (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr); (**b**) disomy for the chromosome 1, from a heterozygote for two Rb metacentrics (Rb[1.3]1Bnr, Rb[9.14]6Bnr); (**c**) disomy for the chromosome 6, from a heterozygote for three Rb metacentrics (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr); (**d**) disomy for the chromosome 4, from a heterozygote for three Rb metacentrics (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr).

Results

The frequencies of spermatocytes II, euploid (one painted chromosome), nullisomic (no painted chromosome) or disomic (two painted chromosomes) for chromosome 1, determined by one-colour FISH in specimens heterozygous for two or three Rb chromosomes are given in Table 1a. The frequencies of spermatocytes II, euploid, nullisomic or disomic for chromosomes 1 and/or 14, determined by dual-colour FISH in specimens heterozygous for two, three or four Rb chromosomes are given in Table 1b. The frequencies of spermatocytes II, euploid, nullisomic or disomic for chromosome 6, determined by one-colour FISH in specimens heterozygous for one or three Rb chromosomes are given in Table 1c. The frequencies of spermatocytes II, euploid, nullisomic or disomic for chromosomes 4 and/or 6, determined by dual-colour FISH in specimens heterozygous for one, three or four Rb chromosomes are given in Table 1d.

Rough data from Table 1a–d allow us to compute the mean values of the nondisjunction rates in meiosis I for chromosomes 1, 4, 6 and 14, which are given in Table 2. Labelled chromosomes show mean values of nondisjunction rate ranging between 7.7 and 16.3%. Double events of nondisjunction are recorded in the columns 0/0, 2/0, 0/2 and 2/2 of Table 1b and d; they have never been observed when chromosomes 4 and 6, which are involved in the same Rb centric fusion (Rb[4.6]2Bnr), were labelled with dual-colour FISH (Table 1d). Therefore mean nondisjunction rate for the whole Rb metacentric (Table 2) can be easily computed adding the values of nondisjunction rate of chromosomes 4 and 6, labelled with dual-colour FISH. Double nondisjunction has been detected when chromosomes 1 and 14 were labelled with dual-colour FISH (Table 1b); its frequency was very close to that expected on the basis of the hypothesis of independent occurrence of single nondisjunction events involving different trivalents (15 vs. 15.63). For this computation data for double, triple and quadruple heterozygotes with dual-colour FISH of chromosomes 1 and 14 were pooled.

Variations in mean nondisjunction rates as functions of trivalent numbers can be seen in Table 2. A very slight decrease of nondisjunction rate, as the number of trivalents increases, was found for chromosomes 4 and 6 (from 0.090 to 0.086 and from 0.102 to 0.093, respectively); a stronger decrease was found for chromosome 1 (from 0.163 to 0.120); a slight increase was found for chromosome 14 (from 0.077 to 0.091). However, in no case did these changes reach significance ($P > 0.05$): the slopes of regression lines were never significantly different

[1] Multiplicative factors leading to an increase of the nondisjunction rate should be better expressed as multiplicative factors leading to a decrease of the "correct" disjunction rate. However, for the values found of the nondisjunction rates and of Ep, the approximation obtained using the increase of the nondisjunction rate is satisfactory and data can be presented in a homogeneous way.

Table 1a. Frequencies of spermatocytes II with 18–22 chromosome arms of mice with a heterozygous karyotype for two (Rb[1.3]1Bnr, Rb[9.14]6Bnr) or three (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr) Rb metacentric chromosomes classified for the number of copies of the chromosome 1 after one-colour FISH painting

Heterozygous Rb metacentric chromosomes	Specimen	No. of chromosome arms	No. of spermatocytes II nullisomic (0), euploid (1) or disomic (2) for chromosome 1		
			0	1	2
Rb[1.3]1Bnr, Rb[9.14]6Bnr	D1	18	1	1	
		19	16	24	1
		20		119	4
		21		11	16
		22			
	D2	18	3	6	
		19	9	28	2
		20	3	117	5
		21		17	7
		22			1
Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr	T1	18	1	4	
		19	11	33	1
		20		113	6
		21	2	19	8
		22		2	
	T2	18	4	5	
		19	11	32	
		20	1	116	3
		21		21	7
		22			2

Table 1c. Frequencies of spermatocytes II with 18–22 chromosome arms of mice with a heterozygous karyotype for one (Rb[4.6]2Bnr) or three (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr) Rb metacentric chromosomes classified for the number of copies of the chromosome 6 after one-colour FISH painting

