Spermatogenesis in several intra- and interspecific hybrids of the lemur (*Lemur*)

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Summary. Spermatogenesis of various hybrids of the genus *Lemur* was studied by testicular biopsy. In hybrids between species whose classification is still much debated, the germinal cells had degenerated after the pachytene stage. This abnormality was variable in intensity depending on the parents mating: *L. fulvus* (2N = 60) subspecies $\times$ *L. macaco* sometimes resulted in fertile offspring while *L. fulvus collaris* $\times$ *L. macaco* never did. We believe that the defect in spermatogenesis is mostly caused by differences in meiotic behaviour in the germinal cells. In hybrids between two species for which there is no taxonomic doubt (*L. fulvus* $\times$ *L. rubriventer*), the gonads were completely devoid of germ cells.

Introduction

Among the lemurs of Madagascar, numerous groups are still engaged in the process of speciation, a phenomenon which poses many problems in the classification of these animals. Such problems are particularly obvious for the genus *Lemur* with special reference to *Lemur fulvus* and *Lemur macaco*. Certain investigators such as Schwartz (1931) based their nomenclature on anatomical or biogeographical characteristics and considered these two animals belonging to the same species, *L. macaco*. On the other hand, some authors (Buettner-Janusch, Swomley & Chu, 1966) considered that each karyotype must inevitably correspond to a different species, which would therefore admit the existence of 4 separate species: *L. macaco* (2N = 44), *L. fulvus* (2N = 60), *L. collaris* (2N = 52) and *L. albocollaris* (2N = 48). The fundamental chromosome number of all species of this genus is the same, i.e. NF = 64, because Robertsonian translocations have played a major role in the chromosomal evolution of lemurs (Rumpler, 1975; Rumpler & Dutrillaux, 1976). Experiments employing hybridizations (Albignac, Rumpler & Petter, 1971) have shown that in captivity hybrids between *L. fulvus*, *L. collaris* and *L. albocollaris* are regularly fertile in both sexes, while hybrids between these 3 species and *L. macaco* are usually sterile. Rumpler (1975) therefore concluded that there exist only 2 species, i.e. *L. macaco* (2N = 44) and *L. fulvus* in which there is chromosomal polymorphism to give rise to the different subspecies: *L. fulvus fulvus*, *L. fulvus rufus*, *L. fulvus albifrons*, *L. fulvus mayottensis* (2N = 60), *L. fulvus collaris* (2N = 52) and *L. fulvus albocollaris* (2N = 48).

The existence of one fertile hybrid (Hamilton & Moses, 1979) between *L. macaco* and *L. fulvus* (out of 9 specimens studied) indicates that sterility after this type of mating is not invariable. Cross-breeding between *L. fulvus mayottensis* (2N = 60) and *L. rubriventer* (2N = 50) (the latter has always been classified in a separate species) also gives rise to hybrids but they are regularly sterile (Saint-Pie, 1970). We therefore evaluated the fertility potential of the different types of hybrids by using testicular biopsies to examine spermatogenesis.

Materials and Methods

We examined groups of male hybrids raised in the Zoological Park of Tananarive, Madagascar, and the School of Medicine, Strasbourg, France. The male parents of these hybrids were included in the controls. Since lemurs have a seasonal reproductive cycle, we have taken samples corresponding to the respective mating season in each hemisphere: December to February in Strasbourg and April to June in Tananarive.

The observations in the present study are based on 9 control specimens (3 *L. fulvus fulvus*, 2 *L. fulvus mayottensis*, 1 *L. fulvus rufus* and 3 *L. macaco*) and 9 hybrids (2 hybrids of *L. macaco* × *L. fulvus albifrons*; 1 hybrid of *L. fulvus rufus* × *L. fulvus albocollaris*; 3 hybrids of *L. macaco* × *L. fulvus collaris*; 3 hybrids of *L. fulvus mayottensis* × *L. rubriventer*) as presented in Table 1. Biopsy specimens were taken at the beginning and the end of the mating season for some animals (Z29 and Z12; Z26 and Z9; Z11 and Z27).

