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Organization of Seminiferous Epithelium in Primates: Relationship to Spermatogenic Efficiency, Phylogeny, and Mating System[1]

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ABSTRACT

The succession in time and space of specific germ cell associations, denoted as spermatogenic stages, is a typical feature of mammalian spermatogenesis. The arrangement of these stages is either single stage (one spermatogenic stage per tubular cross-section) or multistage (more than one spermatogenic stage per tubular cross-section). It has been proposed that the single-stage versus multistage arrangement is related to spermatogenic efficiency and that the multistage arrangement is typical for hominids. In the present work, the arrangement of spermatogenic stages and the spermatogenic efficiency of 17 primate species, comprising Strepsirrhini (Prosimians: Lemuriformes, Lorisiformes), Platyrrhini (New World primates), Catarrhini (Old World primates), and Hominoidea (great apes and humans), were analyzed comparatively by quantitative histological and flow cytometric means. We found a predominant single-stage tubular organization in the Strepsirrhini, indicating that the single-stage form represents the ancestral state. The highest degree of multistage complexity was found in Hominoidea (except orangutan) and in Platyrrhini, but not in Catarrhini. Hence, no direct relationship between single-stage/multistage tubular topography and phylogeny could be established across primates. In fact, the tubule arrangement seen in Platyrrhini and Catarrhini primates is the reverse of what might be expected from phylogeny. Interestingly, spermatogenic efficiency was similar in all species. We found no correlation between single-stage/multistage arrangement and spermatogenic efficiency or mating system. We speculate that the presence of a single-stage/multistage organization might simply reflect germ cell clonal size. Our findings further indicate that sperm competition in primates is not reflected at the level of testicular function.

gametogenesis, male sexual function, sperm, spermatogenesis, testis

INTRODUCTION

Spermatogenesis comprises the proliferation of spermatogonial stem cells, recombination of the genetic information during meiosis, and spermiogenesis. Although the sequence of these events is the same among all mammals, the underlying topography of the germ cell associations (i.e., spermatogenic stages), the functional and topographic relationships between the germ cells and Sertoli cells, and the efficiency of spermatogenesis (i.e., germ cell loss and spermatid production per unit testicular parenchyma) may vary in a species-specific manner (for review, see [1]).

In rodent testes, seminiferous tubular cross-sections contain a single spermatogenic stage (denoted hereafter as single-stage tubules), whereas human tubular cross-sections usually display multiple stages (denoted hereafter as multistage tubules) [2, 3]. Initially, it was thought that the presence of multistage tubules was a feature peculiar to the human testis, but this spermatogenic stage arrangement is also present in chimpanzees [4]. It has been proposed that the human multistage organization corresponds to a helical/spiral arrangement of spermatogenic stages [5], but this view has been challenged [6, 7]. Among other nonhuman primates, single-staged tubules prevail in macaques [8–10], and an intermediate type of organization has been reported for the olive baboon [11]. Interestingly, recent data show that the common marmoset (*Callithrix jacchus*), a neotropical (New World) primate that diverged from the human line some 35×10^6 years ago, also displays a multistaged germinal epithelial organization remarkably similar to that in humans [12, 13]. Among the nonanthropoid species, the only data available so far, to our knowledge, involve the gray mouse lemur (*Microcebus murinus*), in which tubular organization is single staged [14].

Thus, the limited information available presents a rather confusing picture, and to date, no adequate explanation exists either for the variation in tubule arrangement per se or for its pattern and distribution within the primate Order. It was originally suggested that multistage tubular organization, as seen in humans, is associated with low spermatogenic efficiency [1, 15]. However, more recent studies using the optical dissector stereological technique [16] have indicated that human spermatogenesis is more efficient than previously thought, and the finding of a highly efficient spermatogenic process despite a multistage organization in the marmoset suggests that topographic arrangement may not, in fact, be related to efficiency of spermatogenesis [13, 17, 18]. Further data concerning other species displaying a multistage arrangement are needed to confirm this. As an alternative, Aslam et al. [14] proposed that the topography of tubule arrangement in primates may be associated with the workload of the Sertoli cell; that is, Sertoli cells are associated with a higher number of germ cells in testes that show a single-stage arrangement. Again, this needs to be tested with a larger data set, and furthermore, the physio-

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TABLE 1. Germ cell associations (stages I–VI) referring to the criteria previously described for primate spermatogenesis.[a]

Stage					
I	II	III	IV	V	VI
Spermatid d Spermatid a	Spermatid d Spermatid a	Spermatid b	Spermatid b	Spermatid c	Spermatid c Spermatocytes II
Pachytene	Pachytene	Pachytene Preleptotene	Pachytene Leptotene	Pachytene Leptotene	Pachytene Zygotene
B	B			B	B
Apale	Apale	Apale	Apale	Apale	Apale
Adark	Adark	Adark	Adark	Adark	Adark

[a] See [3, 10, 13, 18]. Adark, Apale, B, spermatogonia; Spermatocytes II, secondary spermatocytes; spermatid a–d, spermatids at different steps of development.

logical significance of any such association remains to be determined.

In evolutionary terms, the distribution of tubule-arrangement types among the primates is also difficult to explain. The finding of a single-stage arrangement in the gray mouse lemur (Prosimian) suggests that this is the ancestral characteristic, but why the marmoset has a multiple tube arrangement, which is no longer seen in macaques but reappears in apes and humans, is far from clear. Data regarding more species and additional taxonomic groupings are clearly needed to confirm and explain the taxonomic distribution of tubule topography among primates.

Thus, the initial aim of the present study was to extend the database to attempt a better explanation for the evolutionary significance of the observed variations. This has been achieved through systematic study applying standardized methodology to a total of 17 species representing the major taxonomic groups within the primate Order. A second aim was to investigate to what extent variation in tubule arrangement and organization of germ cell associations is reflected by functional differences in germ cell production (i.e., spermatogenic efficiency or Sertoli cell workload) as determined by flow cytometric and stereological analysis. Finally, we have examined a possible relationship between topography of germ cell association and differing needs for sperm production associated with the contrasting mating systems seen among primates.

