Oogenesis in common shrews homozygous and heterozygous for Robertsonian rearrangements

Autor(en): **Wallace, B.M.N. / Searle, J.B. / Garagna, S.**

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Oogenesis in common shrews homozygous and heterozygous for Robertsonian rearrangements[1]

BY

B.M.N. WALLACE[2], J.B. SEARLE[3] and S. GARAGNA[4]

Summary.–WALLACE, B.M.N., SEARLE, J.B. and GARAGNA, S., 1991. Oogenesis in common shrews homozygous and heterozygous for Robertsonian rearrangements. *In*: J. HAUSSER, ed. The cytogenetics of the *Sorex araneus* group and related topics. Proceedings of the ISACC's Second International Meeting. *Mém. Soc. vaud. Sc. nat. 19.1*: 23-31.

Oogenesis was examined in primigravid common shrews collected from the vicinity of the hybrid zone between the Oxford and Hermitage karyotypic races. There was a substantial pool of oocytes in both homozygotes and simple Robertsonian heterozygotes from the zone, almost certainly sufficient for the maximum natural reproductive output in every female. Germ cell atresia was low in both groups.

The mean number of preantral and antral follicles at the diplotene stage of meiosis was identical in the two karyotypic classes; the mean number of corpora lutea, however, was slightly higher in heterozygotes.

These results support the growing assumption that the differential in fitness between homozygotes and simple Robertsonian heterozygotes is small.

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[2]School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

[3]Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, U.K.

[4]Dipartimento di Biologia Animale and Centro di Studio per l'Istochimica del CNR, Università di Pavia, Piazza Botta 10, I - 27100 Pavia, Italia

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Résumé.–WALLACE, B.M.N., SEARLE, J.B. et GARAGNA, S., 1991. Oogenèse chez des musaraignes carrelet homozygotes et hétérozygotes pour des réarrangements robertsoniens. *In*: J. HAUSSER, dir. The cytogenetics of the *Sorex araneus* group and related topics. Proceedings of the ISACC's Second International Meeting. *Mém. Soc. vaud. Sc. nat. 19.1*: 23-31.

L'oogenèse a été examinée chez des musaraignes carrelet primipares récoltées au voisinage de la zone d'hybridation entre les races chromosomiques Oxford et Hermitage. Tant les homozygotes que les simples hétérozygotes robertsoniens présentent une réserve substantielle d'oocytes, qui peut certainement garantir une reproduction naturelle maximale à chacune des femelles. Le taux d'atrésie des cellules germinale est bas dans les deux groupes.

Le nombre moyen de follicules préantraux et antraux au stade diplotène de la méiose est identique dans les deux groupes; le nombre moyen de corps jaunes est cependant légèrement plus élevé chez les hétérozygotes.

Ces résultats confortent l'opinion de plus en plus généralement acceptée comme quoi la valeur adaptative des simples hétérozygotes robertsoniens est peu différente de celle des homozygotes.

INTRODUCTION

In a Robertsonian chromosomal rearrangement a pair of acrocentric chromosomes join at their centromeres to form a metacentric element. Species such as the common shrew (*Sorex araneus*) and house mouse (*Mus musculus domesticus*) are subdivided into numerous karyotypic races, each characterised by a specific set of Robertsonian metacentric chromosomes in the homozygous state (Fig. 1). Where these different races make contact and hybridise, a hybrid zone is formed in which karyotypic heterozygotes are produced. If the races differ such that for a Robertsonian metacentric in one race the corresponding homologous ancestral acrocentric chromosomes are found in the other race, then when hybridization occurs 'simple' Robertsonian heterozygotes (Fig. 1) will be produced. These should form trivalents at prophase I of meiosis. Robertsonian heterozygotes are expected to be less fertile than homozygotes (WHITE 1978) and there is evidence in favour of this assertion for various mammals (GROPP and WINKING 1981, REDI and CAPANNA 1988, SEARLE 1988).