Biopsies were performed under general anaesthesia and the tissues were fixed by immersion in Bouin's fixative for 24 h. Samples were then dehydrated and embedded in paraffin wax. Sections (5 µm) were cut, mounted on microscope slides and stained in haematoxylin and eosin. We examined spermatogenesis at the level of the seminiferous tubule by counting 1000 cells per specimen and the 7 cell types described by Leblond & Clermont (1952a, b) were identified: (1) spermatogonium type A, having a spherical nucleus with finely granulated chromatin; (2) spermatogonium type B with an ovoid nucleus containing thick, wrinkled chromatin clumps; (3) spermatocyte I in the leptotene stage in which the nucleus and cytoplasm have a conspicuous volume while the chromatin appears in filamentous threads; (4) spermatocyte I in the zygotene stage in which the chromosomes form long cords and begin to thicken; (5) spermatocyte I in the pachytene stage, in which the cell has a nucleus reaching its maximum developmental stage and the chromosomes are short and thick; (6) cells in metaphase of the maturation division, characterized by the chromosomes on the equatorial plate; and (7) round and elongated spermatids. The elements of spermiogenesis were grouped in one class. Counts were made at different planes of sections in each biopsy to ensure that the resulting figures would be representative of spermatogenesis in the entire sample.

Analysis of results

The data are expressed as mean ± s.e.m. The spermatid percentages in relationship to the spermatogenic cells were analysed after angular transformation using the contrast method with the Scheffé test, thus allowing the percentages to be treated like quantitative variables. The following categories were compared: controls with regard to the different types of hybrids *L. fulvus* (2N = 60) × *L. fulvus albocollaris* (2N = 48); *L. fulvus* (2N = 60) × *L. macaco* (2N = 44); *L. macaco* (2N = 44) × *L. fulvus collaris* (2N = 52). The different categories were then compared between themselves.

The index constituted by the relation of the Sertoli cell number to the corresponding number of spermatogenic cells was first studied by two-way analysis of variance with orthogonal comparisons and then by the contrast method with Scheffé's test.

Results

In *L. macaco* (2N = 44) or different subspecies of *L. fulvus* (2N = 60), there was a progressive succession of the various cell stages in moving from the periphery to the lumen of the seminiferous tubule. The percentage of more mature cell types was a prominent feature in the homozygotes; spermatids and spermatozoa represented more than half of the total cell population (Pl. 1, Fig. 1).

In the fertile hybrid (Z12) of *L. fulvus rufus* (2N = 60) × *L. fulvus albocollaris* (2N = 48) the same progression was seen as in the pure controls; the percentage of each cell type was also near

that of the controls (Pl. 1, Fig. 2). The spermatid percentage was not significantly different from that of the controls.

In hybrids resulting from crosses *L. macaco* and *L. fulvus albifrons* ($2N = 60$), these were major variations between individual hybrids. In Hybrid Z9 only a few cells reached maturity. Although spermatogenesis appeared to have begun normally, after the pachytene stage a great majority of cells were degenerating: nuclei were pycnotic or undergoing chromolytic disaggregation and cell lysis (Pl. 1, Fig. 4). Several spermatids and spermatozoa did survive, but the number of cells counted had to be increased by including more tubules. In Hybrid Mt (Pl. 1, Fig. 3) all cell types were present but the number of mature elements was significantly less than that in control tubules. In this hybrid there appeared to be a similar blockage of development beyond the pachytene stage. The percentage of spermatids in relationship to the line cells of this category of hybrid was significantly less than that of the controls ($P < 0.05$).

In the 3 hybrids resulting from *L. macaco* and *L. fulvus collaris* ($2N = 52$), there was no significant variability between individual hybrids (Table 1) and again the more mature cell stages were fewer in number (Pl. 1, Fig. 5). Meiosis rarely continued beyond the pachytene stage and pycnotic nuclei and cells undergoing lysis were frequently seen. The production of spermatids was significantly less than in the controls ($P < 0.01$) but it was not significantly less than in the previous category of hybrids ($P > 0.1$).

