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Rate of meiotic malsegregation in hybrid mice: evaluation by microdensitometric measurement of spermatozoal Feulgen-DNA content

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Biologia. — *Rate of meiotic malsegregation in hybrid mice: evaluation by microdensitometric measurement of spermatozoal Feulgen-DNA content* (*). Nota di MARIA LAURA BELTRAMI (**), EMANUELA FERRI (***) e CARLO ALBERTO REDI (**), presentata (****) dal Socio A. STEFANELLI.

RIASSUNTO. — In tre tipi di ibridi di laboratorio F_1, portatori di eterozigosi strutturali multiple per fenomeni di fusione Robertsoniana, diversi sia nel numero dei metacentrici in eterozigosi (7, 8 e 9 metacentrici) sia per la loro composizione in braccia cromosomiche (Lub 18–24/+, Lub 10–17/+, Lub 1–9/+) rispettivamente, è stato valutato il contenuto in DNA (materiale Feulgen positivo) degli spermatozoi epididimali. I risultuti così ottenuti si sono rivelati in buon accordo con quelli ottenuti da Ferri e Capanna (1979) sullo stesso tipo di ibridi con la tecnica citogenetica di conta delle braccia cromosomiche alla metafase II.

The gametogenesis study of hybrid carriers of multiple structural heterozygosities in which a correct gametogenesis seems to be impeded (for oogenesis see Winking and Groop, 1976; for spermiohistogenesis Stolla and Gropp, 1974; Redi and Capanna, 1978; Hotta *et al.*, 1979) is interesting from two standpoints: first, it points up one of the factors by which natural selection acts in maintaining isolated the different *Mus musculus* populations karyotypically transformed (Capanna *et al.*, 1977) and second, it constitutes an interesting biological model for the characterization of the gametogenetic steps more sensitive to the structural alteration of the genome. The fact that laboratory mice can be produced carrying in homozygous and heterozygous condition different numbers and qualities of chromosomal Robertsonian fusions (Capanna *et al.*, 1976; Gropp et *al.*, 1979) offers the opportunity of studying these problems in favourable conditions. Analysis of the DNA content distribution of a spermatozoal population is one of the more suitable ways of defining the degree of fertility of an individual (Döring *et al*, 1972; Redi and Capanna, 1978). We have investigated in the same way the epididymal spermatozoal population of three different types of hybrids obtained by the crossbreeding of NMRI " all acrocentrics " laboratory mice with:

i) 26-chromosome Lipari mice (Godena *et al.*, 1978); these hybrids have 7 metacentrics in the heterozygous state, i.e. Rb(1.2) 18 Lub, Rb(4.13) 19 Lub, Rb(3.9) 20 Lub, Rb(5.14) 21 Lub, Rb(8.12) 22 Lub, Rb(10.15) 23 Lub and Rb(6.16) 24 Lub.

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ii) 24-chromosome mice from the Apennine ACR population (Capanna *et al*, 1977); these hybrids have 8 heterozygosities, i.e. Rb(1.2) 10 Lub, Rb(5.13) 11 Lub, Rb(3.9) 12 Lub, Rb(4.17) 13 Lub, Rb(6.16) 14 Lub, Rb(8.14) 15 Lub, Rb(10.12) 16 Lub and Rb(11.15) 17 Lub.

iii) 22-chromosome Orobian and Valtellina mice (Capanna and Valle, 1977); these hybrids carry 9 metacentrics, i.e. Rb(1.3) 1 Lub Rb(2.8) 2 Lub, Rb(4.6) 3 Lub, Rb(5.15) 4 Lub, Rb(10.12) 5 Lub, Rb(11.13) 6 Lub, Rb(9.14) 7 Lub, Rb(6.17) 8 Lub and Rb(7.18) 9 Lub.