MATERIALS AND METHODS

Histology

Testes were obtained from the following Prosimians: *Microcebus murinus* (gray mouse lemur; n = 4) and *Otolemur* sp. (greater bushbaby; n = 1); New World monkeys: *Callithrix jacchus* (common marmoset; n = 2), *Saguinus fuscicollis* (saddle-back tamarin; n = 2), *S. oedipus* (cotton top tamarin; n = 2), *Saimiri sciureus* (squirrel monkey; n = 2), and *Cebus apella* (brown capuchin; n = 3); Old World monkeys: *Cercopithecus aethiops* (vervet monkey; n = 3), *Macaca fascicularis* (long-tailed, crab-eating, or cynomolgus monkey; n = 2), *M. thibetana* (tibetan macaque; n = 2), *M. nigra* (celebes black macaque; n = 1), *Papio hamadryas* (hamadryas baboon; n = 4), and *Mandrillus sphinx* (mandrill; n = 2); and great apes: *Pongo pygmaeus* (orangutan; n = 1), *Pan paniscus* (bonobo and pygmy chimpanzee; n = 1), *P. troglodytes* (chimpanzee; n = 2), and *Homo sapiens sapiens* (human; n = 4). The monkey tissues were obtained from various primate-keeping facilities via the German Primate Center (Göttingen).

Four human testis samples were obtained from patients with prostate cancer and were provided via Dr. T.G. Cooper from the Institute of Reproductive Medicine. All men gave informed consent for the use of tissue. Testes of these patients revealed normal spermatogenesis. Analysis was confined to samples from adult male monkeys, which as far as was possible to determine were all in a reproductively active phase and showed normal spermatogenesis. Parts of human and monkey testes were fixed in Bouin solution and stored in 70% ethanol before they were embedded in resin (hydroxethyl-methacrylate; Technovit 7100; Heraeus Kulzer GmbH, Wehrheim, Germany) and prepared for stereological analysis and assessment of germ cell associations.

Analysis of Spermatogenic Stages

To analyze the germ cell associations histologically, thin sections (thickness, 5–7 μm) were prepared and stained with periodic acid-Schiff (PAS) as previously described [13]. To standardize the quantitative analysis of single-stage/multistage tubules, a common staging approach had to be applied for all species to achieve comparable data among different primates. It was decided to adopt the six-stage classification already described for the human testis [18, 19], because this classification has already been used in marmosets [13] and cynomolgus monkeys [10]. Table 1 shows the germ cell association criteria that were used to categorize spermatogenesis into six stages.

One-hundred seminiferous tubules per testis were analyzed and evaluated. Only roundish tubular cross-sections were examined. Among the investigated species, several possible distributions of germ cell association stages were found (predominantly single-staged, predominately multistaged, or a mixture of both) (Fig. 1). If a tubular cross-section contained more than one spermatogenic stage, each stage was counted separately (Fig. 1), yielding more than 100 stages per 100 tubules.

As demonstrated before, this approach allowed us to determine the incidence of each of the six germ cell association stages in a primate testis [13]. In addition, the average number of spermatogenic stages per tubule was calculated, as was the proportion of single-stage and multistage tubules. To compare testicular organization and germ cell association patterns quantitatively among species, the abundance of spermatogenic stages was expressed in terms of the relative stage frequency as follows:

$$RSF_{STAGE} = 100/N \times n_{STAGE} (\%)$$

where RSF_{STAGE} is relative stage frequency, N is the sum of all stages divided by 100 tubular cross-sections, and n_{STAGE} is the number of the specific stage (I–VI) per 100 tubules. All data are presented as the mean ± SD.

Flow Cytometry

Testes (n) from *Microcebus murinus* (gray mouse lemur; n = 4), *Eulemur coronatus* (crowned lemur; n = 4), *Callithrix jacchus* (common marmoset; n = 4), *Saguinus fuscicollis* (saddle-back tamarin; n = 4), *Saguinus oedipus* (cotton top tamarin; n = 7), *Saimiri sciureus* (squirrel monkey; n = 10), *Cebus apella* (brown capuchin; n = 3), *Macaca fascicularis* (long-tailed, crab-eating [or cynomolgus] macaque; n = 4), *M. thibetana* (tibetan macaque; n = 3), *M. silenus* (liontail macaque; n = 5), *Papio hamadryas* (hamadryas baboon; n = 6), and *Mandrillus sphinx* (mandrill; n = 6) obtained from various primate-keeping facilities by the German Primate Center were analyzed. No samples from Hominoidea were available for flow cytometric analysis.

Sample Preparation and Evaluation

Samples of the testes of the animals were snap-frozen in liquid nitrogen and then stored at −80°C until analysis. Pieces of the samples (between 1 and 8.8 mg) dissected from the central part of the testes were thawed, weighed, minced finely in a commercially available staining buffer (Cystain, Partec, Münster, Germany) A) containing 4′-6-diamidino-2-phenylindole (DAPI; Partec), and dissolved in 0.5% (w/v) pepsin solution for 20

