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Biologia. — *Segregation disorders in multiple heterozygous Robertsonian mice.* Nota (*) di EMANUELA FERRI e ERNESTO CAPANNA, presentata (**) dal Socio A. STEFANELLI.

RIASSUNTO. — Il tasso di malsegregazione meiotica, valutato per mezzo del computo del numero delle braccia cromosomiche nelle M 2, è stato studiato in tre sorta di ibridi di laboratorio in topi multipli eterozigoti Robertsoniani, differenti sia per il numero di metacentrici in eterozigosi (7, 8 e 9 metacentrici), sia per la loro composizione (Lub 18–24/+, Lub 10–17/+ e Lub 1–9/+ rispettivamente). I valori di malsegregazione meiotica sono risultati simili nei tre tipi di ibrido e molto elevati, con frequenza di aneuploidia gametica compresa tra il 65.5% e il 67.5%.

Segregational disorders due to Robertsonian heterozygosity produce a fertility impairment in mice being of marked degree in multiple metacentric heterozygotes (Tettenborn and Gropp, 1970; Capanna, 1975; Winking and Gropp, 1976). This impairment is more severe in the heterogametic sex (Searle *et al.*, 1978). In fact the disjunction of trivalents in the diakinesis gives rise to a different malsegregation rate in males and females, easily revealed by computing the haploid fundamental number - i.e. number of chromosomal arms—in the meiotic M 2 (Winking and Gropp, 1976). The percent of unbalanced gametes originating from the meiotic process of hybrids is higher in the cases of double (Evans, 1975, Gropp, 1974; Gropp and Kolbus, 1974) and/or multiple metacentric heterozygosity than in those of chromosomal heterozygosity for single Robertsonian metacentrics. Nevertheless, it should be stressed that the observations of Ford (1972), Ford and Evans (1973) and Cattanach and Moseley (1973) demonstrated that the rate of malsegregation depends also, in Robertsonian single heterozygous mice, upon the metacentric being in the heterizygous state. In fact the values of the percent of the eu-haploid cells arising from the segregational process of such hybrids varies from 86.3 %, for the hybrid Rb (9.14) 6 Bnr/+, to 65.0 %, for the hybrid Rb 11.13) 4 Bnr/+, (Cattanach and Moseley, 1973).

It appears, therefore, that the rate of malsegregation in Robertsonian hybrid mice is controlled by both the "quantity" and the "quality" of the genoma present in the heterozygous state. Investigations were thus carried out to study the malsegregation rate in 3 kinds of multiple metacentric heterozygous mouse hybrids carriers of metacentric chromosomes different as far as the number and the Robertsonian arrangements are concerned. The following laboratory hybrids were studied:

i) hybrids by crossbreeding of 26-chromosome Lipari mice (Godena *et al.*, 1978) with NMRI all acrocentric laboratory mice; these hybrids have

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7 metacentrics in the heterozygous state, i.e. Rb (1.2) 18Lub, Rb (4.13) 19Lub, Rb (3.9) 20Lub, Rb (5.14) 21Lub, Rb (8.12) 22Lub, Rb (10.15) 23Lub and Rb (6.16) 24Lub [1].

ii) hybrids obtained by crossbreeding 24-chromosome mice from the Apennine ACR population (Capanna *et al.*, 1977) with the all-acrocentric NMRI mice which have 8 heterozygosities for the following metacentrics: Rb (1.2) 10Lub, Rb (5. 13) 11Lub, Rb (3.9) 12 Lub, Rb (4.17) 13Lub, Rb (6.16) 14Lub, Rb (8.14) 15Lub, Rb (10.12) 16Lub and Rb (11.15) 17Lub.

iii) hybrids obtained from 22-chromosome Orobian and Valtellina mice (Capanna and Valle, 1977) and NMRI laboratory mice showing 9 heterozygosities for the Robertsonian metacentrics: Rb (1.3) 1Lub, Rb (2.8) 2Lub, Rb (4.6) 3Lub, Rb (5.15) 4Lub, Rb (10.12) 5Lub, Rb (11.13) 6Lub, Rb (9.14) 7Lub, Rb (6.17) 8Lub and Rb (7.18) 9Lub.

The specimens investigated for each kind of hybrid were in all instances litters of the same couples and of approximately the same age. The number of specimens investigated is given in Table I. Male meiosis was investigated by means of the air-drying method of Evans *et al.* (1964). At least 200 2nd metaphases were counted for each kind of hybrid on account of the number of chromosomal arms—i.e. haploid fundamental number—and the number of metacentrics shown. All-acrocentric homozygous mice were also investigated. The chi-square (χ^2) test was used to check the sample (homogeneity and symmetry within the groups) and to test the differences between the groups. Results of the analysis are shown in Table I, and the values of the χ^2 are reported in Table II.