One may expect simple Robertsonian heterozygotes to show physiological differences from homozygotes as a result of genic heterozygosity at particular loci (SEARLE 1988); there is some evidence that heterosis influences gametogenesis in both male and female shrews (GARAGNA *et al.* 1989, SEARLE 1990). Alternatively or additionally, the gametogenic process may be affected as a result of the presence of unusual heteromorphic configurations at the first division of meiosis. Firstly, trivalents may undergo anaphase I nondisjunction (giving rise to aneuploid gametes) due to incorrect orientation of the chromosomes on a spindle adapted to bivalents. Failure of chromosome pairing and chiasma formation at prophase I will have a similar effect. Secondly, if trivalents are incompletely paired during prophase I (and, in the

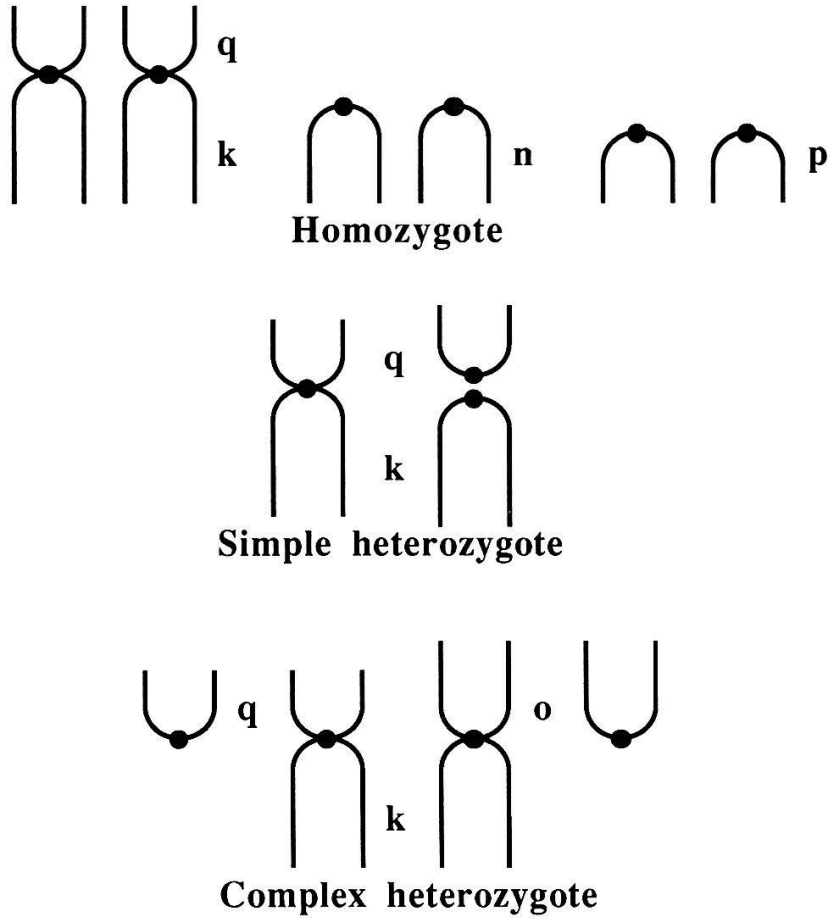

Figure 1.–Diagrams of various possible partial karyoptypes. The diagrams represent individuals that are, (top) homozygous metacentric for *kq* and homozygous acrocentric for chromosomes *n* and *p*, (middle) a single 'simple' Robertsonian heterozygote for arm combination *kq* and (bottom) a 'complex' Robertsonian heterozygote metacentrics *kq* and *ko*.

male, interact illegitimately with the sex body), there may be expression of genes inappropriate to this stage of meiosis (see BURGOYNE and BAKER 1984, SEARLE 1988 for reviews). The consequence of this behaviour is a general physiological disruption of meiosis leading to germ cell death.

Studies of the fertility and fitness of common shrews homozygous and heterozygous for Robertsonian rearrangements are essential for a thorough understanding of the structure of karyotypic hybrid zones, the extent to which gene flow between karyotypic races is interrupted and the consequences for speciation, in this species. Spermatogenesis has been studied in the common shrew for the vicinity of the hybrid zone between the Oxford and Hermitage races (GARAGNA *et al.* 1989). In and around this zone there are Robertsonian homozygotes, simple heterozygotes and 'complex' heterozygotes (whose karyotypes include different metacentrics with monobrachial homology, fig. 1). Here we present data on oogenesis of common shrews from the same hybrid zone, concentrating on the comparison of various homozygotes and simple heterozygotes.