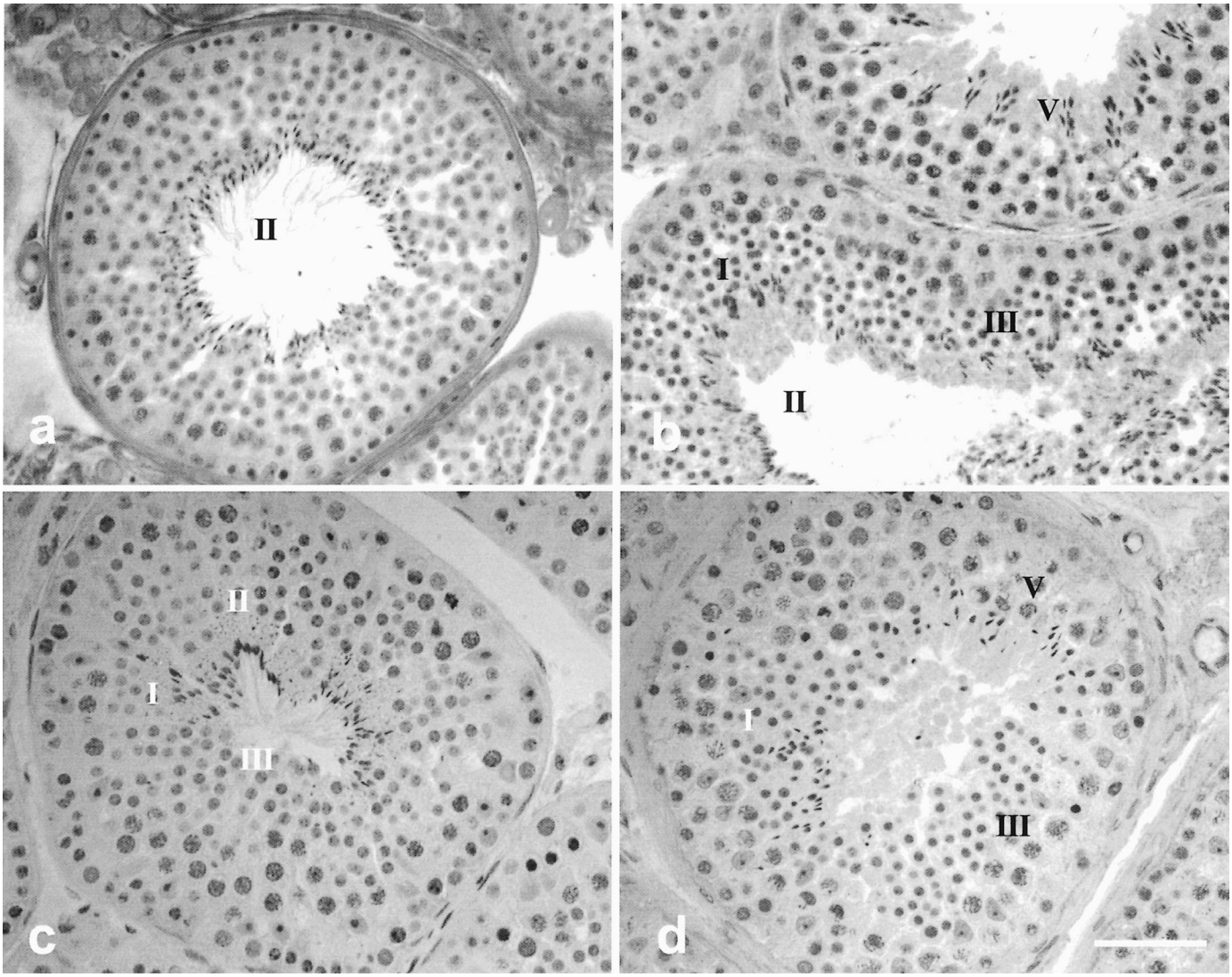

FIG. 1. Distribution of spermatogenic stages (single-stage/multistage). **a**) Predominantly single-staged arrangement (*Otolemur* sp.). **b**) Mixed arrangement between single-stage (V) and multistage (I, II, and III) tubular cross-sections (*Papio hamadryas*). **c** and **d**) Predominately multistage arrangement in *Saguinus fuscicollis* (**c**) and *Pan paniscus* (**d**). The numbers indicate the germ cell association stage situated at this site of the tubulus. If a tubular cross-section contained more than one spermatogenic stage, each stage was counted separately. Bar = 50 μm.

sec. The DAPI was chosen for DNA staining because flow background signals despite the use of frozen testicular tissue [20, 21] remains low. The DAPI staining and Partec flow cytometer were specifically chosen, because this approach had been validated previously for frozen testicular tissue and for similarity of yield of all cell populations [22–24].

The DAPI-stained cells were analyzed using the PAS III flow cytometer (Partec) equipped with mercury arc lamp for ultraviolet excitation for measurement of cellular DNA and the FlowMax software (Partec) for data acquisition and analysis. Hacker-Klom et al. [25] previously described the composition of a testis DNA histogram. Four peaks, representing the various testicular developmental cell stages, can be separately discriminated by their DNA content [25, 26]. The first peak contains haploid elongated spermatids with condensed chromatin. The second peak contains haploid round spermatids. The third peak comprises the diploid cells (spermatogonia, preleptotene spermatocytes, and Sertoli and Leydig cells), and the fourth peak contains mainly primary spermatocytes (4C) synthesizing DNA [25, 26]. The numbers of cells per gram testicular tissue were computed. Furthermore, a meiosis index (number of cells that entered the meiotic phase divided by total cell count) were computed. Data are expressed as percentages, per gram testicular parenchyma, and per testis.

Stereological Analysis

In terms of single-stage/multistage organization, chimpanzees and New World monkeys were closest to humans in the present work. To characterize further the spermatogenic process quantitatively, stereological analysis was performed for those species with testicular tissue available for plastic embedding and optical dissector analysis and for which corresponding stereological data were not available. Testes from *Saguinus oedipus*, *S. fuscicollis*, and *Saimiri sciureus* were prepared as previously described [13], and three sections per testis were evaluated using a systematic, uniform, random sampling approach [16]. Sections (thickness, 25 μm) were stained with periodic acid and hematoxylin as previously described [13].

Using the optical dissector method [16, 17], the number of germ cells per testis was determined (assuming that nuclear number equals cell number). A 100× oil-immersion lens with a numerical aperture of 1.3 was utilized (Axioscop; Zeiss, Oberkochen, Germany) equipped with an ocular net grid and a device to measure section thickness. Microscopic fields for counting were selected using a systematic, uniform, random sampling scheme [27]. Sixty frames were evaluated per animal. The upper surface of the section was brought into focus, and the first 3 μm were ignored to avoid interference from surface imperfections. The next 10–18 μm were then examined by counting the cells when the nuclei came sharply into focus according to the dissector principle as previously described [28]. The ratio between testis volume and weight was assumed to be nearly 1:1 [29], and the numerical density of each cell type was calculated by dividing the number of enumerated cells by the volume of all dissectors [13]. The germ cells were grouped as previously described [13]. Type A and type B spermatogonia, preleptotene to zygotene spermatocytes, pachytene spermatocytes, round and elongated spermatids, and Sertoli cells were

TABLE 2. Single and multistage tubule topography in selected species across the primate order.

Species	No. of subjects/ testes	No. of tubules	Average no. of stages per tubule[a]	Multistage tubules (%)[a]	Mating system (category)[b]
Prosimians					
Otolemur sp.	1/2	200	1.11 ± 0.06	10.5 ± 6.36	MM (2)
Microcebus murinus	4/4	400	1.28 ± 0.09	24.5 ± 7.9	MM (2)
New World monkeys					
Callithrix jacchus	2/2	200	2 ± 0.1	73.25 ± 3.89	MON (1)
Saguineus fuscicollis	2/2	200	1.66 ± 0.04	56.67 ± 3.06	PolyA (2)
Saguinus oedipus	2/2	200	1.91 ± 0.13	72 ± 5.66	PolyA (2)
Saimiri scireus	2/2	200	1.71 ± 0.07	59 ± 1.41	PolyG (2)
Cebus apella	3/6	600	1.65 ± 0.12	55.5 ± 7.61	PolyA (2)
Old World monkeys					
Cercopithecus aethiops	3/6	600	1.34 ± 0.08	32.5 ± 7.65	MM (2)
Macaca fascicularis	2/2	200	1.22 ± 0.09	20.5 ± 7.77	MM (2)
Macaca nigra	1/2	200	1.36 ± 0	32.5 ± 2.12	MM (2)
Macaca tibetana	2/4	400	1.32 ± 0.01	30.5 ± 1.91	MM (2)
Mandrillus sphinx	3/3	300	1.43 ± 0.05	39 ± 2.65	MM (2)
Papio hamadryas	4/8	800	1.45 ± 0.08	39.375 ± 6.36	PolyG (1)
Great apes/humans					
Pan troglodytes	2/2	200	2.26 ± 0.12	88 ± 2.83	MM (2)
Pan paniscus	1/2	200	2.26 ± 0.16	91 ± 4.24	MM (2)
Pongo pygmaeus	1/1	100	1.69	55	DIS (2)
Homo sapiens	4/4	400	2.03 ± 0.05	79.5 ± 3.11	MON/PolyG (1)

[a] Data are expressed as the mean ± SD.
[b] Mating systems: MON, Monogamous; MM, multimale; PolyG, polygynous; PolyA, polyandric; DIS, dispersed. Mating categories: 1, Monandry; 2, polyandry.

counted separately. The data obtained were normalized using Sertoli cell numbers, because Sertoli cells do not divide in the adult testis. The ratio of all germ cells to Sertoli cell number represents the germ cell load.