Heterozygous Rb metacentric chromosomes	Specimen	No. of chromosome arms	No. of spermatocytes II nullisomic (0), euploid (1) or disomic (2) for chromosome 6		
			0	1	2
Rb[4.6]2Bnr	S1	18	1		
		19	2	15	
		20		136	
		21		5	8
		22			1
	S2	18	1	3	
		19	8	14	
		20	1	131	2
		21		7	9
		22			
Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr	T5	18	1	1	
		19	1	28	
		20	2	106	5
		21		16	12
		22			3
	T6	18	6	8	
		19	8	32	
		20	2	126	2
		21		14	4
		22		1	1

Table 1b. Frequencies of spermatocytes II with 18–22 chromosome arms of mice with a heterozygous karyotype for two (Rb[1.3]1Bnr, Rb[9.14]6Bnr), three (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr) or four (Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr, Rb[16.17]7Bnr) Rb metacentric chromosomes classified for the number of copies of the chromosomes 1 and 14 after dual-colour FISH painting

Heterozygous Rb metacentric chromosomes	Specimen	No. of chromosome arms	No. of spermatocytes II: nullisomic (0), euploid (1) or disomic (2) for chromosome 14 (left side of the slash) or chromsome 1 (right)								
			0/0	0/1	1/0	0/2	1/1	2/0	1/2	2/1	2/2
Rb[1.3]1Bnr, Rb[9.14]6Bnr	D3	18		1	3		8				
		19		4	6		14				
		20		1			114		2	1	
		21					11		13	7	
		22							1		
	D4	18	2		1		6				
		19		5	13		42				
		20			1		110		5	1	
		21					10		14	8	
		22									1
Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr	T3	18		1	2		9				
		19		12	5		28				
		20			2		105		2	3	
		21					23		3	9	
		22					2				1
	T4	18	3	2	1		4				
		19	1	7	14		16				
		20		1	3	1	106	1	4	1	
		21					19		8	5	
		22							1		
Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr, Rb[16.17]7Bnr	Q1	18	2	4	3		5				
		19		6	9		42				
		20			2	1	94	1	2	4	
		21					29		6	5	
		22				1	3		1	1	
	Q2	18		2	2		11				
		19		4	9		28				
		20			2		113		2	2	
		21					16		3	6	
		22					1		5		

Table 1d. Frequencies of spermatocytes II with 18–22 chromosome arms of mice with a heterozygous karyotype for one (Rb[4.6]2Bnr), three (Rb[1.3]1Bnr), (Rb[4.6]2Bnr), (Rb[9.14]6Bnr) or four (Rb[1.3]1Bnr), (Rb[4.6]2Bnr), (Rb[9.14]6Bnr), (Rb[6.17]7Bnr) Rb metacentric chromosomes classified for the number of copies of the chromosome 4 and 6 after dual-colour FISH painting

Heterozygous Rb metacentric chromosomes	Specimen	No. of chromosome arms	No. of spermatocytes II: nullisomic (0), euploid (1) or disomic (2) for chromosome 4 (left side of the slash) or 6 (right)								
			0/0	0/1	1/0	0/2	1/1	2/0	1/2	2/1	2/2
Rb[4.6]2Bnr	S3	18		1			2				
		19		9	8		13				
		20					129		2		
		21							9	10	
		22									
	S4	18									
		19		4	11		3				
		20					133				
		21					1		8	8	
		22									
Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr	T7	18		1	1		5				
		19		8	6		34		1	1	
		20			2		113		3	2	
		21					17		6	5	
		22							1	1	
	T8	18		1	1		6				
		19		3	6		28				
		20					108		2	2	
		21					19		9	7	
		22					1		1	1	
Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr, Rb[16.17]7Bnr	Q3	18		4	3		3				
		19		5	10		37				
		20		1			108		3		
		21					18		7	6	
		22					5		1	1	
	Q4	18		2	1		15				
		19		6	6		27				
		20		2			98		6	5	
		21					28		2	4	
		22					5				

Table 2. Mean nondisjunction rate in meiosis I of specific arms of specific trivalents (corresponding to chromosomes 1, 4, 6, 14) and of whole, specific trivalents (involving the Rb chromosome (Rb[4.6]2Bnr) evaluated in spermatocytes II of specimens heterozygous for one, two, three or four Rb metacentrics. Nondisjunction rate was estimated on the basis of data from one-colour and dual-colour FISH for chromosomes 1 and 6, on the basis of data from dual-colour FISH for chromosomes 4, 14 and (Rb[4.6]2Bnr). The estimated Ep values (multiplicative epistasis coefficients) are given in the last row.

