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MINI REVIEW

VARIATIONS IN GENOME MASS

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Abstract—1. Genome size varies considerably among vertebrates, ranging from less than 1 pg to more than 200 pg; the amount of DNA differing among individuals in a population can equal the amount in the entire structural gene complement.

2. Recent technological advances permit evaluation of genome size variation at several levels including sub-chromosomal, chromosomal and cellular.

3. Genome size variation may also be viewed from taxonomic levels, and across evolutionary time frames.

4. As sources of genome size variation are identified and studied, the conundrum of the C-value paradox (lack of correlations among genome size, genomic complexity and phylogenetic status of organisms) may prove to be more apparent than real.

5. For example, the limited and relatively constant genome size of avians may be related to the physiological constraints of flight.

INTRODUCTION

Genome size usually refers to the DNA content of a resting haploid cell. The biological significance of genome size has been a subject of interest since Mirsky and Ris (1951) discovered differences in the DNA content of cells from diverse species. Based on their early observations they asked whether DNA content increased from the more primitive to the more advanced species; whether related organisms had similar amounts of DNA; and whether the evolutionary transition from water to land was accompanied by changes in DNA content.

In fact, differences in DNA content (DNA mass) do not reflect evolutionary relationships. DNA mass does not increase with increasing genetic complexity. Genome size has not steadily increased or decreased throughout evolutionary history, and increases and decreases each seem to have occurred within various groups. The bony fishes, for example, can lay claim to the largest (>200 pg) and smallest (<1 pg) values for genome size among the vertebrates. Similar findings pertain to the plant kingdom (Price, 1976).

It is also well-established that the quantity of DNA present greatly exceeds the quantity of DNA that is expressed, even in the smallest of vertebrate genomes. The variable, and seemingly unpredictable, nature of genome size has been perplexing and has been characterized as paradoxical. Although the explanation for variation in genome mass remains obscure, the variation itself can be studied from several perspectives. Here we address the variation in genome size found at different levels of organization. We emphasize the study of vertebrates in general and birds in particular.

DIVERSITY AND DISTRIBUTION OF GENOME SIZE

The DNA mass of free-living organisms ranges from 0.007 picograms (pg) in bacterial cells to more than 200 pg (1 pg = 10^{-12} g) in lungfish. The cells of most eukaryotes have a diploid DNA content of from 2 to 10 pg—about 300 times the DNA content of a bacterial cell. But, as noted above, some eukaryotes have considerably more—about 6000 times the DNA content of a bacterial cell. A similar range is found among plants: from 0.09 pg in certain fungi to >100 pg in ferns and certain psilopsids.

The distribution of genome size among several taxa is given in Table 1. Note that large and small values both occur within groups, and that even simple organisms such as *Gonyaulax* can have massive genomes. This underscores the fact that, with few exceptions, (e.g. *Saccharomyces*) most of the DNA in the genome does not code for protein. in fact, only 0.002% of the *Gonyaulax* genome codes for protein; only 0.7% of the tobacco plant genome; and only 9% of the human genome (see review in Cavalier–Smith, 1985b).

METHODS USED IN THE STUDY OF GENOME SIZE

A variety of techniques with different levels of precision have been used to estimate genome size

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Table 1. Diploid DNA content of selected species

Kingdom	Scientific name	Common name	Diploid genome (pg)	Base pairs DNA ($\times 10^9$)
Monera	*Escherichia coli*	Gut bacterium	0.02	0.02
Protista	*Dictyostelium discoideum*	Slime mold	0.07	0.07
	Gonyaulax polyedra	Algae	200.0	196.0
Fungi	*Saccharomyces cerevisiae*	Fission yeast	0.02	0.02
	Neurospora crassa	Bread mold	0.04	0.04
Plantae	*Nicotiana tabaccum*	Tobacco	7.8	7.64
	Fritillaria davisii	Angiosperm	295.0	289.0
Animalia	*Drosophila melanogaster*	Fruit fly	0.36	3.53
	Caenorhabditis elegans	Nematode	0.18	1.72
	Protopterus aethiopicus	Lungfish	284.0	278.0
	Ictalurus punctatus	Channel catfish	1.98	1.94
	Tetraodon fluviatilis	Pufferfish	0.78	7.64
	Rana catesbeiana	Bullfrog	15.0	14.7
	Cryptobranchus alleganiensis	Salamander	110.0	107.8
	Gallus domesticus	Chicken	2.54	2.49
	Homo sapiens	Human	7.0	6.86