For the Sertoli cell/spermatogenic cell index, there was no significant difference between *L. fulvus* ($2N = 60$) $\times$ *L. macaco* and *L. macaco* $\times$ *L. fulvus collaris* ($P < 0.01$). The three categories of

Table 1. The numbers of Sertoli and germinal cells counted and the percentage of various cell types in testicular biopsies in lemurs

Animals studied*	No. of cells counted		Germinal cell† %						
	Sertoli cells	Germinal cells	SpA	SpB	Lep	Zyg	Pachy	Met	Sptid
Z2 LFF	24	1405	2.5	1.8	5.4	8.6	19.3	2.6	59.5
Z3 LFF	29	1194	4.2	4.5	1.1	1.0	25.7	0	63.1
Z23 LFF	28	1211	4.5	6.2	5.2	4.1	20.6	0	59.1
Z25 LFR	30	1096	1.5	1.9	8.2	3.7	20.1	0	64.4
A1 LFM	77	1337	1.4	1.0	2.6	3.0	18.9	0.3	72.5
A2 LFM	103	1086	2.5	4.0	0.7	1.1	15.0	0	76.5
Z4 LMA	104	1250	1.9	3.4	0.8	1.6	18.8	0.2	73.0
Z7 LMA	148	1178	1.2	1.2	1.1	0.5	33.1	0	62.7
Z20 LMA	256	967	1.9	1.3	10.4	2.4	28.5	0.2	55
Z29 LFR $\times$ LFA	81	1166	1.8	2.2	1.8	0.7	13.2	0	80.1
Z12 LFR $\times$ LFA	71	1174	0.5	0.8	2.2	1.7	41.3	0	53.1
Z26 LMA $\times$ LFL	340	613	12.2	11.7	22.0	7.8	41.2	0	4.8
Z9 LMA $\times$ LFL	245	1002	5.3	5.1	9.3	13.3	66.6	0	0
Mt LMA $\times$ LFL	206	851	3.5	4.2	6.1	6.9	39.3	0.5	39.2
St LMA $\times$ LFC	204	809	5.9	6.3	6.6	6.7	54.3	17.4	2.4
Z10 LMA $\times$ LFC	205	958	7.5	6.2	8.3	2.8	69.7	0.7	4.5
Z11 LMA $\times$ LFC	295	846	0.4	0.8	13.9	23.8	59.1	1.7	0
Z27 LMA $\times$ LFC	299	757	4.6	4.7	13.4	6.3	60.7	8.8	1.1
A3 LRU $\times$ LFM	100	0	0	0	0	0	0	0	0
A4 LRU $\times$ LFM	100	0	0	0	0	0	0	0	0
A5 LRU $\times$ LFM	100	0	0	0	0	0	0	0	0

* LFF = *Lemur fulvus fulvus*; LFR = *Lemur fulvus rufus*; LMA = *Lemur macaco*; LFA = *Lemur fulvus albocollaris*; LFL = *Lemur fulvus albifrons*; LFC = *Lemur fulvus collaris*; LFM = *Lemur fulvus mayottensis*; LRU = *Lemur rubriventer*.

† SpA = spermatogonia A; SpB = spermatogonia B; Lep = spermatocyte I, leptotene; Zyg = spermatocyte I, zygotene; Pachy = spermatocyte I, pachytene; Met = metaphase II; Sptid = Spermatid.

controls, *L. fulvus* (2N = 60) $\times$ *L. albocollaris*, and the above group differed overall ($F_{1,4}^2 = 4.54$, $P < 0.05$), but Scheffé's test indicated a difference only between the controls and *L. macaco* $\times$ *L. fulvus collaris*.

The cause of sterility in hybrids of *L. rubriventer* $\times$ *L. fulvus fulvus* is evident. The seminiferous tubules of those hybrids were completely depleted of germ cells and contained only Sertoli cells (Pl. 1, Fig. 6). The pathology in this case appears analogous to that known as the 'Sertoli cell only syndrome' observed in man (del Castillo, Trabucco & de la Balze, 1947).

Discussion

The histology of testicular biopsies of the hybrid between *L. fulvus albocollaris* (2N = 48) and *L. fulvus rufus* (2N = 60) was very similar to that of the controls and this type of hybrid is fertile in the present conditions of captivity.