Heterozygous Rb metacentric chromosomes	Mean nondisjunction rate (± SE) of specific arms of specific trivalents and of whole, specific trivalents and estimated epistasis coefficients				
	1	14	4	6	Rb[4.6]2Bnr
Rb[4.6]2Bnr			0.090±0.019	0.102±0.011	0.199±0.015
Rb[1.3]1Bnr, Rb[9.14]6Bnr	0.163±0.013	0.077±0.001			
Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr	0.135±0.024	0.119±0.008	0.080±0.008	0.111±0.009	0.177±0.008
Rb[1.3]1Bnr, Rb[4.6]2Bnr, Rb[9.14]6Bnr, Rb[16.17]7Bnr	0.120±0.008	0.091±0.023	0.086±0.006	0.093±0.021	0.179±0.015
Ep[a]	*0.850*	*1.067*	*0.986*	*0.993*	*0.962*

[a] Ep values are never significantly different from 1 (absence of epistatic interactions).

from 0 (Fig. 2) and, as a consequence, the values of Ep, given in Table 2, were never significantly different from 1. Therefore the existence of epistatic interactions among Rb heterozygous rearrangements, affecting the nondisjunction rates of the tested chromosomes, was not verified.

The overall frequency of aneuploidy spermatocytes II increases as the number of trivalents increases (20.12% in heterozygotes for one Rb rearrangement, 33.47% in heterozygotes for two Rb rearrangements, 37.07% in heterozygotes for three Rb rearrangements, 47.21% in heterozygotes for four Rb rearrangements), as expected.