This Table is based in part on data in Cavalier-Smith (1985b).

since the pioneering work of the 1940s. These include DNA reassociation kinetics (Britten and Davidson, 1971); fluorimetric methods (Hinegardner and Rosen, 1972); Feulgen staining (Rasch, 1985); and flow cytometry (see following discussion). Estimates made by analysis of reassociation kinetics of DNA (measurement of the rate of renaturation after heating) provide physical data on genome size (Britten and Davidson, 1971), but offer low precision. Fluorometric determination of total DNA standardized to a known number of cells has been used to estimate genome size in hundreds of species with a measurement error of about 5% (Hinegardner, 1976). Microdensitometry of Feulgen-stained nuclei can yield estimates of genome size precise enough to differentiate male and female in birds, based on ZZ/ZW sex chromosome heteromorphism (Rasch, 1985). Flow cytometry offers the greatest resolution, however, and current procedures may permit detection of specific chromosome deletions or duplications of the order of 7300 kilobases (McConnell *et al.*, 1989). According to this method, the cells are lysed, and stained with a DNA-specific fluorochrome. Then, by use of an argon-ion laser (for example), the nuclei are analyzed one-at-a-time for fluorescence, which is directly proportional to the amount of DNA that they contain. Several thousand nuclei can be analyzed in a few seconds. The fluorescence of each nucleus is converted to an analog signal, and the signal is transmitted to a computer where it is digitized to generate pulse-height histograms. In these studies the DNA mass of the target cells is established relative to a standard DNA mass in cells from a reference species. And, because genome size estimated by one technique may not be comparable to that estimated by another technique, it is often necessary to normalize values in relation to a particular standard when relating one study to another (Tiersch *et al.*, 1989). The chicken has been especially valuable as a reference species (Rasch *et al.*, 1971).

VARIATIONS IN GENOME MASS

DNA aneuploidy and chromosomal polymorphism

DNA aneuploidy, meaning abnormal quantity of DNA in cells, is often associated with malignancy (Dressler *et al.*, 1988; Murphy *et al.*, 1986). For that reason flow cytometric evaluation of DNA ploidy is widely used in cancer diagnosis. Now it is possible to use flow cytometry to analyze and identify individual chromosomes; the procedure is called "flow karyotyping". In this procedure, blood cells are cultured and arrested in metaphase, and the chromosomes are released by the use of detergent. The chromosomes are stained with a fluorescent dye, and the DNA content is measured as each chromosome passes through a laser beam that stimulates fluorescent emission by the dye. Chromosomal rearrangements such as duplication, deletion, and translocation have been identified by this procedure (McConnell *et al.*, 1989).

Chromosomal polymorphism has been documented in humans by flow karyotyping. In the study by Trask *et al.* (1989) for example, chromosomes Y, 21, 22, 15, 16, 13, 14, and 19 were found to be the most heteromorphic, and chromosomes 2 through 8 and X were found to be the least heteromorphic. In the study by Harris *et al.* (1986) chromosomes 1, 9, 16 and Y were the most variable, followed by chromosomes 13, 14, 15, 21 and 22. Variability in individual chromosomes was substantial; the difference between the largest chromosome 1 and the smallest chromosome 1 was 20.8%, or almost 1% of the total genome—a mass greater than that of chromosome 21. The Y chromosome was especially variable with a difference of 85.9% found between the largest Y and the smallest. Microspectrophotometric analysis of banded Y chromosomes revealed a variation of 70%, attributed to the number of discrete blocks of heterochromatin present (Wall and Butler, 1989).

Table 2. Variation in DNA mass in human subjects with chromosome aneuploidies

Subject	Condition	Karyotype	No. Cases	DNA content Mean ± SE
Male	Standard	46,XY	1	1.0000
Male	Normal	46,XY	15	0.9997 ± 0.0005
Female	Normal	46,XX	15	1.0167 ± 0.0004
Female	Turner syndrome	45,X	5	0.9911 ± 0.0012
Female	Down Syndrome	47,XX + 21	3	1.0299 ± 0.0039
Female	Trisomy-13	47,XX + 13	1	1.0421
Male	Down syndrome	47,XY + 21	1	1.0091
Male	Triple-X	48,XXXY	1	1.0488
Male	Klinefelter syndrome	47,XXY	1	1.0181
Male	Double-Y	47,XYY	1	1.0056

Based on data on Elias *et al.* (1988).

