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Genome Sizes in Afrotheria, Xenarthra, Euarchontoglires, and Laurasiatheria

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Abstract

Topical literature and Web site databases provide genome sizes for ~4,000 animal species, invertebrates and vertebrates, 330 of which are mammals. We provide the genome size for 67 mammalian species, including 51 never reported before. Knowledge of genome size facilitates sequencing projects. The data presented here encompassed 5 Metatheria (order Didelphimorphia) and 62 Eutheria: 15 Xenarthra, 24 Euarchontoglires (Rodentia), as well as 23 Laurasiatheria (22 Chiroptera and 1 species from Perissodactyla). Already available karyotypes supplement the haploid nuclear DNA contents of the respective species. Thus, we established the first comprehensive set of genome size measurements for 15 Xenarthra species (armadillos) and for 12 house-mouse species; each group was previously represented by only one species. The Xenarthra exhibited much larger genomes than the modal 3 pg DNA known for mammals. Within the genus *Mus*, genome sizes varied between 2.98 pg and 3.68 pg. The 22 bat species we measured support the low 2.63 pg modal value for Chiroptera. In general, the genomes of Euarchontoglires and Laurasiatheria were found being smaller than those of (Afrotheria and) Xenarthra. Interspecific variation in genome sizes is discussed with particular attention to repetitive elements, which probably promoted the adaptation of extant mammals to their environment.

Introduction

The word *genome* was coined in 1920 by Hans Winkler (1877–1945) while he was studying parthenogenesis in animals and plants. He defined *genom* (German spelling) as the haploid set of chromosomes and referred to the hypothesis that they are the sole carriers of hereditary factors. Today, the term is used in two distinct ways to indicate either the total number of genes or the whole amount of nuclear DNA. Considering both Winkler's definition and the fact that in the majority of organisms, only a small fraction of DNA comprises coding genes, we prefer for the definition of the total amount of DNA.

Early cytogeneticists quantified genomes by analyzing the partition in chromosomes that were counted in cytological preparations. However, the majority of nuclei remain in interphase, but the development of microphotometry allowed DNA measurements throughout the cell cycle. Thus, Hewson Swift introduced the C-value as a shortcut for any (haploid) genome. His first paper on animal nuclei, written for his PhD dissertation, discussed two competing ideas: Vendrely and Vendrely (1948) had proposed a hypothesis about DNA constancy, whereas Schrader and Leuchtenberger (1949) emphasized variability after they had recorded different DNA content in cell nuclei of the same species. In contrast to both positions, Swift (1950a) distinguished "DNA classes," which could increase by a factor of two from a fundamental class for a given species. Swift (1950b) expanded his discovery and showed that Feulgen DNA in corn nuclei was distributed in 1C, 2C, 4C, 8C, and 16C levels. He observed the DNA content 1C only at the end of meiosis (Greilhuber et al. 2005).

Genomes vary in size. This realization came from the pioneers of microphotometry shortly before the structure of DNA was discovered (Mandel et al. 1951; Marshak and Marshak 1953; Mirsky and Ris 1951; Swift and Kleinfeld 1953; Vendrely and Vendrely 1948).

The recognition that genome sizes (*GS*) are widely distributed prompted the formulation of the C-value paradox

(Cavalier-Smith 1985; Thomas 1971). The most popular ideas are that *GS* is neither related to gene numbers nor to morphologic complexity, even though Van Nimwegen (2003) suggested scaling laws in the functional content of genomes in relation to the design of organisms. The completion of the euchromatic sequence of the human genome is an instructive example, resulting in some 20–25,000 protein-coding genes that represent less than 2% of all sequences (IHGSC 2004). The identification of repetitive sequences has only partially resolved the C-value paradox.

In the present study we evaluated the *GS* of several mammals of particular interest for comparative genomics (Hedges and Kumar 2002) and molecular phylogeny. Particularly, we focused on the whole spectrum of mice actually used in biomedical research, on the Xenarthra and the Chiroptera. From the first group, only the *GS* of the house mouse, *Mus* (*musculus*) *domesticus*, appears in databases. In current databases the Xenarthra are represented by only one species, the ant-eater, *Myrmecophaga tridactyla*, whereas the Afrotheria are represented just by the aardvark, *Orycteropus afer*.