It appears from the data obtained (Tables I and II) that the segregational disorders affecting the meiotic preocess of the 3 kinds of hybrids studied here are very similar, reaching high values of gamete aneuploidy i.e. 66.5 % in Rb 1–9Lub/+, 64.5% in Rb 10-17Lub/+ and 67.5 % in Rb 18–24Lub/+. Moreover the rate of malsegregation found in the present research also corresponds to the segregational disorder revealed by Capanna (1975) in mice heterozygous for 9 Robertsonian metacentrics, i.e. in hybrid mice obtained by crossbreeding 22-chromosome mice from the Apennine population CD (Capanna *et al.*, 1974) with 40-chromosome mice; the Robertsonian repatterning of these 22-chromosome mice is quite different and is characterized by the metacentrics Rb 1–9Rma (see Capanna *et al.*, 1976). Consequently, diversity is not revealed by comparison of the malsegregation rate in different multiple metacentric heterozygous mice. It has to be assumed that the segregational disorder due to the high number of Robertsonian heterozygosities is so marked that the

(1) Nomenclature of the Robertsonian metacentrics is according to the rules of the Committee on standardized genetic nomenclature for mice ("J. Heredity", *63*, 69, 1972 and "Nouse News Letter", *50*, 1974). The arrangement of the acrocentric arms of different Robertsonian metacentrics is according to the paper of Gropp, Winking and Noack, "Mouse News Letter", *57*, 1976).

TABLE I.

HYBRID	Code	Age days	Arm counts: —19 hypomodal	Arm counts: 19 hypomodal	Arm counts: 20 = n	Arm counts: 21 hypermodal	Arm counts: +22 hypermodal	Total
Lub 18–24/+	Hy 19	83	4	3	3	3	—	13
	Hy 20	83	1	4	3	2	1	11
	Hy 21	83	1	3	2	5	—	11
	Hy 22	83	1	7	10	5	6	29
	Hy 23	83	2	4	5	3	2	16
	Hy 25	60	1	2	5	3	3	14
	Hy 26	60	11	21	33	21	10	96
	Hy 28	60	3	2	2	2	1	10
	Total . .		24	46	63	44	23	200
	Percent . .		34.5%		32.5%	33.0%		
Lub 10–17/+	Hy 29	46	4	4	8	5	4	25
	Hy 30	46	—	2	12	7	4	25
	Hy 31	46	1	3	2	3	—	9
	Hy 32	52	1	3	4	3	2	13
	Hy 33	63	21	14	40	19	17	111
	Hy 34	63	—	4	6	1	1	16
	Hy 35	63	2	9	9	5	5	27
	Total . .		29	39	81	33	33	226
	Percent . .		30.1%		35.5%	35.4%		
Lub 1–9/+	Hy 1	52	1	2	2	6	3	14
	Hy 2	57	1	3	3	2	3	12
	Hy 3	57	13	7	25	11	10	66
	Hy 4	57	2	—	3	—	2	7
	Hy 5	63	3	1	6	2	3	15
	Hy 6	66	1	4	10	—	2	17
	Hy 7	66	2	2	6	5	6	21
	Hy 8	73	1	2	2	4	2	11
	Hy 9	76	2	3	2	1	1	9
	Hy 10	73	3	2	4	5	3	17
	Hy 11	73	3	3	6	5	4	21
	Total . .		32	29	69	41	39	210
	Percent . .		28.0%		33.5%	38.5 %		
Homozygous All-acroc. MNRI	C 18	60	1	—	14	1	—	16
	C 37	60	1	2	13	1	—	17
	C 38	60	—	1	34	1	—	36
	C 39	60	—	—	55	3	—	58
	C 40	60	—	3	65	5	—	73
	Total . .		2	6	181	11	—	200
	Percent . .		4.0%		90.5%	5.5%		

TABLE II.

Chi-square values.