We have performed the Feulgen reaction on epididymal spermatozoal smears according to the method of Itikawa and Ogura (1954); fixation in 10% formalin for 10 min; 1h in 5N HCl; 45 min in Schiff reagent; dehydration and mounting. The DNA content (Feulgen-positive material) was evaluated by

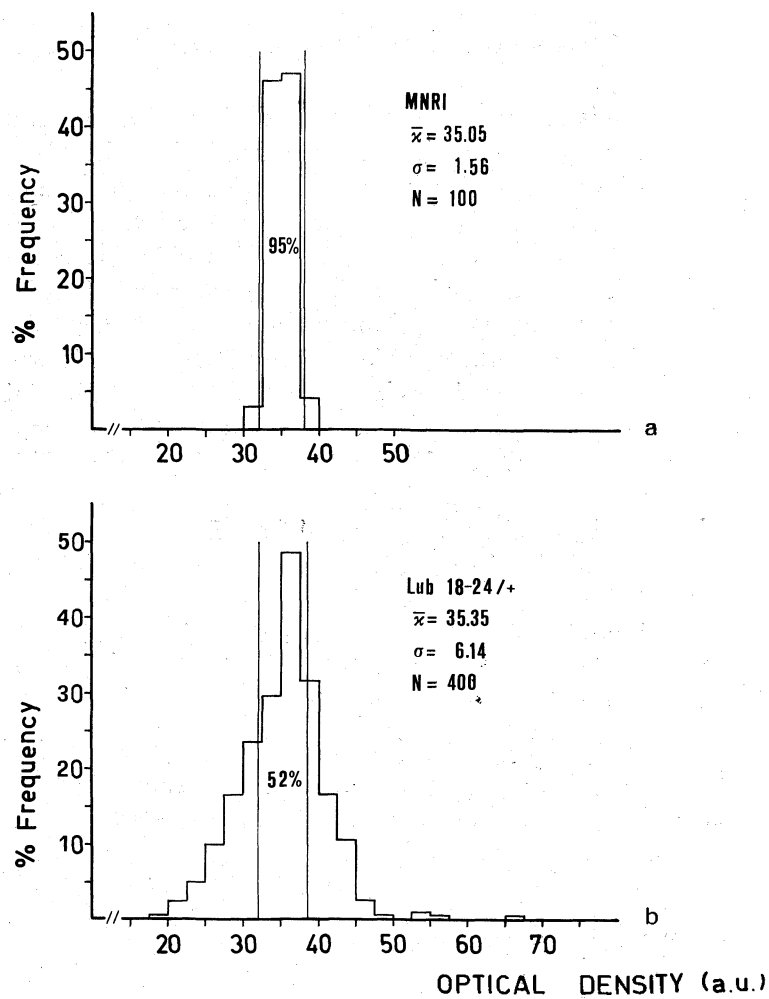

Fig. 1.

a Vickers M86 integrating microdensitometer at a wavelength of 545 ± 5 nm. As the comparisons of the Feulgen-DNA content all fall within the values normally referred to spermatozoa, we can here accept as homogeneous the