Statistics

Statistical analyses were performed by Student *t*-test, one-way ANOVA, and post-hoc Tukey test.

RESULTS

Arrangement of Spermatogenic Stages

Table 2 summarizes the quantitative analysis of single-stage and multistage tubules. On average, the two Prosimian species exhibited the lowest number of stages per tubule, with fewer than 25% of tubules displaying multiple-stage arrangements. Old World species showed a higher proportion of multistage tubules (>30%, except for *Macaca fascicularis*), but single-stage tubules predominated. The mean number of stages per tubule was highly consistent within the four genera examined (range, 1.2–1.4). In contrast, all New World species studied were predominantly multistaged (56–73%), with values for the mean number of stages per tubule ranging between 1.65 (*Cebus apella*) and 2.04 (*Callithrix jacchus*). Of the two major taxonomic groups represented, the two highest values were for callitrichid species, although this trend was not continued in *Saguinus fuscicollis*, which exhibited values comparable to those of the two cebids (Table 2). All hominoid species (great apes and humans) were also predominantly multistaged. Values for *Pongo pygmaeus*, however, were considerably lower than for chimpanzee species and humans.

Relative frequencies for each of the six stages of germ cell association are shown in Table 3. Stage 1 occurred most frequently in almost all the New World and Old Word species examined. In contrast, stage 3 predominated among the great apes and humans. The overall tendency was for multistage arrangements to be associated with a predominance of later spermatogenic stages (III–VI) (Fig. 2a), although no significant correlation was found between the two parameters. Generally, a high degree of similarity was observed within the major taxonomic groupings of the New and Old World monkeys, with the exception of *Cercopithecus aethiops*, in which stage 4 was much more frequently represented than in any of the other species. The two Prosimians showed clear differences between each other. *Microcebus murinus* had an exceptionally high proportion of stage 1 tubules, whereas stage 2 predominated in the *Otolemur* sp. (Table 3). No obvious relationship was found between pattern of germ cell association and taxonomic grouping. The main points in this area are: 1) Prosimians show marked differences to most other species, 2) macaques show consistent differences to the great apes and humans as well as to *C. jacchus* and *S. oedipus*, and 3) a high degree of similarity generally was found between species within the Old and New World taxonomic groupings, with the exception being some differences between *Mandrillus sphinx* and *Macaca fascicularis*. No obvious relationship was observed between the average number of stages per tubule and the various mating strategies. The three species with a predominantly monandrous mating system fell well within the range of values obtained for those species in which females regularly mate with multiple males (i.e., polyandrous) (Table 2 and Fig. 2b).

Expressing the data in terms of a correlation between the mean number of stages per tubule and the percentage of multiple stages allows the relationship between tubule arrangement and taxonomic grouping to be visualized more easily (Fig. 3). Data points are clustered according to taxonomy (with the exception of *Pongo pygmaeus*, *Otolemur* sp., and *S. fuscicollis*), but little overlap is found. Within the anthropoid species, the relative positions for Old World monkeys and New World monkeys, however, are opposite to what would have been expected from their phylogenetic position.

Meiotic Index, Efficiency Index, and Testicular Cell Composition

Cell counts per gram tissue were similar in all species examined (200–350 $\times$ 10^6 cells/g), with the exception of