Recent molecular studies (Delsuc et al. 2002; Madsen et al. 2001; Murphy et al. 2001, 2004) divide placentals into the Southern Hemisphere clades Afrotheria (elephants, hyraxes, aardvarks) and Xenarthra (sloths, ant-eaters, armadillos), opposite to the monophyletic Boreoeutheria in the Northern Hemisphere, which are composed of the Laurasiatheria clade (cetaceans, carnivores, bats) and the Euarchontoglires clade (rodents, lagomorphs, and primates). Statistical tests identified three early divergences with almost equal likelihood; these are a basal Afrotheria hypothesis, an Afrotheria plus Xenarthra alternative, and a basal Xenarthra hypothesis.

The available data and those we evaluated speak well for a general evolutionary tendency toward smaller *GS* in Boreoeutheria, in which the vast majority shows a *GS* around 3 pg. In contrast, the Xenarthra genomes have around 4.5 pg DNA, and the Afrotherian aardvark possesses 5.86 pg. We discuss possible mechanisms responsible for changes in *GS*. Quantitative variation of noncoding DNA sequences might not only obey intrinsic properties but could also be triggered by environmental signals. The resulting *GS* variations display nucleotypic effects in cell functions, and the organisms in question are exposed to natural selection forces. Such a scenario could render less enigmatic to understand the panoply of *GS* in mammals.

Materials and Methods

Mammals

This investigation of nuclear DNA contents comprised 5 metatherian and 62 eutherian mammals. Most of the animals were caught in the wild using live traps at several localities in Argentina and Venezuela during the period 2000–2003. They were released after donating a drop of peripheral blood. Coordinates of the collection places are available on request. The zoos of Buenos Aires and Caracas gave blood smears from *Priodonte maximus* and *Tapirus terrestris*, respectively. Michael Potter (National Cancer Institute, Bethesda, MD) provided samples of *Mus caroli*, *M. castaneus*, *M. cervicolor*, *M. cookii*, *M. spicilegus* (also named *M. hortulanus*), *M. molossinus*, and *M. spretus*. The other *Mus* species came from animals housed at the Dipartimento di Biologia Animale, University of Pavia (Italy). Our intention was to use two slides for each of two animals per species, but only one animal was available from *Marmosops fuscatus*, *Cabassous centralis*, *Dasypus kappleri*, *Priodonte maximus*, *Zaedyus pichiy*, and *Oecomys concolor*.

Feulgen Procedure

Air-dried specimens were fixed in 10% formaldehyde aqueous solution for 20 min. The Feulgen reaction included hydrolysis in 5 M HCl at room temperature for 60 min and staining with Schiff's reagent (basic fuchsin; BDH) for 45 min. Several batches had to be processed, therefore it was important that each batch comprised slides bearing DNA standards. The standards were erythrocytes of the chicken (*Gallus gallus*) and sperms and lymphocytes of *M. domesticus* with 2.54, 3.4, and 6.8 pg nuclear DNA, respectively. Weak fading and minor sensitivity to DNA base composition are advantages of Feulgen staining.

Microphotometry

Twenty-five nuclei of lymphocytes and monocytes were measured from each slide. Thus, most samples had 100 nuclei, so that both interindividual and intraindividual (technical) variability was taken into account. Six samples were limited to 50 nuclei when only one animal was available (see previous description).

Nuclear DNA contents were recorded with a scanning microscope photometer 03 and the APAMOS program (Zeiss). The wavelength for maximum absorbance was determined at 550 ± 5 nm instead of expected 560 nm. A planapochromat 100× objective (n.a. 1.3) opened the measuring diameter to 0.5 μm and the illuminated field to 10 μm, both in the plane of the specimen. Therefore, scanning steps were set to 0.5 mm in both dimensions and for all measurements.