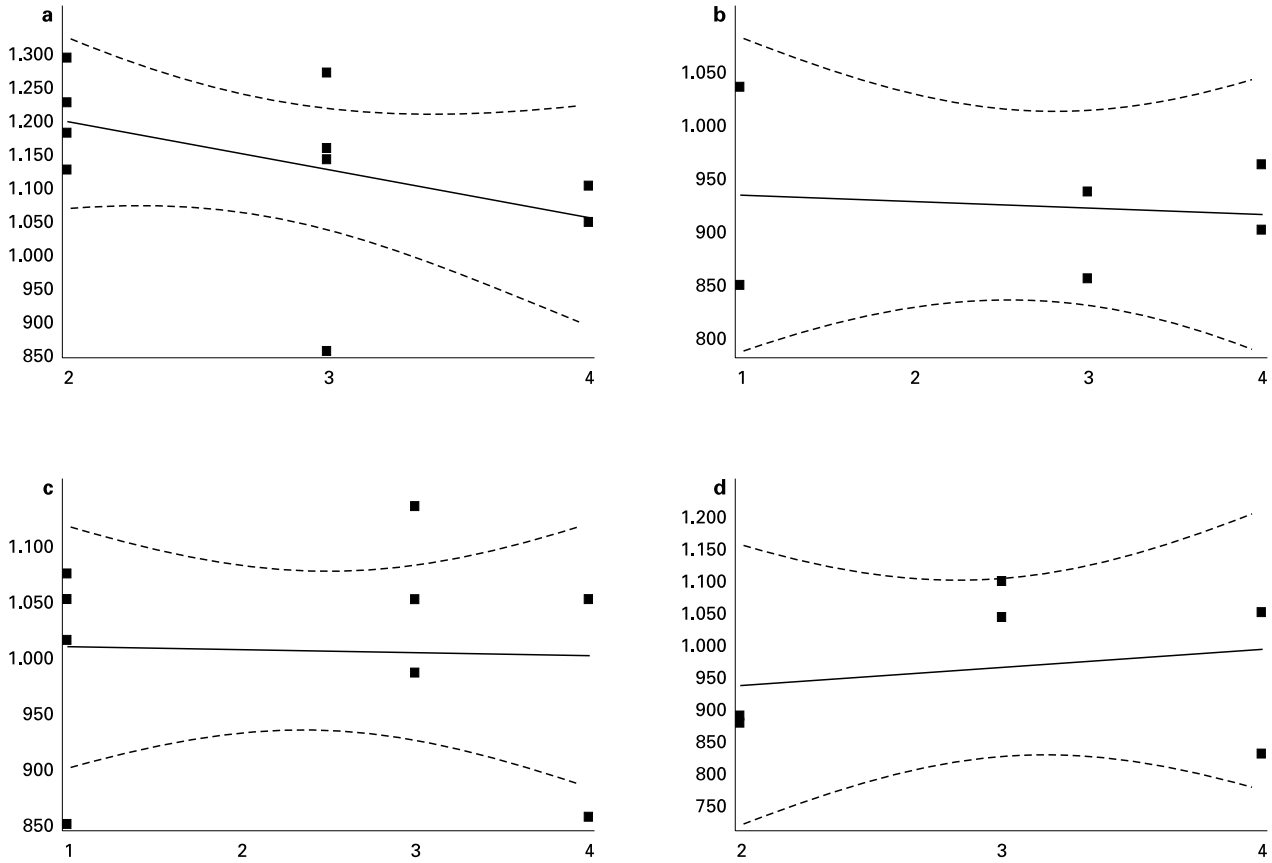

Fig. 2. Linear regression between the number of trivalents (in abscissa) and the log of the nondisjunction rates of specific chromosomes, expressed as percentages (in ordinate): (**a**) chromosome 1; (**b**) chromosome 4; (**c**) chromosome 6; (**d**) chromosome 14. Straight lines represent regression lines; full squares represent log of nondisjunction rate of single specimens; dashed curves represent 95% confidence values of ordinate values. The slopes are never significantly different from zero.

Discussion

Analysis of spermatocytes II by FISH allowed us to understand the contribution of individual chromosomes, involved in different Rb metacentric fusions, to the total amount of the observed aneuploidy in different karyotypes. The mean nondisjunction rate is rather similar for the different labelled chromosomes, although literature data suggest that this similarity could not be extended to the whole karyotype: in double heterozygous mice for the Rb metacentrics Rb(9.19)163H and Rb(16.17)7Lub, there is a remarkable difference in nondisjunction frequencies between the trivalents, from about 0.2% for the former, up to about 36% for the latter (Gropp et al., 1975; Winking and Gropp, 1983; Winking et al., 2000). Nondisjunction rates were estimated for the Rb metacentrics Rb(1.3)1Bnr and Rb(4.6)2Bnr in wild mice, heterozygous for single Rb metacentrics, caught in Northern Italy (Hauffe and Searle, 1998). The nondisjunction rate found for the latter (12%) was lower than the value observed in the present paper for the same chromosome (18–20%). Nondisjunction rates found for the former (14%) corresponds to the value observed in the present paper for one arm only – corresponding to chromosome 1 (12–16%). These differences might be due to a lower nondisjunction rate in wild than in laboratory heterozygous mice, but also to the staining technique.

As expected, nondisjunction cannot involve simultaneously both arms of an Rb trivalent: such a trivalent does not migrate, as a whole, to one meiosis I pole. This is demonstrated by the lack of any double nondisjunction involving chromosomes 4 and 6, which are involved in the same Rb centric fusion (Rb[4.6]2Bnr). On the other hand, nondisjunction events involving different trivalents are reciprocally independent, as testified by the frequency of double nondisjunction involving two trivalents (chromosomes 1 and 14).

The mating scheme by which we obtained the F_1 heterozygous specimens analysed in the present work allowed us to understand the effects of the one by one addition of Rb metacentrics in a similar genetic background on nondisjunction associated with a whole trivalent or with one arm. The nondisjunction rates for the tested chromosomes were not significantly affected by the number of trivalents in meiosis I. Epistatic interactions do not seem to play a significant role in nondis-

junction. However, data from wild mice caught in hybrid zones is required to ascertain the existence of possible epistatic interactions on nondisjunction rates and to evaluate their evolutionary role.

The frequencies of aneuploid spermatocytes II based upon the count of haploid and hyperhaploid cells (see Results) are rather low, if compared to the nondisjunction rates of specific, labeled chromosomes (Table 2). The use of hyperhaploid cells only to estimate aneuploidy is recommended to avoid overestimations of aneuploidy frequency due to the enrichment of hypohaploid cell populations scored due to artifactual chromosome loss from euploid cells. On the other hand, an unnegligible amount of such a loss may affect hyperhaploid cells too, so that some underevaluation of aneuploidy cannot be avoided. However, the greater accuracy of FISH methods suggests that results obtained by this method are much more reliable. Aneuploidy estimates present in literature based on traditional staining methods should be, therefore, reconsidered.