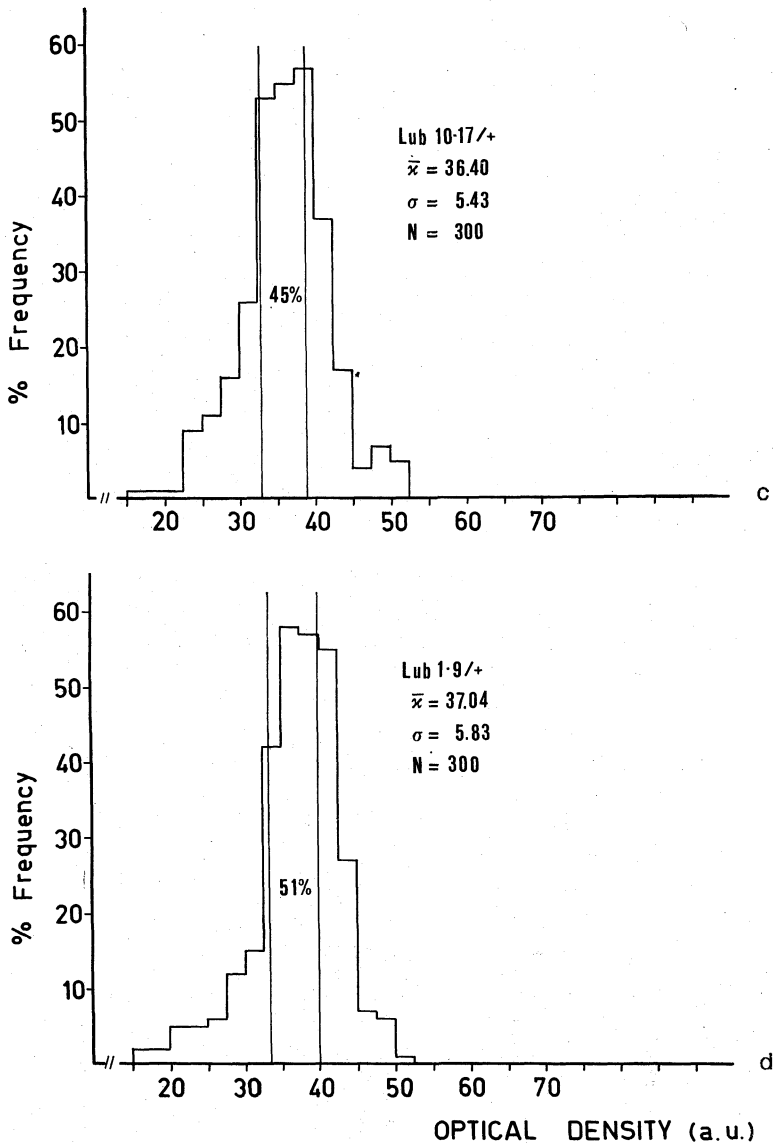

Fig. 2.

Figs. 1-2. – Percent spermatozoal DNA content distributions of: *a*) homozygous mice "all acrocentrics" NMRI; *b*) 4 hybrid mice Lipari ×NMRI; *c*) 3 hybrid mice Ancarano × NMRI; *d*) 3 hybrid mice Orobie ×NMRI. The thin lines cover the range of $\bar{x} \pm 2\sigma$ that we assume as "euploidy range" for the NMRI as well as for the hybrids. The euploidy range for hybrids is computed on the basis of their own means $\pm 2\sigma$ of the control population. Abscissa: optical density in arbitrary units. Ordinate: percent frequencies.

performance of the Feulgen reaction on a chromatin such as that of the spermatozoa, i.e. characterized by a high degree of packing able to mask a certain number of active sites for the hydrochloric-hydrolysis (for a discussion see Manfredi Romanini *et al.*, 1979). The distribution values of 100 spermatozoa of an MNRI male are used as a control. The percent distribution values of the spermatozoa DNA content for the three kinds of hybrids are shown in Figs. 1 and 2. The figures show that there is non statistically significant difference among the means even if there is a wider distribution of the values around the means of hybrids. It should be noted that the control distribution is a normal one and that 95 % of the values lie within the range of the mean plus or minus times the standard deviation.

Assuming the range of the mean of hybrids plus or minus 2σ as that with euploidy significance, the number of the spermatozoa outside these ranges is related to malsegregation rates.

TABLE I.

Percent frequencies of spermatozoa with an "euploidy" DNA content for each individual investigated. The hypo and hyper contents refer to spermatozoa with higher or lower Feulgen-positive material.

HYBRID		% HYPO	% « EUPLOID »	% HYPER
Lub 18–24/+ Lipari	1	31	57	12
	2	26	48	26
	3	23	55	22
	4	26	53	21
Lub 10–17/+ Ancarano	1	32	31	37
	2	28	50	22
	3	33	53	15
Lub 1–9/+ Orobie	1	26	45	29
	2	26	50	24
	3	27	58	15
Homozygous all-acroc. NMRI		2	95	3

In Table I we analytically report the number of cells with a DNA content falling outside the assumed euploid range. Whereas the data of malsegregation rates on individual variability is clearly present, this aspect of the problem is at the moment being investigated. In the three different kinds of hybrids, 50% of the cells produced lie on the average within the range of "euploidy" and there is no preferential production of spermatozoa with high or low DNA content. These results roughly correspond to those obtained by Ferri and Capanna (1979) in the same kind of hybrids by the cytogenetic method of counting the chromosomal arms in metaphase II preparations. Another point to be stressed is that differences in both number and quality of metacentrics influence fertility to the same extent.

It is possible to conclude that a cytochemical method is suitable for a fast screening of the male malsegregation rate. On the same type of male hybrids an investigation by flow cytofluorimetry is presently in progress in order to increase the statistical significance of these results, thereby also reducing timeconsuming steps in the method.

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