Sex-chromosome differences

Differences in DNA mass due to sex-chromosome–heteromorphism are readily detected in species with X and Y, or Z and W sex chromosomes. In the study by Elias *et al.* (1988) differences between populations of XX and XY cells were identified consistently in the human. When a value of 1.0000 was assigned to the DNA mass of leukocytes from a normal male reference (46,XY), the mean value ± SEM for 15 normal males was 0.9997 ± 0.0005 and that for 15 normal females was 1.0167 ± 0.0004 ($P < 0.0001$, t-test). In other words, the mean relative DNA content of white blood cells from women was found to be greater by 1.7% than that in leukocytes from men. There was no overlap between the values for men and women. Similar findings were reported for the horse (Kent *et al.*, 1988) and for many species of birds having distinct Z and W sex chromosomes (Nakamura *et al.*, 1990).

Chromosomal aneuploidy

In their study of the DNA content of human leukocytes, Elias *et al.* (1988) also evaluated various chromosome abnormalities including 13 cases of aneuploidy (a condition in which the chromosome number is not an exact multiple of the haploid number). As described in the preceding paragraph, DNA mass was estimated by comparison with the DNA mass (= 1.0000) in cells from a "standard" 46,XY male. The average DNA mass of leukocytes from five females with the 45,X karyotype (Turner syndrome) was 0.9911 ± 0.0012, and the average for three females with 47,XX + 21 (Down syndrome) was 1.0299 ± 0.0039. Thus the values for the five 45,X females were significantly lower than those for normal 46,XX females ($P < 0.001$), and the values for the three 47,XX + 21 females were significantly greater than those for normal females ($P < 0.01$, non-parametric Wilcoxon test). In addition, the DNA mass in cells from a female with 46,XX + 13 was 1.0421. In each of the nine aneuploid females, the values fell outside the range of values for normal 46,XX females (Table 2).

Four aneuploid males also were studied: 47,XY + 21 (Down syndrome), 47,XXY (Klinefelter Syndrome), 48,XXXY and 47,XYY. The DNA values for these subjects were: 1.0091, 1.0181, 1.0488 and 1.0056 respectively, each one outside the range of DNA values for normal 46,XY males (Table 2).

Within-species variation

Genome size has been called the "C-value" in reference to the suggestion that each species has a constant and distinctive cellular DNA mass (Swift, 1950; Manfredi-Romanini, 1985). This idea has served as a paradigm for research in genome size, and although many species have been studied, conclusions often are based on the study of only one or two animals. For example, in birds genome size has been studied in more than 130 species, yet only four are represented by analysis of 10 or more animals (Tiersch and Wachtel, 1991). Recent studies, however, have shown levels of genome size variation as large as 101% within species (e.g. Lockwood and Derr, 1992; Sherwood and Patton, 1982) (Table 3),

Table 3. Within-species variation for selected species

Scientific name	Common name	N	Percent variation (%)	Reference
Coregonus nasus	Broad whitefish	111	101.2*	Lockwood and Derr, 1992
Carollia brevicauda	Neotropical bat	9	33.3	Burton *et al.*, 1989
Trachemys scripta	Slider turtle	82	31.9	Lockwood *et al.*, 1991
Thomomys bottae	Pocket gopher	127	30.7	Sherwood and Patton, 1982
Oncorhynchus clarki	Cutthroat trout	28	16.0	Johnson *et al.*, 1987
Notemigonus crysoleucas	Golden shiner	10	12.7	Gold and Amemiya, 1987
Aphelocoma coerulescens	Scrub jay	39	4.6	Tiersch and Mumme, 1993
Salmo salar	Atlantic salmon	31	4.4	Johnson *et al.*, 1987
Anthias squamipinnis	Wreckfish	22	3.0	Wachtel *et al.*, 1991
Coturnix japonica	Japanese quail	49	2.5	Tiersch and Wachtel, 1991
Ictalurus punctatus	Channel catfish	115	2.5	Tiersch *et al.*, 1990

*Calculated as range divided by the mean.

and study of genome size variation at the population level is just beginning to receive attention.