Open Access Databases

Several Web sites host databases on or providing links to animal (and plant) genome resources (last visit December 15, 2004).

- Animal Genome Size Database offers C-values from about 1,300 invertebrates and 2,500 vertebrates; it is maintained by Ryan Gregory, University of Guelph, Canada: www.genomesize.com.
- Cancer Genomics and a Mouse Expression Atlas are provided (among others) from the British Columbia Genome Sciences Centre, directed by Marco Marra and under the auspices of the BC Cancer Agency, Vancouver, Canada: www.bcgsc.ca.
- DBA Mammalian Genome Size Database has 237 data sets managed by Daniele Formenti, Dipartimento di

Biologia Animale, Pavia University, Italy: www.unipv.it/webbio/dbagsdb.htm.

- DOGS, the Database of Genome Sizes covers 301 organisms; it is directed by Sören Brunak, Center for Biological Sequence Analysis, Technical University of Denmark in Lyngby: www.cbs.dtu.dk/databases/dogs.
- EBI, the European Bioinformatics Institute, gives access to completed genomes and genome shotgun sequences; it is at Wellcome Trust Genome Campus, Hinxton, Cambridge, UK: www.ebi.ac.uk/genomes.
- ENSEMBL is a joint project between the EBI, Cambridge, and the Sanger Institute, London, to develop a software system for automatic annotation on metazoan genomes: www.ensembl.org.
- GOLD: the Genomes Online Database represents a Web resource for genome projects worldwide referring to 463 eukaryotic genomes; it is kept by Nikos C. Kyrpides, Lawrence Berkeley National Laboratory, Berkeley, CA: www.genomesonline.org.
- HGMP, the Human Genome Mapping Project provides links to genome databases; it is managed by the Rosalind Franklin Centre for Genomics Research, Medical Research Council, Hinxton, Cambridge, UK: www.hgmp.mrc.ac.uk/genomeweb.
- JGI, the Joint Genome Institute, is operated by the University of California for the U.S. Department of Energy. It offers a Genome Portal to download sequences; details on human chromosomes 5, 16, 19; and entry to the Evolutionary Genomics Department. JGI Production Genomics Facility, Walnut Creek, CA: www.jgi.doe.gov.
- KEGG, the Kyoto Encyclopedia of Genes and Genomes, provides (among other things) a database about genome projects with 243 entries; it was set up by Minoru Kanehisa at Bioinformatics Center, Institute for Chemical Research, Kyoto University, Tokyo: www.genome.ad.jp/kegg.
- Plant DNA C-values Database was initiated by Michael Bennett and Ilja Leitch and got release 3.0 in December 2004. Royal Botanic Gardens Kew, Richmond, Surrey, UK: www.rbgkew.org.uk/cval.
- VEGA, the Vertebrate Genome Annotation browser, makes its focus on human, mouse, and zebrafish; it is operated by the Wellcome Trust Sanger Institute, London: www.vega.sanger.ac.uk.

Results

We measured the nuclear DNA contents of 5 metatherian and 62 eutherian mammals (Table 1). Their *GS* were calculated in picograms of 1C DNA and also converted into Mbp using a factor of 978 (Dolezel et al. 2003). In parentheses, 16 already available *GS* were added from databases (*Material and Methods*). Our estimates agree with 14 of these data points, but there were remarkable differences for *Didelphis marsupialis* (21%) and *Mus poschiavinus* (23%).

Subclass Metatheria

Five species of opossums, family Didelphidae, could be investigated. The smallest *GS* of 2.85 pg DNA was found in *M. fuscatus*, whereas the largest, 5.22 pg, came from *Monodelphis brevicaudata*. Thus, the genomic range for pouched mammals was expanded, because the 26 species already studied had occurred between 3.0 and 4.9 pg DNA. Their modal *GS* was more than the average, slightly above 3 pg, as known for mammals in general.