Tests of homogeneity and symmetry ($m \times n$)				Test of significant differences (2×2)		
Within the group	χ^2	Degrees of freedom	Probability	Between the groups	χ^2	Probability
Lub 1–9/+ . . .	14.98	20	$0.75 < P < 0.90$	Lub 1– 9/+ – All-acrocentr.	139.6	$P < 0.001$
				Lub 10–17/+ – All-acrocentr.	136.2	$P < 0.001$
Lub 10–17/+ . .	9.12	12	$0.50 < P < 0.75$	Lub 18–24/+ – All-acrocentr.	139.6	$P < 0001$
				Lub 1– 9/+ – Lub 10–17/+	0.013	$00.75 < P < 0.90$
Lub 18–24/+ . .	11.10	14	$0.50 < P < 0.75$	Lub 1– 9/+ – Lub 18–24/+	0.011	$0.75 < P < 0.90$
				Lub 10–17/+ – Lub 18–24/+	0.113	$0.50 < P < 0.75$
Homozygous All-acrocentr. . . .	11.92	8	$0.10 < P < 0.25$	Lub 18–24/+ – Bnr 1–7/+	17.02	$0.001 < P < 0.005$
				Lub 1– 9/+ – Rma 1–9	0.71	$0.25 < P < 0.50$

differences due to the dissimilarity of the genomas in heterozygous state are cancelled (Fig. 1). Nevertheless some significant differences are observed from the comparison of the frequency of unbalanced gametes produced by the Lipari×MNRI hybrids (7 heterozygosities, 18–24Lub/+) with of that the Poschiavo×40-chromosome hybrids (7 heterozygosities 1–9Bnr/+). In fact, according to the data of Tettenborn and Gropp (1970), the rate of malsegregation of these hybrids corresponds to 50% (Fig. 1). Although it is always difficult to compare data obtained by different research groups— on account of both the different methods employed and the different 40-chromosome material used in the mouse hybridization— these differences could

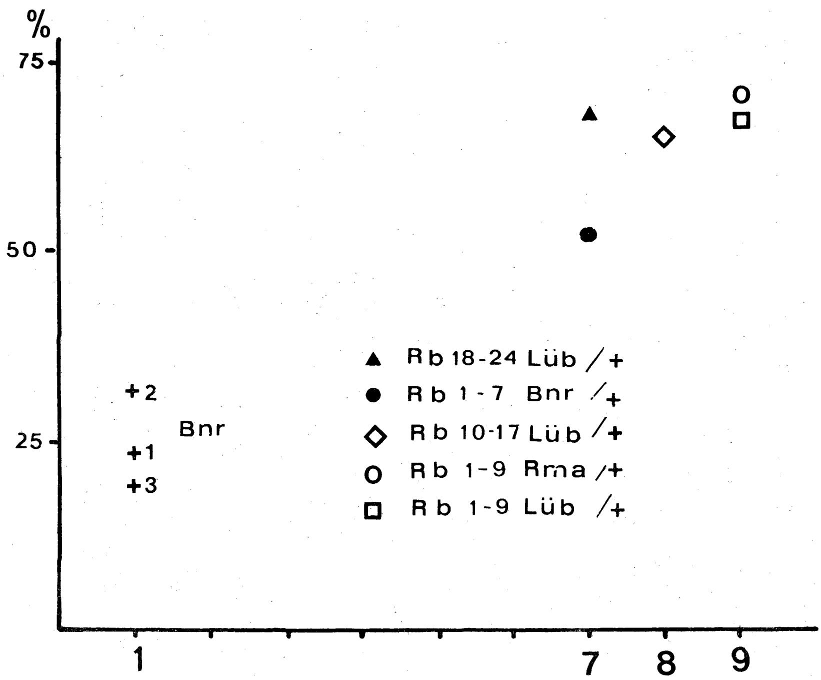
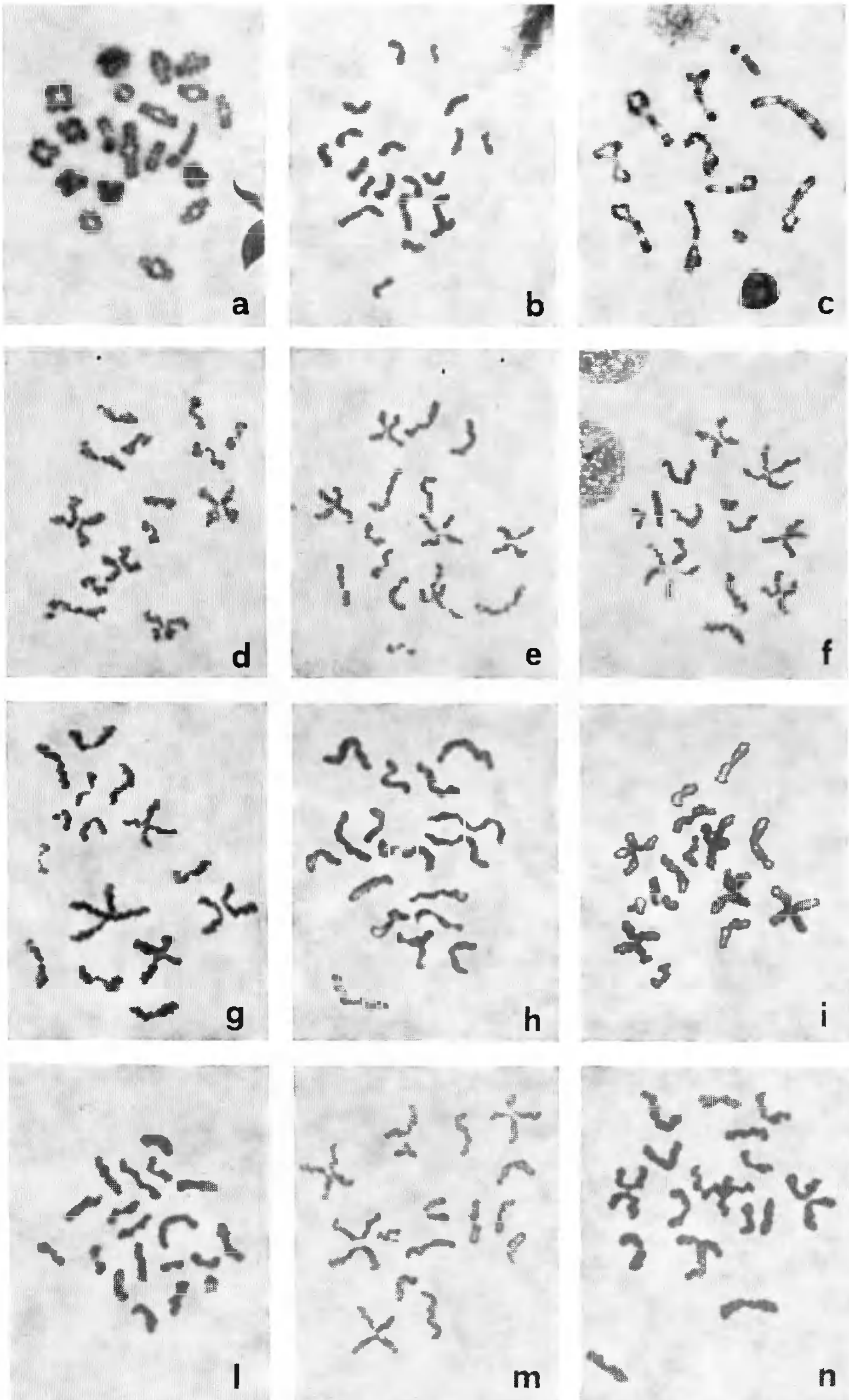