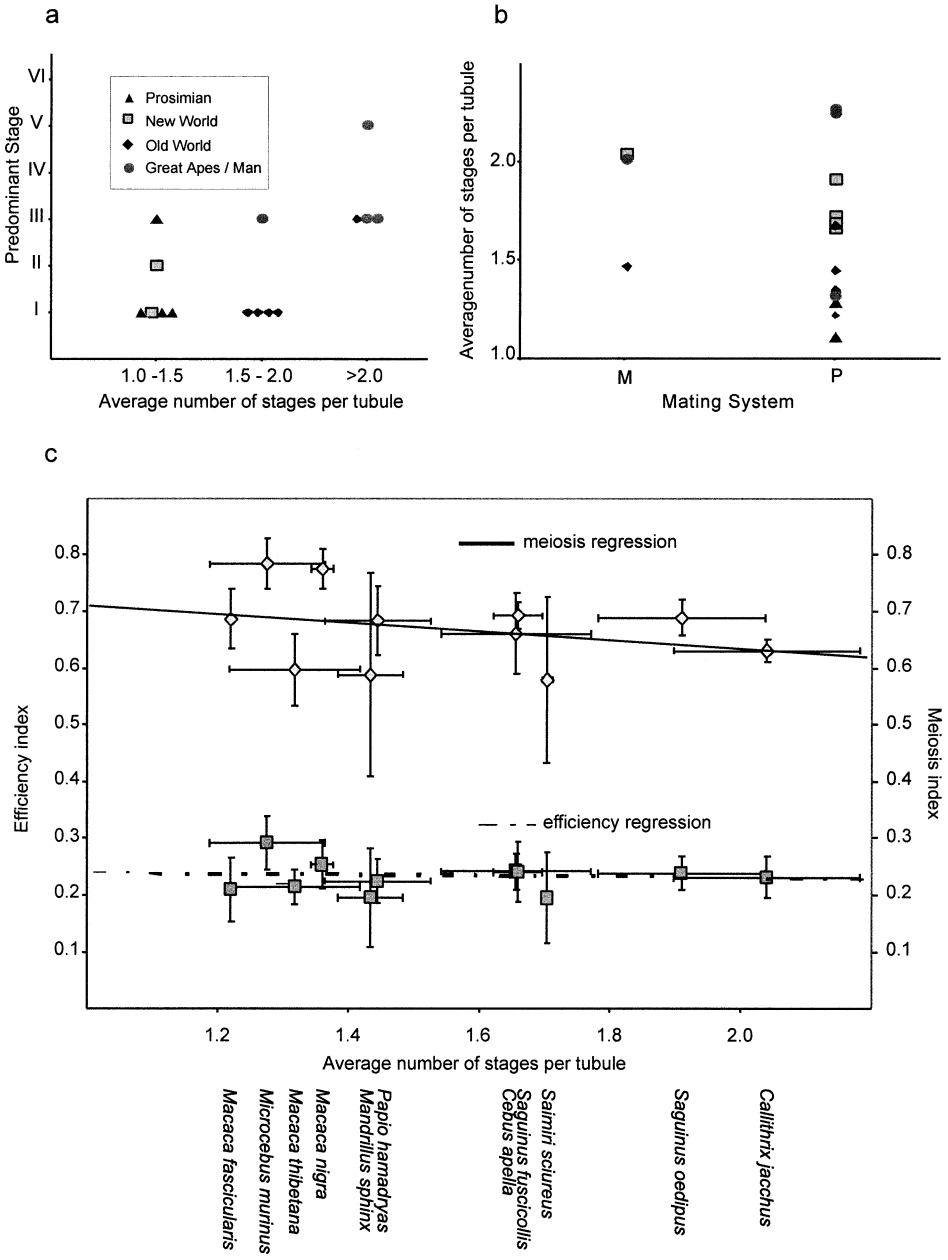

FIG. 2. **a**) Correlation of the mean number of stages per tubule and the predominantly abundant stage reveals clusters according to the taxonomic groups. Only a few exceptions were found. Strepsirrhini have predominantly single-stage tubular organization, and stage I was most abundant. In the Catarrhini, stage I is paralleled with an average of approximately 1.5 stages per tubule. Hominoidea showed multistage organization and predominant stage III or V, whereas Platyrrhini combine mainly multistage organization and stage I. **b**) No correlation was observed when monandric (M) or polyandric (P) mating strategy and the mean number of stages per tubule were related to each other. **c**) The correlation reveals that organization of the germinal epithelium does not influence meiosis index or spermatogenic efficiency, which therefore are independent from tubule arrangement and similar in all investigated species, as shown by regression analysis. Efficiency index is defined as number of elongated cells per total cell number. Meiosis index is defined as the number of haploid cells divided by the total cell number. The error bars give SD.

Microcebus murinus, in which numbers were clearly elevated and more than 600×10^6cells/g were found (Fig. 4a). Values for the efficiency index (number of elongated, postmeiotic germ cells divided by the total cell count) and the meiosis index (number of cells that entered the meiotic phase divided by total cell count) were similar in all species examined. Although the efficiency index in *Microcebus murinus* was higher than in the others, the difference was not statistically significant. Furthermore, no relationship was found between either parameter tested and tubule arrangement by statistical analysis using Student *t*-test (Fig. 2c).

The two Prosimian species examined differed markedly from each other. The proportions of elongated and round spermatids were considerably lower in *Eulemur coronatus* than in *Microcebus murinus*, whereas the number of diploid cells in *E. coronatus* exceeded that in *M. murinus* by more than 4-fold. Cell stage distribution in both species, however, was different from that seen in the two anthropoid groups (Fig. 4b).

A close similarity in developmental cell stage distribution was found within the Catarrhini species. In all cases, round spermatids were present in the highest proportion (40–55%), and approximately 20% of testicular cells were represented by elongated spermatids. The small differences seen in the proportions of diploid cells were mainly evident within the macaques (15% in *Macaca thibetana* to 25% in *M. fascicularis*) rather than between the different genera. In general, a similar pattern was seen among the Platyrrhini species, with round spermatids also being the most abundant cells in all species. Proportions of both elongated and diploid cells were similar both between species and compared with each other (20–25%), whereas primary spermatocytes varied slightly (between 10% and 15%). Similar relationships were also found when the data were expressed as total cell numbers per gram testicular tissue (Fig. 4c).

Stereological Analysis

The numbers of the various germ cells (spermatogonia type A [A], spermatogonia type B [B], preleptotene to

TABLE 3. Relative stage frequency of germ cell associations in selected species across the primate Order.[a]

Species	Stage frequency (%)					
	I	II	III	IV	V	VI
Prosimians						
Otolemur sp.	19 ± 2.3	**23.5 ± 1.3**	18.5 ± 2.2	9.9 ± 3.3	19.4 ± 0.9	9.6 ± 2.5
Microcebus murinus	**44.3 ± 7.5**	17 ± 3.2	8.5 ± 2.7	8.6 ± 3.9	15.68 ± 3	6 ± 1.5
New World monkeys						
Callithrix jacchus	26 ± 3.5	8.8 ± 0.3	**27.6 ± 3**	11.5 ± 1.4	16.4 ± 0.6	7.1 ± 2.9
Saguinus fuscicollis	**31.3 ± 5.3**	6.6 ± 0.5	17.8 ± 5	13.6 ± 2.9	24.1 ± 0.3	6.4 ± 1.5
Saguinus oedipus	**33.3 ± 2.6**	16.7 ± 2.3	21.1 ± 1.5	7.5 ± 2.2	16 ± 4.3	6.2 ± 1.9
Saimiri scireus	**31.7 ± 4**	5 ± 1.2	22.9 ± 10.9	12.3 ± 2.4	21.4 ± 2	6.7 ± 1.2
Cebus apella	**32.9 ± 6.3**	11.3 ± 3.8	17.9 ± 5.7	11 ± 4.3	20.5 ± 3.5	6.5 ± 1.7
Old World monkeys						
Cercopithecus aethiops	**27.2 ± 4.1**	14.2 ± 5.8	10.7 ± 2	25.2 ± 5.9	18.2 ± 4.4	4.2 ± 1.1
Macaca fascicularis	**39.8 ± 7.7**	15.5 ± 4.6	13.3 ± 2.2	9.3 ± 2.2	17.2 ± 2.2	6.5 ± 1.8
Macaca nigra	**30.1 ± 4.9**	16.1 ± 2.1	18.1 ± 3.7	11.5 ± 0.5	16.9 ± 3.1	5.6 ± 2.6
Macaca thibetana	**29.9 ± 5.4**	16.2 ± 1.9	19.8 ± 2.9	12.5 ± 2.6	16.7 ± 3.3	5.7 ± 1.8
Mandrillus sphinx	21.9 ± 3.5	8.9 ± 4.3	**28.1 ± 6.5**	12.8 ± 4.5	22.3 ± 2.9	5.8 ± 0.9
Papio hamadryas	**30.6 ± 5.2**	14.1 ± 3.2	19.3 ± 2.9	11.2 ± 2.7	18.8 ± 4.2	6.3 ± 2.2
Great apes/humans						
Pan troglodytes	28.4 ± 1.8	4.9 ± 1.7	**31.7 ± 5.3**	12.4 ± 1.9	18.5 ± 1.8	3.7 ± 2
Pan paniscus	16.7 ± 1.7	3.8 ± 1.1	30 ± 1.1	12 ± 0	**32.8 ± 3.5**	5.1 ± 1.3
Pongo pygmaeus	22.5	11.83	**28.4**	14.8	17.2	5.9
Homo sapiens	15.5 ± 2.6	4.4 ± 1.3	**34 ± 3.3**	11.2 ± 2.7	30.3 ± 3.2	4.8 ± 1.8