Subclass Eutheria

Xenarthra. The order of extra-jointed animals, endemic to Central and South America, had been represented in the databases only by the giant ant-eater *Myrmecophaga tridactyla*. Its *GS* had been given 4.15 pg DNA, for which the present measurement was 4.49 pg. *Tamandua tetradactyla* is another representative of the Myrmecophagidae, which showed here 4.11 pg DNA. The first record for three-toed sloths, family Bradypodidae, was 4.23 pg from *Bradypus variegatus*; it lies in the order of the Myrmecophagidae. In addition, we considered 12 species of armadillos, family Dasypodidae, which ranged between 3.98 pg in *Cabassous centralis* and 5.76 pg in *Dasypus sabanicola*. Thus, their *GS* appeared much larger than the modal value known for mammals.

Rodentia. This order has the largest number of species. Their *GS* scattered to a greater extent than those of the other orders in consideration. *Heteromys anomalus* and *Microryzomys minutus* displayed the smallest genomes of 2.77 pg and 2.78 pg, whereas the largest, 6.21 pg and 6.25 pg, were found in *Proechymis guairae* and *P. trinitatis*, respectively. However, variability was less evident at the level of families and appeared particularly low in Muridae.

We measured the *GS* of several house-mouse species, of which 10 were unknown. The values reported hitherto were from *M. musculus* and *M. poschiavinus*. The latter refers to the house mouse from the Poschiavo valley in Switzerland. But this naming should be avoided, because these animals represent just one of the many variants from Robertsonian chromosomes within the wild-living short-tailed mice in Western Europe (Redi and Capanna 1988). The taxonomy of the house mouse species assigned the *poschiavinus* animals to *M. musculus domesticus* (Marshall and Sage 1981); we prefer the abridged version *M. domesticus*. Furthermore, *M. musculus musculus*—or better, *M. musculus*—is the correct term for the wild-living long-tailed mice in Eastern Europe. Unfortunately, the literature does not completely comply with Marshall's and Sage's suggestions. *GS* databases likewise indicated *M. musculus*, even though the animals were *M. domesticus*. Such incorrect terminology puts a risk to biomedical research and genomic studies.

The value we estimated for *poschiavinus* animals was 3.22 pg DNA. This is different from the former report of 3.97 pg, but rather close to 3.35 pg for *M. domesticus*. The largest murine genome of 3.68 pg DNA turned out in our coherent approach with *M. spretus*. Its *GS* exceeded that of other mice, probably due to the amplification of minor satellite sequences (Garagna et al. 1993).

Table 1. Genome sizes of 67 mammals (Numerical scatter given as SD)