MATERIAL AND METHODS

a. *Animals*

Pregnant female common shrews (*Sorex araneus*) of the Oxford karyotypic race were collected in Oxfordshire and Berkshire, U.K. during May 1983 and maintained under routine conditions for up to five days before they were killed (SEARLE 1983). All the animals used in this study were considered to be in their first breeding season and first pregnancy (SEARLE 1984).

b. *Karyotypes*

Chromosome preparations were made from bone marrow cells of the pregnant female by the method of FORD (1966) and stained with toluidine blue:resin (BRECKON and EVANS 1969). A G-banding staining method was also used to aid interpretation (SEARLE 1984).

c. *Counts of Follicles and Corpora Lutea*

The ovaries of each pregnant female were dissected out into culture medium or physiological saline, fixed in Bouin's fluid and embedded in paraffin wax. Serial sections 5-7 µm thick were cut through each ovary and stained with haematoxylin and eosin.

Sections were examined and follicles classed as follows (classification adapted from PETERS and MC NATTY 1980, see also fig. 2): Primordial follicles are those in which the oocyte is surrounded by one layer of flattened granulosa cells. Very early growing and early growing follicles had 1-2 and 2-5 layers of cuboidal granulosa cells surrounding the oocyte, respectively. Preantral and antral follicles were characterised by many layers of granulosa cells and antral fluid in spaces between the cells; for antral follicles, there is a large cavity composed of such fluid.

An estimate of the number of primordial oocytes was made by counting the nuclei of those cells appearing in each section and applying Abercrombie's correction to allow for nuclei which appeared in more than one section (ABERCROMBIE 1946).

Growing follicles were counted by tracing individual follicles through the ovarian sections, a process which was aided by drawing a diagram of every tenth section.

Corpora lutea were scored by examining sections of both left and right ovaries. Follicles, however, were scored in the left ovary only.

RESULTS

The ovaries of six pregnant female common shrews have been analysed; five of these animals represent a subset of the data given by SEARLE (1984). Four females were simple Robertsonian heterozygotes for one or two of the Oxford