Fig. 1. – The frequencies of aneuploid cells are plotted against the number of Robertsonian heterozygosities (abscissa). The symbols are explained within the figure. Bnr 1, 2 and 3, single heterozygosity for different Poschiavo Metacentrics (Ford and Evans 1973).

be interpreted as a consequence of a true genoma difference of the Lipari and Poschiavo population.

As far as is concerned the pattern of the metacentric segregation in the diakinesis, revealed by their presence in the M 2, a symmetrical arrangement can be observed around a mode number of metacentrics equal to half of the metacentrics present in the hybrid.

In conclusion, it is possible to see that the segregational disorders due to the heterozygosity for different multiple Robertsonian metacentrics are similar independently of the "*quality*" of the genoma present in the heterozygous state. These differences in the malsegregation rate related to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the quality of the heterozygosity—detectable in the case of single metacentric heterozygosity—are masked by a more important disorder i.e. the large *quantity* of Robertsonally rearranged genoma present in the heterozygous state in the hybrids.

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EXPLANATION OF PLATE I

a) Diakinesis of 40-chromosome homozygous all-acrocentrics. *b*) M 2 of an eu-haploid cell of an all-acrocentric homozygous. *c*) Diakinesis of a Lub 1–9/+ hybrid showing 9 trivalents. *d*) ipo-haploid M 2 from Lub 1–9/+ hybrid. *e*) eu-haploid M 2 from Lub 1–9/+ hybrid. *f*) ipo-haploid M 2 from Lub 1–9/+ hybrid. *g*) ipo-haploid M 2 from a Lub 10–17/+ hybrid. *h*) eu-haploid M 2 from a Lub 10–17/+ hybrid. *i*) iper-haploid M 2 from a Lub 10–17/+ hybrid. *l*) ipo-haploid M 2 from a Lub 18–24/+ hybrid. *m*) eu-haploid M 2 from a Lub 18–24/+ hybrid. *n*) iper-haploid M 2 from a Lub 18–24/+ hybrid.