Variation in genome size within a species can be greater than the variation found among species (Sherwood and Patton, 1982) and according to Johnson *et al.* (1987), measurements from single individuals, or even single populations, are insufficient for assigning a DNA value to a species. While it should be emphasized that a large variation can exist and that adequate sampling should be employed, it should also be noted that genome size variation can be quite small (Table 3). Thus we found no significant difference among DNA values in 14 populations of *Ictalurus punctatus*, the channel catfish (Tiersch *et al.*, 1990). Within-population variation averaged 1.5%, and the total variation for 115 fish was 2.5%, which is the lowest value reported among vertebrates for a sample of this size. Indeed, 2 pg represented, in the strict sense, a constant ("C") values for the species. This may reflect artificial stabilization of genome size by human intervention, or alternatively, evolutionary conservatism within the genome of the channel catfish.

Species–species variation

Much of the work on species-level variation in genome size has been concentrated within certain families. The groups most studied include the salmonid and cyprinid fishes (Johnson *et al.*, 1987; Gold *et al.*, 1990; Lockwood and Derr, 1992), plethodontid salamanders (Sessions and Larson, 1987), neotropical bats (Burton *et al.*, 1989), primates (Pellicciari *et al.*, 1982) and pocket gophers (Sherwood and Patton, 1982).

In a study of 29 species of neotropical bats by Burton *et al.* (1989) relatively few significant differences were found among the mean values for DNA content of species, genera and families. No significant correlations were found between DNA content and cytogenetic traits such as diploid chromosome number, fundamental number (of chromosome arms) and rate or pattern of chromosomal evolution.

Ragland and Gold (1989) observed that DNA values within and among different species of centrarchid and cyprinid fishes were continuous and distributed normally. The authors suggested that changes in DNA quantity in these species were small, involved gains and losses, and were cumulative and independent in effect. They concluded that changes in genome size might be concentrated in speciation events in these fishes. Study of armored catfishes of the genus *Corydoras* revealed a range in diploid number of chromosomes of 46–102, and a range in values of genome size of 1.39–8.75 pg (Oliveira *et al.*, 1992). The cytogenetic differences were related to chromosomal rearrangements and to differences in genome size, and the authors suggested that different groups of species within this genus followed distinct evolutionary trends.

The variation observed among species is at the heart of what is perceived as the C-value paradox, but it would seem that this variation is continuous and overlapping in nature, and despite whatever major trends that might exist, the genome encounters gains and losses of DNA over time.

Polyploidy and heteroploidy

Mammals are unable to tolerate polyploidy, a condition responsible for a significant frequency of embryonic deaths in man and agricultural animals. Other vertebrates can tolerate polyploidy; adult triploid, tetraploid and pentaploid forms have been described in birds (Ohno *et al.*, 1963), reptiles, amphibians and fishes (Vrijenhoek *et al.*, 1989).

Heteroploidy, the occurrence of cells of different ploidy within an organism, is not uncommon in vertebrates, and has been described in mammals. An example is diploid–triploid heteroploidy described in the human by Ellis *et al.* (1963) and in the cat by Chu *et al.* (1964). The domestic chicken has exhibited a striking array of heteroploid forms including haploid–diploid, haploid–triploid, haploid–diploid–triploid, diploid–triploid and diploid–tetraploid (Fechheimer and Jaap, 1978; Thorne *et al.*, 1987). These combinations can involve complex origins, as in the case of the haploid–diploid chicken embryo derived entirely from three paternal haploid genomes (Fechheimer and Jaap, 1978).

Diploid–triploid mosaicism occurs in wild populations of turtles (Bickham *et al.*, 1985) and is widespread in hybrids of minnows of the genus *Phoxinus* (Dawley and Goddard, 1988). In these minnows, studied by flow cytometry, the ratio of diploid to triploid cells formed a continuum of values between the pure diploid and triploid forms.