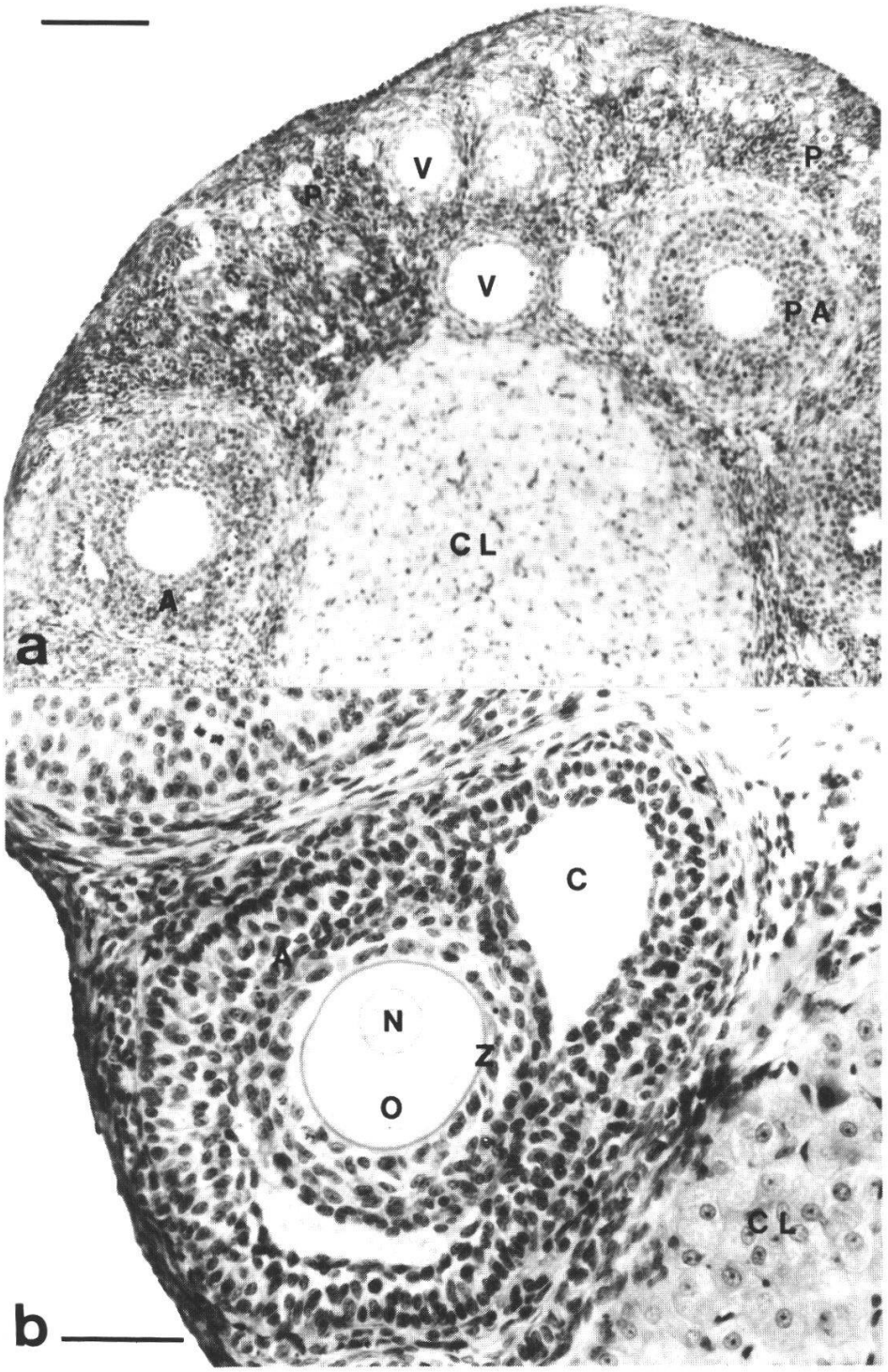

Figure 2.–Light micrographs from ovaries of common shrews. (a) Follicles at different stages of development in a homozygous shrew. Part of a corpus luteum is visible in the lower half of the section. Scale bar: 100 μm. (b) Antral follicle from a simple Robertsonian heterozygous shrew. Scale bar: 50 μm.

P: primordial follicle, V: very early growing follicle, PA: preantral follicle, A: antral follicle, CL: corpus luteum, C: antral cavity, N: nucleus, O: oocyte, Z: zona pellucida.

race metacentrics (*no*, *pr*) and would be expected to form one and two trivalents, respectively, at prophase I of meiosis. None were heterozygous for the Oxford race metacentric *kq*. The remaining two females were Robertsonian homozygotes; both were homozygous metacentric for the Oxford race chromosomes *kq* and *no* and one homozygous metacentric for chromosome *pr* and the other homozygous acrocentric for the chromosomes *p* and *r*. (See Fig. 1 for terminology.)

Inspection of the ovarian sections indicated that there was no substantial difference in appearence between those from left and right ovaries. Both ovaries of all shrews looked 'healthy' with little sign of atresia at any stage of folliculogenesis. The primordial follicles and the antral follicles measured approximately 20 μm and 300 μm in diameter, respectively. No binucleate or polyovular follicles were found amongst the growing follicles, in contrast to data for the human (GOUGEON 1981, B.M.N. Wallace unpublished results). The general appearance of the ovaries was in agreement with the description given by BRAMBELL (1935).

a. *Follicles and oocytes*

Mean follicle numbers at the different stages of folliculogenesis are presented in Table 1. Preantral and antral follicles are grouped together as the distinction between them was frequently not clear-cut; only those at the diplotene stage of meiosis were included.