(No)		Genome (1C, pg)	SD (pg)	Genome (1C, Mbp)	Karyotype (2n)	Karyotype authors
	Mammalia: Metatheria order Didelphimorphia Didelphidae					
1	*Monodelphis brevicaudata*	5.22	±.26	5105	18	Hsu and Benirschke 1971
2	*Marmosa robinsoni*	3.94	±.23	3853	14	Hsu and Benirschke 1971
3	*Marmosops fuscatus*	2.85	±.38	2787	nd	
4	*Micoureus demerarae*	4.88	±.37	4773	14	Carvalho and Mattevi 2000
5	*Didelphis marsupialis (3.90)*	3.21	±.42	3139	22	Hsu and Benirschke 1968
	Mammalia: Eutheria Xenarthra order Xenarthra Bradypodidae					
6	*Bradypus variegatus*	4.23	±.33	4137	54/55	Jorge 1981
	Dasypodidae					
7	*Cabassous centralis*	3.98	±.24	3892	62	Hsu and Benirschke 1969
8	*Chaetophractus vellerosus*	4.46	±.21	4362	62	Hsu and Benirschke 1969
9	*Chaetophractus villosus*	4.18	±.14	4088	60	Hsu and Benirschke 1974
10	*Dasypus hybridus*	4.89	±.34	4782	64	Saez et al. 1964
11	*Dasypus kappleri*	4.92	±.24	4812	64	Goldschmidt and De Almeida 1993
12	*Dasypus novemcinctus*	5.41	±.27	5291	64	Beath et al. 1962
13	*Dasypus pilosus*	4.32	±.17	4225	64	Goldschmidt and De Almeida 1993
14	*Dasypus sabanicola*	5.76	±.32	5633	64	Goldschmidt and De Almeida 1993
15	*Dasypus septemcinctus*	5.17	±.25	5056	64	Barroso and Seuanez 1991
16	*Euphractus sexcinctus*	4.16	±.41	4068	58	Jorge et al. 1977
17	*Priodonte maximus*	4.47	±.34	4372	50	Benirschke and Wurster 1969
18	*Zaedyus pichiy*	4.21	±.26	4117	62	Merrit et al. 1973
	Myrmecophagidae					
19	*Myrmecophaga tridactyla* (4.15)	4.49	±.28	4391	60	Hsu 1965
20	*Tamandua tetradactyla*	4.11	±.36	4020	54	Hsu 1965
	Euarchontoglires order Rodentia Muridae Murinae					
21	*Mus bactrianus*	3.08	±.05	3012	40	See Marshall and Sage 1981
22	*Mus caroli*	3.02	±.09	2954	40	Makino 1951
23	*Mus castaneus*	3.07	±.06	3002	40	See Marshall and Sage 1981
24	*Mus cervicolor*	2.98	±.06	2914	40	Hsu & Benirschke 1971
25	*Mus cookii*	3.07	±.05	3002	40	See Marshall and Sage 1981
26	*Mus (musculus) domesticus* (3.34)	3.35	±.08	3276	22–40	Capanna et al. 1976
27	*Mus macedonicus (M. abbotti)*	3.10	±.08	3032	40	See Marshall and Sage 1981
28	*Mus molossinus*	3.08	±.07	3012	40	See Makino 1951
29	*Mus (musculus) musculus*	3.28	±.10	3208	40	Hsu and Benirschke 1967
30	*Mus poschiavinus* (3.97)	3.22	±.06	3149	26	Gropp et al. 1970
31	*Mus spicilegus (M. hortulanus)*	3.07	±.60	3002	40	See Marshall and Sage 1981
32	*Mus spretus*	3.68	±.07	3599	40	See Marshall and Sage 1981
	Sigmodontinae					
33	*Calomys hummelincki*	3.36	±.25	3286	60	Pérez-Zapata et al. 1987
34	*Microryzomys minutus*	2.78	±.12	2719	58	Pérez-Zapata et al. 1996
35	*Neacomys tenuipes*	3.28	±.16	3208	56	Pérez-Zapata et al. 1995
36	*Oecomys concolor*	3.06	±.32	2993	80	Gardner and Patton 1976
37	*Oryzomys talamancae*	3.41	±.22	3335	34	Pérez-Zapata et al. 1986
38	*Oryzomys albigularis*	3.78	±.17	3697	66	Aguilera et al. 1995
39	*Rhipidomys venezuelae*	3.69	±.26	3609	nd	
40	*Zygodontomys brevicauda*	3.81	±.37	3726	84	Gardner and Patton 1976
	Heteromyidae					
41	*Heteromys anomalus*	2.77	±.21	2709	60	Schmid et al. 1992