[a] For the stage I–VI germ cell associations, the relative stage frequency was determined from 100 tubules. Data are presented as the mean ± SD. The highest abundancies (intraspecies) are printed bold, and the lowest are underlined.

zygotene [Pl-Z], pachytene spermatocytes [P], round spermatids [Rs], elongated spermatids [Es], and Sertoli cells [SC]) were evaluated in the three Platyrrhini species: *Sanguinus fuscicollis*, *S. oedipus*, and *Saimiri sciureus*. For all developmental cell stages, *S. sciureus* showed the highest total cell counts ($\times 10^6$) per testis (A: 45 ± 10; B: 81 ± 3.5; Pl-Z: 194 ± 146; P: 522 ± 256; Rs: 895 ± 56.5; Es: 1016 ± 652; and SC: 133 ± 41.7), *S. oedipus* the lowest (A: 13 ± 2.8; B: 26 ± 0.7; Pl-Z: 43 ± 7; P: 153 ± 17.7; Rs: 344 ± 7.8; Es: 365 ± 12; and SC: 29 ± 2.8), and *S. fuscicollis* an intermediate organization (A: 47 ± 5.7; B: 33 ± 1.4; Pl-Z: 89 ± 0.7; P: 279 ± 82; Rs: 406 ± 132; Es: 499 ± 48; and SC: 51 ± 3.5). Similarly, by expressing germ cell numbers per Sertoli cell (relative germ cell load; SC workload), the lowest ratios were found for *S. sciureus* (20.9 ± 2.1), the highest ratios for *S. oedipus* (32.7 ± 2.4), and intermediate ratios for *S. fuscicollis* (26.2 ± 3.4). If the single germ cell stages were expressed per Sertoli cell to assess the ratios for the gradually maturing germ cells, only nonsignificant variations were observable.

DISCUSSION

Given the single tubular organization in rodents [1] and a comparable arrangement in both major taxonomic groups

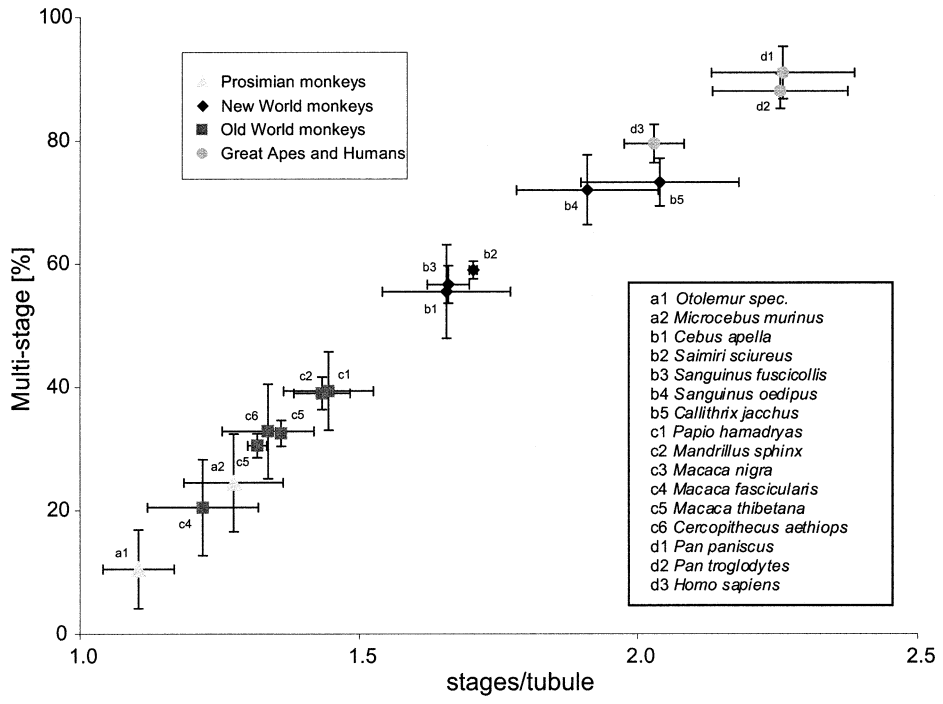

FIG. 3. Correlation between the percentage of multistage tubules and the average number of stages per tubular cross-section. The linear correlation elucidates that the species belonging to a certain systematic entity are grouped together. Overlapping is rare. The error bars give SD.