Appendix

Evolutionary effects of epistatic interactions affecting nondisjunction

In spite of the lack of evidence, in the present paper, of epistatic interactions among trivalents affecting the nondisjunction rate, it was investigated whether and to what extent possible epistatic interactions of this kind affect the karyotypical stability of small populations with Rb metacentric chromosomes and the gene flow occurring between these populations and larger populations with the standard karyotype, in order to evaluate their potential role in chromosomal speciation.

A classical two-population model was used. One population is infinitely larger than the other ("continent-island" model). Before the contact, the larger population is monomorphic for the standard karyotype and the small one is monomorphic for two Rb metacentrics. Then migration starts and occurs at a constant rate each generation. Continent-island models are fit to represent the early stages of speciation, when small, peripheral populations accumulate more and more genetic differences from the main, central population. It is assumed that epistatic interactions involve female germ line to the same extent as the male germ line.

The stability of a chromosomal race was measured as the value of the critical migration rate (mc) over which the metacentric chromosomes are lost in the small population (Spirito et al., 1991), on the basis of deterministic simulations. The values of mc were found on the basis of the nondisjunction rate of the trivalent in a single heterozygote (s) – as an approximation to the coefficient of selection against heterozygotes for a chromosomal rearrangement. The fitness of a double heterozygote is $(1 - Ep \cdot s)^2$, assuming the same value of s for both trivalents; Ep = 1, when there is no epistatic interaction.

An approximated equation was used to test how epistatic interactions between two Robertsonian rearrangements affecting nondisjunction rate modify the gene flow for biallelic, neutral genes. This gene may have different distances to either centromere, which is the rearrangement site. The reduction in gene flow is expressed as the equivalence to a reduction in the migration rate (Spirito et al., 1987):

$$MRE = r\,[1 - Ep \cdot s]^2/[1 - 0.5(1 - Ep \cdot s)^2(1 - r)]\,(0.5[1 - s][1 - r]/ [1 - (1 - s)(1 - r)] + 0.25[1 - s]/[1 - 0.5(1 - s)] + 0.5)$$

where MRE = migration reduction equivalent; s = nondisjunction rate of the trivalent in a single heterozygote (assuming the same value of s for both trivalents in the double heterozygote); r = recombination rate between the neutral locus and the centromere of a Rb rearrangement; this equation may be used for very low migration rates.

The s value chosen to evaluate mc and MRE was s = 0.2, on the basis of experimental data (Table 2); the values chosen of the multiplicative epistatic interaction (Ep) were Ep = 0.85, corresponding to the larger deviation observed in the present paper from the lack of epistatic interactions, i.e. Ep = 1 and its reciprocal (Ep = 1.18). Results are given in Table 3.

Table 3. Evolutionary effects of epistatic interactions among Rb heterozygous rearrangements affecting nondisjunction rate: critical value of the migration rate (m_c) between a small population, initially monomorphic for two Rb metacentric chromosomes, and a much larger one monomorphic for the standard karyotype, below which two Rb metacentric chromosomes are maintained in the small population and efficiency of the barrier against gene flow for a neutral gene, measured as the equivalence to a reduction of the migration rate (MRE), iin the presence of different values of recombination rate (r) between that gene and one of the rearrangements as functions of the values of the epistatic coefficient (Ep). Both m_c and MRE were estimated for s = 0.2 (s is the coefficient of selection against heterozygotes for a single chromosomal rearrangement).

Ep	mc	MRE			
		$r = 0.5$	$r = 0.1$	$r = 0.02$	$r = 0.004$
0.85	0.024	0.485	0.212	0.051	0.012
1	0.027	0.444	0.190	0.049	0.010
1.18	0.030	0.400	0.168	0.043	0.009

If Ep > 1, i.e. nondisjunction rate of a specific trivalent increases as the number of trivalents increases, the values of mc increase, leading to a stronger stabilization of the chromosomal race, while the values of MRE decrease, revealing a reduction in gene flow of a neutral gene between the small population and the larger one with the standard karyotype for any recombination rate between such a gene and the site of the chromosomal rearrangement, i.e. the centromere. This "positive" epistatic interaction would lead to a greater efficiency of underdominant chromosomal rearrangements as isolating factors. If, on the contrary, Ep < 1, i.e. nondisjunction rate of a specific trivalent decreases as the number of trivalents increases, the values of mc decrease and those of MRE increase. This "negative" epistatic interaction would weaken the efficiency of underdominant rearrangements as isolating factors.

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