Table 1. Continued

(No)		Genome (1C, pg)	SD (pg)	Genome (1C, Mbp)	Karyotype (2n)	Karyotype authors
	Echimyidae					
42	*Proechimys cayennensis*	5.47	±.36	5350	40	Reig et al. 1979a
43	*Proechimys guairae* (6.25)	6.21	±.34	6073	42–62	Reig et al. 1980
44	*Proechimys trinitatis* (6.30)	6.25	±.42	6113	62	Reig et al. 1979b
	Laurasiatheria order Perissodactyla Tapiridae					
45	*Tapirus terrestris*	3.78	±.42	3697	80	Houck et al. 2000
	order Chiroptera Microchiroptera Phyllostomidae Carollinae					
46	*Carollia brevicauda (2.93)*	2.98	±.16	2914	20/21	Patton and Gardner 1971
47	*Carollia perspicillata (3.06)*	2.92	±.15	2856	20/21	Baker 1967
	Glossophaginae					
48	*Anoura caudifera*	2.73	±.22	2670	30	Yonenaga 1968
49	*Anoura geoffroyi*	2.64	±.21	2582	30	Baker 1967
50	*Glossophaga soricina* (2.78)	2.78	±.09	2719	32	Baker 1967
	Stenodermatinae					
51	*Artibeus glaucus*	2.58	±.14	2523	30/31	Gardner 1977
52	*Artibeus jamaicensis* (2.74)	2.63	±.11	2572	30/31	Hsu and Benirschke 1968
53	*Platyrrhinus umbratus*	2.77	±.24	2709	30	Eisenberg 1989
54	*Platyrrhinus vittatus*	2.65	±.16	2592	30	Baker 1973
55	*Sturnira erythromos*	2.82	±.21	2758	30	Gardner and O'Neil 1969
56	*Sturnira lilium* (2.84)	2.54	±.18	2484	30	Baker 1967
57	*Sturnira ludovici*	2.82	±.13	2758	30	Baker 1967
58	*Sturnira tildae*	2.68	±.22	2621	30	Baker and Hsu 1970
59	*Vampyressa pusilla* (2.73)	2.80	±.21	2738	18–24	Baker et al. 1982
	Phyllostominae					
60	*Lonchorhina aurita (2.56)*	2.41	±.16	2357	32	Baker and Hsu 1970
61	*Phyllostomus hastatus*	2.62	±.18	2562	32	Yonenaga 1968
	Desmodontinae					
62	*Desmodus rotundus*	2.66	±.08	2601	28	Hsu and Benirschke 1967
	Vespertilionidae					
63	*Eptesicus brasiliensis*	2.55	±.07	2494	50	Baker and Patton, 1967
64	*Eptesicus diminutus*	2.74	±.13	2680	50	Williams 1978
65	*Eptesicus furinalis (2.43)*	2.63	±.18	2572	50	Baker and Patton 1967
66	*Myotis keaysi (2.65)*	2.34	±.26	2289	44	Baker and Patton 1967
	Mormoopidae					
67	*Pteronotus parnellii (2.67)*	2.71	±.14	2650	38	Baker 1967

Notes: Figures in brackets were taken from www.genomesize.com. Each 1C (haploid) DNA content in picograms (pg) and in Mbp. Odd figures for 2n (diploid) chromosomes represent male karyotypes with XY1Y2 sex chromosomes.

Perissodactyla. *Tapirus terrestris*, the South American tapir, is the third species in this order, from which the *GS* became known. Its value of 3.78 pg DNA is situated between 3.15 pg for the horse and 4.12 pg for the donkey. The database presentation of even-toed ungulates surpasses that of perissodactyls, because most of the 18 *GS* for Artiodactyla have been obtained from domestic animals.

Chiroptera. The *GS* databases hitherto included 50 bat species. This investigation added 12 original records that confirmed the minor variability of *GS* already known for this order. *Myotis keaysi* showed the minimum *GS* with 2.34 pg, whereas *Carollia brevicauda* the maximum with 2.98 pg. The majority of bat values ranged around 2.6 pg DNA.

Discussion

Sizing Mammalian Haploidy

The research community experiences and produces a growing need to analyze new genomes. Sequencing projects, however, demand not only hefty budgets but also a wide overview on the breadth of genomic capacities.