Crosses between *L. macaco* (2N = 44) and the *L. fulvus* (2N = 60) subspecies yield offspring which are usually sterile although Hamilton & Moses (1979) reported one case in which such a hybrid successfully mated three times. The histological variation in spermatogenesis between these hybrids, indicates why certain specimens may have a less serious disruption of spermatogenesis and may even be fertile. Furthermore, as in men with sterility problems, it is possible that a male with a relatively low degree of fertility can have progeny if he mates with a particularly fecund female.

Crosses between *L. macaco* (2N = 44) and *L. fulvus collaris* (2N = 52) produce sterile offspring. Spermatogenesis is strongly disrupted and, in the three hybrids studied, the majority of cells degenerated shortly after the pachytene stage; spermatids and spermatozoa were therefore rare in the seminiferous tubules.

The seminiferous tubules of hybrids between *L. fulvus mayottensis* $\times$ *L. rubriventer* show a complete absence of germinal cells. However, it is not possible to tell from these histological specimens whether the germinal cells degenerated in the tubules post-natally (Lutz-Ostertag, 1965) or during embryonic development.

The 5 varieties of lemurs studied differ among themselves at the chromosomal level by displaying Robertsonian translocations (Rumpler, 1975; Rumpler & Dutrillaux, 1976). A relation could exist between the number of chromosomal rearrangements which have occurred and the disruption of spermatogenesis, especially as the different subspecies of *L. fulvus* with 2N = 60 regularly produce fertile hybrids (Rumpler, 1975). Other factors, however such as the genome of the individual hybrid or even the gene component of the translocated chromosomes (Cattanach & Moseley, 1973; White, Crandall, Raveché & Tjio, 1978) may contribute to the condition. It is surprising that the existence of 6 centric fusions between *L. fulvus rufus* (2N = 60) and *L. fulvus albocollaris* does not perturb spermatogenesis, as has been reported for other species, and the phenomenon of increased chromosomal rearrangements existing between subspecies of *L. fulvus* (2N = 60) and *L. macaco* cannot alone explain spermatogenic malfunction in those hybrids.

The chromosomal pairing in hybrids between *L. fulvus* (2N = 60) subspecies and the different species or subspecies of which the karyotype was derived from that of *L. fulvus* (2N = 60) by centric fusions, includes trivalents. In spite of the relatively high proportion of trivalents the spermatogenesis of these hybrids is nearly normal (*L. fulvus rufus* $\times$ *L. fulvus albocollaris*) or disturbed, but to a varied degree in some specimens (*L. fulvus albifrons* $\times$ *L. macaco*) such that at least some fertile hybrids can occur (Hamilton & Moses, 1979).

However, spermatogenesis is strongly disrupted and regularly results in sterility of the hybrids between the different species or subspecies of which the karyotype is derived from that of *L. fulvus* (2N = 60), i.e. *L. fulvus collaris* $\times$ *L. macaco*. The Robertsonian translocations which occurred in the different karyotypes did not concern the same chromosomes, and the chromosomes form elongated chains during meiotic pairing in these hybrids (Dutrillaux & Rumpler, 1977) as has been reported by others (Gropp, Tettenborn & Von Lehmann, 1970; Forejt, 1979). Therefore, for

PLATE 1

Testicular sections of *Lemur fulvus fulvus* (Fig. 1), *L. fulvus rufus* $\times$ *L. fulvus albocollaris* (Fig. 2), *L. fulvus albifrons* $\times$ *L. macaco* (Figs 3 and 4), *L. fulvus collaris* $\times$ *L. macaco* (Fig. 5) and *L. fulvus mayottensis* $\times$ *L. rubriventer* (Fig. 6). Spermatogenesis has progressed further in Fig. 3 than in Fig. 4 despite the fact that both hybrids are of similar type. There is a total absence of germinal cells in Fig. 6. H & E; $\times$ 40.

hybrids between two discrete species for which there are clear taxonomic relationships, e.g. *L. fulvus* and *L. rubriventer*, spermatogenesis is null. When the germinal cells are completely lacking, the resulting sterility is not caused by a defect in meiosis due to an accumulation of centric fusions, but rather to genetic factors.

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