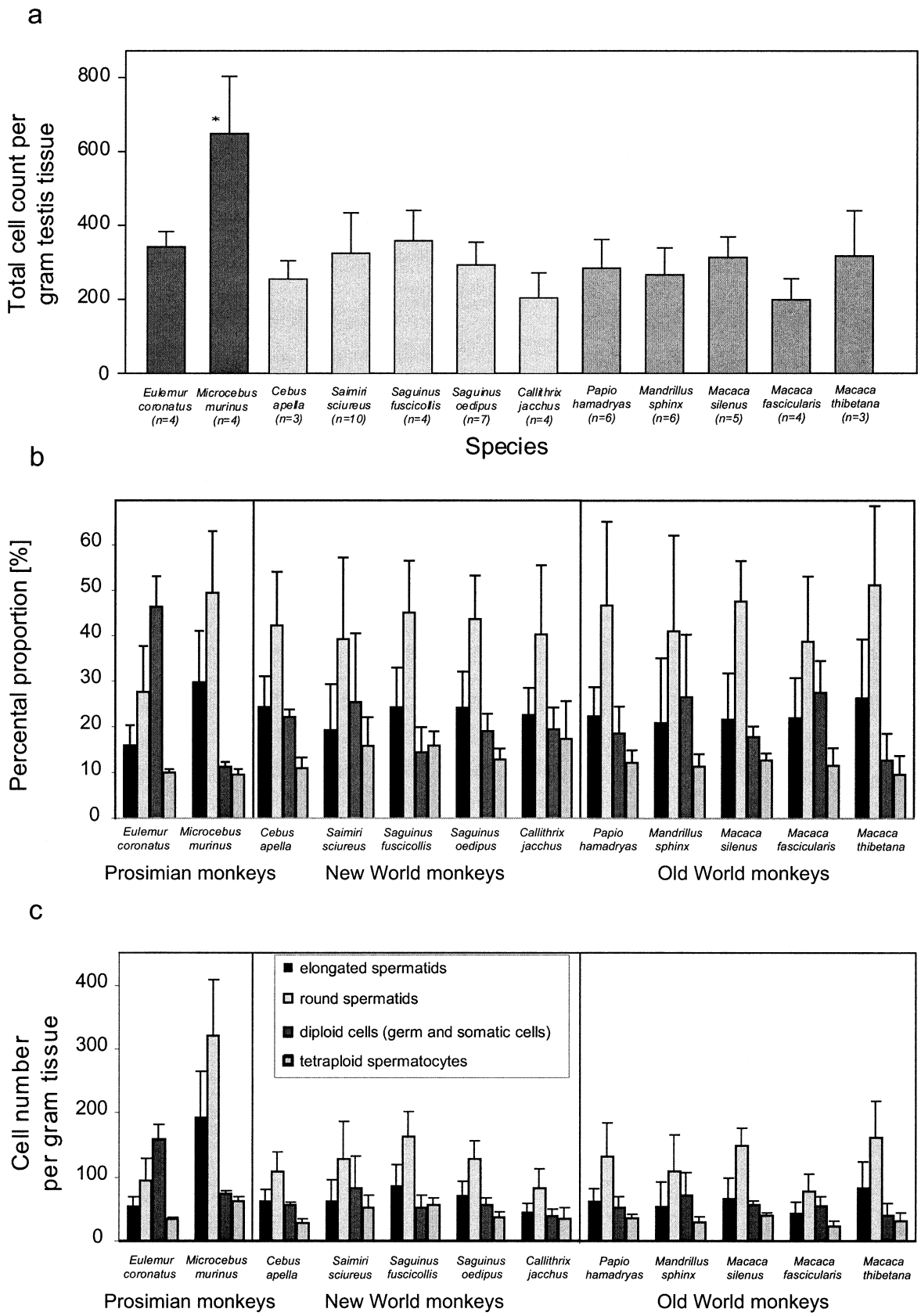

FIG. 4. Testicular cells as revealed by flow cytometry. **a**) A similar distribution is seen among the various species and systematic entities if data are expressed as total cell counts per gram testicular tissue. **b**) Percentage testicular cells as revealed by flow cytometry. Only among the two Prosimian species was a different pattern found, whereas the Old and the New World monkeys showed similar proportions in the various fractions. **c**) Total germ cell numbers per gram testis as revealed by flow cytometry. Only in testes from *Microcebus murinus* was the number of cells significantly elevated, whereas the other species revealed a similar amount of cells. The depicted legend of the testicular cell composition in **c** defines the columns of **b** and **c**. The error bars give SD.

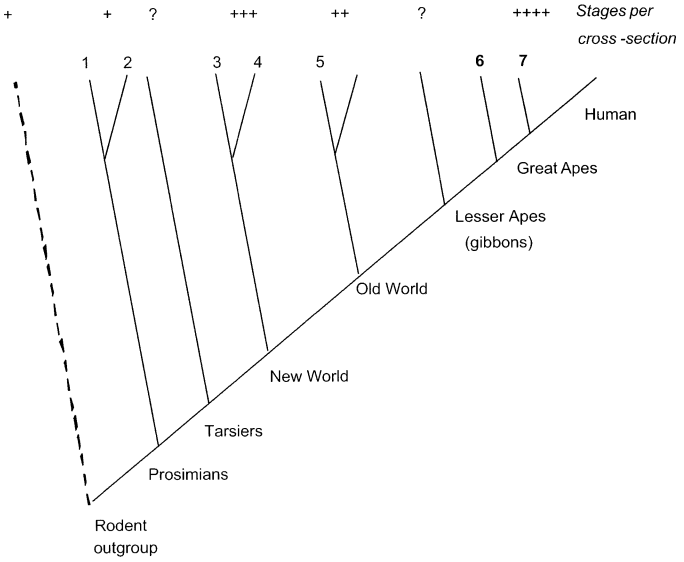

FIG. 5. Arrangement of spermatogenic stages in relation to primate phylogeny. Taxonomic groups represented in the present study are: 1, Lemuriformes (*Microcebus*); 2, Lorisiformes (*Otolemur*); 3, Cebidea (*Saimiri* and *Cebus*); 4, Callitrichidea (*Callithix, Saguinus*); 5, Cercopithecinae (macaques, baboon); 6, Hominoidea (*Pongo*); and 7, Hominoidea (*Pan*). The + symbols indicate relative degree of tubule complexity, corresponding to the mean number of stages per tubular cross section, as shown in Table 2 (i.e., Prosimians < Old World monkeys < New World monkeys < great apes). The general tendency is for an increase in the number of stages per tubular cross section. Note that the organization of the New and Old World monkeys is the reverse of what would be expected from phylogeny.

of the Prosimians, it can be concluded with reasonable certainty that the single-stage form represents the ancestral state of spermatogenic stage topography among primates (Fig. 5). From this, it appears that the general trend in primate evolution is that of an increasing proportion of multiple tubule stages, with the highest degree of tubule complexity being found in the hominid branch of the great apes (chimpanzees and humans). Our data, however, also clearly indicate that no simple (i.e., direct) relationship exists between seminiferous tubule organization and phylogeny among the primates, because the New World primates (see also [12, 13]) and great apes (but not orangutan) both display a more complex pattern of organization than do Old World monkeys. Thus, the tubule arrangement seen in Old and New World monkeys is the reverse of what would be expected based on phylogeny. It was of particular interest to note that all neotropical primate testes had a distinct multistage arrangement.

This could have occurred in two main possible ways (Fig. 5). The first possibility is that a gradual increase in tubule complexity took place along the main branch of anthropoid evolution, with two further separate increases occurring in the lineages to the New World monkeys (++ to +++) and hominids (+++ to ++++). The second possibility is that the character evolved more quickly, with multitubule arrangement (+++) already predominating before the branch to the New World monkeys, which was followed by a subsequent reduction (to ++) in the Old World lineage and a further increase (to ++++) in the lineage to the hominids. Both options are equally parsimonious, with each requiring two changes. Discrimination between these two, however, requires additional samples, in particular from the Tarsiers and Asian hominoid (i.e., gibbon) lineages, both of which were not available for analysis.

It had been assumed that the human multistage arrangement is associated with low spermatogenic efficiency and that single-stage rodent testes are associated with an opposite spermatogenic state [1, 30]. However, stereological analysis by the optical dissector approach revealed that human spermatogenesis is, in fact, highly efficient [17, 18]. Spermatogenesis in the multistage neotropical marmoset is also highly efficient [13]. Furthermore, our comparative flow cytometric analysis across 12 primate species provided strong evidence that spermatogenic efficiency and single-stage/multistage topography are entirely unrelated. All testicular samples were analyzed in a single run, thus further increasing comparability. Indices of meiotic and spermatogenic efficiency were derived, and it was obvious that neither of these indices was related to tubular stage topography. Flow cytometry also revealed that the testicular cell proportions were highly similar across primates, with *Microcebus* sp. being the only exception. The latter observation confirms the results from a previous study [14].