The overall mean number of follicles (oocytes), both growing and non-growing was greater in homozygotes (2168) than heterozygotes (1586). However, considering growing follicles the mean was greater in heterozygotes (211.0) than homozygotes (146.0).

Although the number of preantral and antral follicles varied between individuals, the mean was identical in homozygotes and heterozygotes (21.0). The nuclei of the oocytes in these follicles were at the diplotene stage of meiosis prior to chromosome condensation and spindle formation. They are assumed to be the next cohort of oocytes to be recruited for ovulation. A number of oocytes had nuclei that had reached metaphase II, but from the appearance of the follicles, many of these were clearly atretic.

In both the simple Robertsonian heterozygotes and homozygotes there was a large pool of non-growing primordial follicles (Table 1) available for recruitment.

b. *Corpora lutea*

Corpora lutea were counted in sections of the right and left ovaries (Table 2); the values obtained are in agreement with counts made on the same ovaries by the 'fresh ovary method' (SEARLE 1984, 1990).

The number of corpora lutea varied between right and left ovaries and between individuals. The mean number was slightly higher in heterozygotes than homozygotes (Table 2) and agrees well with the mean number of

implants in homozygotes (7) and heterozygotes (8). This suggests that only one ovulation, represented by a corpus luteum, has failed to result in an implant in both the homozygous and heterozygous groups of shrews studied.

Table 1.–Mean number of follicles at different stages of folliculogenesis in the left ovary of homozygous and heterozygous common shrews.

Shrew category	Mean number of follicles			
	Primordial*	Very early growing	Early growing	Preantral and antral
Homozygotes	2022	111.0	14.0	21.0
Heterozygotes	1375	171.5	18.5	21.0

* Calculated using Abercrombie's correction for a section thickness of 7 μm

Table 2.–Total and mean number of corpora lutea and implants in homozygous and simple heterozygous common shrews

Shrew category	Number of corpora lutea			Number of implants
	Left ovary	Right ovary	Total	
Homozygotes (N=2)	11	4	15	14
Mean			7.5	7
Heterozygotes (N=4)	17	16	33	32
Mean			8.25	8

DISCUSSION

Our observations on the structure of the ovary and follicles in the common shrew concur well with those of BRAMBELL (1935). Large numbers of primordial follicles occupied the cortex while growing follicles of all stages were found deeper in the ovary. The pattern of development of the follicles followed the typical mammalian pattern.

There was no obvious difference in the appearance of the ovaries between homozygous and simple Robertsonian heterozygous shrews. However, the total oocyte pool was found to be larger in homozygotes, almost entirely due to greater numbers of non-growing oocytes. In contrast, the number of

growing oocytes tended to be greater in heterozygotes. Although these results are to some extent contradictory, it is clear that in both homozygotes and heterozygotes there were ample numbers of growing follicles to provide sufficient ovulations for the maximum productivity of the common shrew in Britain, which we estimate to be 3 or 4 litters of up to 9 or 10 young each (see BRAMBELL 1935, CROWCROFT 1957).

Thus, there is no evidence that, in terms of oocyte availability, simple Robertsonian female shrews from the hybrid zone between the Oxford and Hermitage karyotypic races are at any disadvantage relative to homozygotes from the same zone. The data for corpora lutea (Table 2) suggest that heterozygotes may actually ovulate more oocytes at their first oestrus than homozygotes and the same trend can be seen in a larger data set (SEARLE 1990). In contrast, there appears to be a higher frequency of anaphase I nondisjunction in female simple Robertsonian heterozygotes than in homozygotes (SEARLE 1990). However, overall, litter sizes are unlikely to differ much between female simple heterozygotes and homozygotes from the Oxford-Hermitage hybrid zone (SEARLE 1990). Similarly, there are unlikely to be large differences in fertility of male simple heterozygotes and homozygotes from the same zone (GARAGNA *et al.* 1989, MERCER *et al.* 1991). Therefore, in our attempts to understand the structure and evolution of the Oxford-Hermitage hybrid zone and its influence on gene flow, we must presume that the fitness differential between homozygotes and simple heterozygotes of both sexes, is small.

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