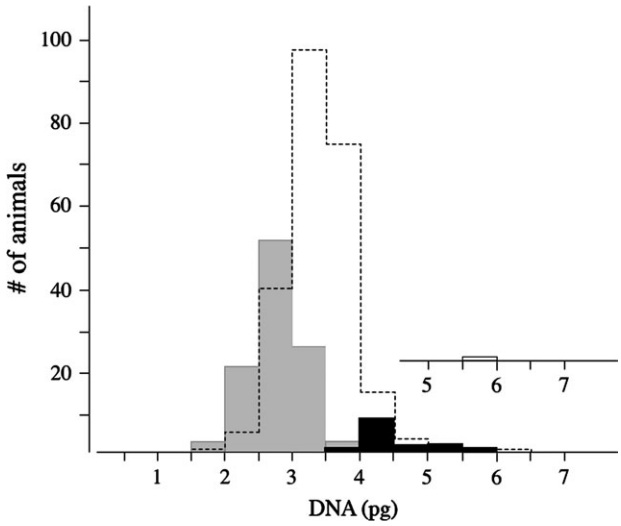

Figure 1. Distribution of the 373 eutherian *GS* known, as from the present study and the *GS* databases illustrated in *Materials and Methods*: Afrotheria (1, shown in the insert), Xenarthra (15, dark gray), Laurasiatheria (112, light gray), and Euarchontoglires (245, white, dashed line).

Consider the practical interest in the correct *GS* of a species. The nine-banded armadillo, *Dasypus novemcinctus*, was selected for sequencing, and the BAC library now under construction has been based on a presumptive *GS* of 3.0 pg DNA (www.genome.gov). However, we estimated a much larger *GS* of 5.41 pg. Knowledge of *GS* facilitates a suitable and efficient sequencing strategy. It is of particular relevance considering the frequency with which genomes are entering sequencing projects (www.genomesonline.org); these were 76 just from July 29 to October 6, 2003, and 296 from then to June 11, 2004.

Knowledge about the extent of *GS* conservation and divergence will help unravel the genetic and evolutionary mechanisms that shape genomes. Therefore, we attempted to fill in some gaps in the mammalian *GS* database. This enterprise encompassed 67 species from Metatheria as well as Eutheria, where hitherto a paucity of data prevailed (Table 1).

Recent molecular data split the placental orders into four phylogenetic clades. Afrotheria, and Xenarthra occupy basal positions, followed by the Boreoeutheria, which embrace Euarchontoglires and Laurasiatheria (Delsuc et al. 2002; Madsen et al. 2001; Murphy et al. 2001, 2004). The actually known data for 373 eutherian mammals attribute the largest genomes to the aardvark (*Orycteropus afer*, Afrotheria) and to Xenarthra (Figure 1). Both types of results shall converge by the assumption that there has been a shift toward smaller genomes during the transition of basal clades to the Boreoeutheria that conquered the Northern Hemisphere. This suggestion requires extensive exploration of Afrotherian genomes (Fronicke et al. 2003; Svartman et al. 2004).

Compacted Genomes

The genome of the verspertilionid *Myotis capaccinii* is composed of just 1.9 pg DNA (Capanna and Manfredi Romanini 1971). Here, an additional 21 bat species were found to possess rather small *GS* (<3 pg; Table 1); thus, they represent valuable models for comparative genetics to find essential features.

Because the *GS* correlates positively with nuclear and cellular volumes, small genomes appear well adapted to the metabolic requirements for flight (Hughes and Hughes 1995). This correlation holds also for birds, where larger genomes were risky for extinction (Vinogradov 2004a) and continue only in running species (Tiersch and Wachtel 1991). Bats achieve their small genomes thanks to a minimum of repetitive DNA elements (Van den Bussche et al. 1995). This characteristic might facilitate the identification of regulatory sequences in noncoding DNA.

An extreme example of small *GS* is the tetraodontoid fish *Fugu rubripes*. Its 1C content of 0.41 pg DNA was estimated to possess nearly the same number of genes as humans (www.genome.jgi-psf.org/fugu). Although most of the human genome is made up of noncoding DNA, the *Fugu* carries only a handful of giant genes containing long introns. Nevertheless, some 10% of sequences are repetitive. These findings suggest that even the smallest genomes need a minimum of repetitive DNA to correctly express genes responsible for complex functions. By engulfing a genome, these sequences may participate in the "eurygenic" system of gene regulation, which requires sectors of noncoding DNA (Vinogradov 2004b; Zuckerkandl and Hennig 1995).

Redundancy in Genomes

In the majority of mammals, protein-coding exons contribute merely 2% to the *GS*. The rest is composed in almost equal portions by repetitive DNAs and by unique sequences of mainly unknown function (Sogayar et al. 2004). Particularly the ubiquitous repetitive elements, cytologically detectable or not, account for varying C-values even among closely related taxa (John 1988; Manfredi Romanini 1985).