Sertoli cell workload (germ cell:Sertoli cell ratio) in *Saguinus fuscicollis* and *Saimiri sciureus* in the present study was nearly identical to that of *Callithrix jacchus* (14 and 17.5, respectively, vs. 16.5 in the marmoset [13]), whereas data for *Saguinus oedipus* were similar to those for predominantly single-stage cynomolgus monkeys (24.6 vs. 22.5–34.8 [17, 31, 32]). However, in terms of quantitative single-stage/multistage arrangement, marmoset and *S. oedipus* were nearly identical (1.91 vs. 2.0). It is also pertinent to consider that the quantitative single-stage/multistage arrangement is identical for marmosets and humans (2.0 vs. 2.03), whereas Sertoli cell load is different (16.5 vs. 10.2 and 10.9, respectively [17, 18]). These data further corroborate the view that the arrangement of spermatogenic stages is not directly related to quantitative measures of spermatogenic cell production.

This investigation did not aim to reveal the underlying causes for a single-stage versus multistage topography. Schulze and Rehder [5] brought up the very appealing concept that a helical/spiral arrangement of spermatogenic stages along tubules explains the human multistage tubule organization. It must be pointed out, however, that others have been unable to confirm the existence of a complete wave (spiral/helix) of spermatogenesis in the human testis [5, 6]. Irrespective of whether multistage organization truly reflects a complete helical arrangement, it is likely that the clonal size of every spermatogenic stage could be associated with single-stage versus multistage organization. It is well established that spermatogenesis follows a clonal organization; that is, all germ cells within a given spermatogenic stage represent a single clone [33]. The topographical importance of this phenomenon has become apparent from germ cell transplantation studies [34–36]. The size of a spermatogenic clone quite likely will bear a relation to single-stage/multistage arrangements, with a putative inverse relationship for clone size and number of stages per tubular cross-section. Recent data from studies regarding selective elimination and repopulation of rat testis germinal epithelium indirectly lend support to this view [37]. In these studies, spermatocytes were eliminated by chemical treatment, and following depopulation of the germinal epithelium, repopulation was followed by spermatid-specific immunocytochemistry analysis. Although rat testis is entirely single staged, multistage tubules were encountered with small numbers of stained spermatids of different stages. However,

to further investigate this important question, serial sectioning and clonal analysis in specific germ cell transplantation studies are required.

The issue is further complicated in that the frequency of a spermatogenic stage has also been related to its duration [38, 39]. Among the primate species studied so far, the duration of one spermatogenic cycle was longest for humans (16 days [40]), followed by chimpanzees (14 days [4]). Among various macaques, *P. cynocephalus*, *C. jacchus*, and *S. sciureus*, cycle duration was similar and ranged between 9.5 and 11.6 days [12, 40]. Thus, it is unlikely that stage frequencies are significantly related to primate testicular single-stage/multistage evolution or germ cell production. In the present work, we applied the human six-stage classification [38] to all species. It is important that whenever a comparison with previous studies was possible (human, chimpanzee, cynomolgus monkey, marmoset, and gray mouse lemur), the stage frequencies obtained using this stage system were highly comparable.

Stemming from initial observations by Short [41] and by Harcourt et al. [42, 43] that males of species with a polyandrous mating system have much larger testes in relation to body size compared to those in which females usually mate with a single male, it is now generally accepted that sperm competition, whereby gametes from different males compete for access to the female gamete within the female tract, represents an important component of intrasexual competition among primates. In line with the sperm competition theory, larger testes have the capacity to produce larger and/or more frequent ejaculates and would be selected for in species with polyandrous mating systems. Although evidence indicates that increased relative testis size may also be paralleled by an increase in the ratio of tubules to interstitial tissue, at least in some Old World monkeys [42, 44], the extent to which tubule organization and/or functional aspects of spermatogenesis may also differ according to mating system has not, to our knowledge, been investigated. Although somewhat limited (particularly in terms of monandrous species), the present data provide no evidence that the variation in tubule topography seen among primates is related to the number of male partners and the likely prevalence of sperm competition. Furthermore, the high degree of similarity in meiotic and efficiency indices among species with contrasting mating systems provides an additional indication that functional aspects of the spermatogenic process itself have not been extensively molded by selective forces operating in the context of sperm competition. Interestingly, however, the species with the highest spermatogenic efficiency is the gray mouse lemur, in which competition for estrous females is particularly intense and several males are likely to mate with a single female within her very brief period of receptivity [45, 46]. This species was also found to have unusually long and highly motile sperm, and it is possible that differences in sperm morphology and/or motility characteristics (see, e.g., [47, 48]) are, in fact, more likely to be related to differing mating systems than are testicular architecture or spermatogenic efficiency.

Because the sperm production rate is similar in all other investigated species, larger testes must produce more sperm per time interval. If the number of mating partners is correlated with the testis size, then sperm competition in a multimale mating system would be conceivable. The parameters of body weight, testis volume, and spermatogenesis have been suggested to be androgen dependent [49], and it has been shown that the testosterone levels differ across primate species and are slightly elevated in cebids and callithrichids. It was not clear whether those variations reflect phylogenetic differences in testicular function or are related to differences in body size [50, 51]. However, the testis size and the ratio of testis to body weight might be the parameters that reflect competition because of the obviously identical relative efficiency; a larger testis would produce more sperm than a smaller one. Also, the conditions of competition could lead to different quality of sperm [48]. Thus, sperm competition might exist in primates but seems not to influence the testis as such. Obviously, the parameters examined in our study do not reflect competition. Such a competition might be evident only at the level of the mature sperm [48] or might also be present on the female side, such as by variations in female mating behavior and preferences, which could influence the mating success as reported in *Cebus apella* [52]. Variations in the size and volume of the female reproductive tract, causing dilution of ejaculate, have also been considered to be related to sperm competition [53].

In summary, the present work revealed that the single-stage/multistage tubular organization in primates is not related to spermatogenic efficiency and that neither tubular organization nor spermatogenic efficiency are related to phylogeny or mating systems. The data set on tubular organization and spermatogenic efficiency confirms initial work on marmosets [13] and humans [17, 18] and substantially extends these findings across a variety of primate species. The observations that tubular organization and spermatogenic efficacy are apparently uncoupled from phylogeny and mating systems are novel and had not been predicted from previous related work.

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