Despite earlier negative attributes, repetitive DNAs are nowadays not regarded as useless (Beaton and Cavalier-Smith 1999). They rather provide an efficient mechanism for genomic shuffling. Makalowski (2000) has bridged the difference of opinion by his concept of a genomic scrap yard, from which evolution may serve itself.

The long interspersed nucleotide element LINE-1 is an autonomous retroelement that makes up about 16% of the human genome. LINE-1 can jump to chromosomes with broken DNA strands and then slip into and repair the damage (Morrish et al. 2002).

Repetitive sequences contribute not only to *GS* variation (Petrov 2001; Vinogradov 1998), but also ensure the maintenance of a definite three-dimensional chromatin order, which is a prerequisite for its correct and efficient functioning (Cremer et al. 1993; Spector and Gasser 2003). Heterochromatin is an acknowledged stronghold of repetitive DNAs, which interact with surrounding sequences and nearby genes. Repetitive DNA sequences may serve as recombination hot spots or become part of protein coding regions. They have specific functions in dosage compensation, sister chromatid

cohesion, and telomere maintenance. More general effects appear in the repression of gene activities, position effect variegation, DNA elimination, differential DNA endoreplication, and concurrent variations in *GS* (Grewal and Moazed 2003; Redi et al. 2001). In addition, when examining samples from many individuals of the same species, the amount of heterochromatin at specific loci may not be constant. The grasshopper *Atractomorpha similis* is an impressive example, in that all 10 chromosomes of the haploid set can be affected so that more than 250 cytotypes have been detected (John and King 1983). Such intraspecies heterochromatin polymorphisms are apparently without phenotypic expression.

We are challenged to deviate from a narrow gene-centered view, because coding sequences alone neither tell the whole story of life nor account for the organismal complexity. To understand genome functioning better, the linear genome map must be supplemented with studies on the epigenetic mechanisms of gene regulation and expression. The so-called nucleotypic effect allots a role to *GS* itself (Bennett 1972; Gregory 2001; Olmo et al. 1989; Olmo 2003); particularly considering the clear negative correlation with metabolic rates (Vinogradov 1998). We might also learn how genomic elements allow cross-talk between environmental signals and genomic receptors.

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Signal transduction networks convey information about extracellular and intracellular environments to the nucleus, while coordinated relocation of large DNA sections is feasible thanks to natural genetic engineering systems (Shapiro 1997). The rapid restructuring of the maize genome in response to the "genome shock" is a classical example, and ciliate macronuclear development is another. Furthermore, phytochrome-mediated shade-avoiding and light-seeking responses in flowering plants have been proven to be based on a family of regulatory multigenes in *Arabidopsis thaliana* (Callahan et al. 1997). Thus, repetitive DNA elements appeared as appropriate candidates for being the physical basis that couples the nucleus and its environment.

A recent example of such inside-outside cross-talk is the finding that human Alu element retrotransposition can be induced by exposure to the topoisomerase II inhibitor etoposide that is mediated in *trans* by endogenous LINEs (Hagan et al. 2003). Alu elements are quite abundant, accounting for about 10% of the human genome. The fact that retrotransposition can be induced by genotoxic stress supports the scenario of *GS* variations triggered by environmental signals.

Documented molecular mechanisms for amplification and dispersion throughout the genome comprise not only transposable elements (Kidwell 2002) but also illegitimate recombination, unequal crossing over, deletion, and duplication of larger genomic segments. These processes of DNA metabolism are capable of producing quantitative as well as qualitative rearrangements. They could prove to be key events in generating significant functional variability, which will be faced with selection in the Darwinian world.

When exploring new ecological niches, the outcome organism's *GS* variation must withstand examination of what might be likely to survive: the *GS* variation will be selected for new favorable physiological answers. At the risk of oversimplification, we suggest that the exons of a genotype *plus the GS per se* might be the new paradigm to explain, in a more comprehensive manner, the determination of a phenotype.

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