Multiploidy, an extreme form of heteroploidy, has been described in succulent plants in which multiples corresponding to 2, 4, 8, 16, 32 and 64 times the haploid genome complement were observed in a tissue-specific fashion and at characteristic developmental stages in leaves (De Rocher *et al.*, 1990).

Technical variation

Interpretation of studies on genome size can be complicated by disparities in technique (Rasch and Rasch, 1973). For example, different species are used as reference standards by different laboratories for comparisons between species (Rasch, 1985). In addition, different values have been reported for the species used as standards, including the domestic chicken, the most used standard, for which the values of 2.54 pg (Rasch *et al.*, 1971) and 3.45 pg (Bachmann *et al.*, 1972) have been reported. This can lead to systematic differences in values, and can artificially increase the observed variation. In a similar fashion, the use of different techniques can yield different values, although Feulgen densitometry and flow cytometry have been shown to yield similar results (Michaelson *et al.*, 1991).

EVOLUTIONARY ASPECTS OF GENOME SIZE

In vertebrates

On the basis of his study of genome mass in fishes, Hinegardner (1976) developed several points, including the following.

(i) Within major taxa, the distribution of DNA content is often asymmetrical; the majority of species have values skewed to the low end of the curve.
(ii) The DNA mass of a particular species may or may not be related to the number of chromosomes.
(iii) Most eukaryotic species have a diploid DNA content of more than 0.8 pg.
(iv) Within certain taxa, highly-specialized forms have less DNA than do less-specialized forms.

These points would seem to hold true throughout the taxa that have been studied. For example, certain deep-sea fishes and others (e.g. sea horse) having unusual or even bizarre body forms have smaller genomes than do the more well-known species with bodies more commonly recognizable as "fish-like" (e.g. catfish, salmon, etc.). The universality of this point has been questioned, however; see Cavalier-Smith (1985a).

The important study by Sessions and Larson (1987) was the first in which genome size and developmental parameters were analyzed jointly in a phylogenetic framework. Among 27 species of salamanders with genome sizes ranging from 14 to 76 pg, the rate of differentiation in regenerating tissue was inversely proportional to the mass of nuclear DNA. The authors suggested that genome size may be an important constraint in development and limb regeneration in plethodontid salamanders, and may play a role in the evolution of traits such as neoteny, the retention of juvenile characteristics in sexually mature adults.

Olmo *et al.* (1989) Provided a more mechanistic interpretation of genome size evolution in vertebrates. These authors cited divergences between amniotes and anamniotes in DNA quantity and composition which would involve changes in genome control and organization. Changes such as these would yield a reduced and relatively stable genome size in amniotes, and a less restricted modulation of genome size in anamniotes allowing a greater range of adaptations to different environments.

In birds

We studied genome size in 55 species of birds representing 12 different orders. We then compared our values with those obtained in other laboratories, normalizing all of the data in relation to a standard diploid value of 2.5 pg DNA for the domestic chicken. We surveyed 135 species overall, representing 17 orders. The mean value for DNA mass was 2.82 ± 0.03 pg (SEM), and the range of values was 2.0–3.8. These and other observations (Cavalier-Smith, 1978) show that birds have the smallest amount of species–species variation of any vertebrate class. The variation within avian species is also minimal. For example the mean DNA mass in Japanese quail (*Coturnix coturnix*) is 2.81 ± 0.02 pg, with a range of 2.78–2.85, and a variation of 2.5% (Table 3). Yet despite the small size and constancy of the avian genome, birds are ecologically diverse and speciose.

There are at least two explanations for the size of the bird genome: (a) DNA content was gradually reduced after the emergence of birds from a reptile precursor and during their subsequent radiation; (b) birds *inherited* a reduced genome directly from the reptile precursor, and retained it throughout their radiation (Fig. 1). Cell size is tightly correlated with genome size, and selection for an advantageous cell surface-to-volume ratio could thus influence DNA content in a taxon (Cavalier-Smith, 1978; Szarski, 1974).

Evidently birds are monophyletic; i.e. they are evolved from a single precursor. A flesh-eating dinosaur about the size of a chicken would make an excellent candidate for the precursor (Carroll, 1988; Feduccia, 1980), and it follows that a small genome attendant to the vigorous predatory lifestyle of the little dinosaur could act as a crucial adaptation for the evolution of endothermy and flight (Fig. 1).

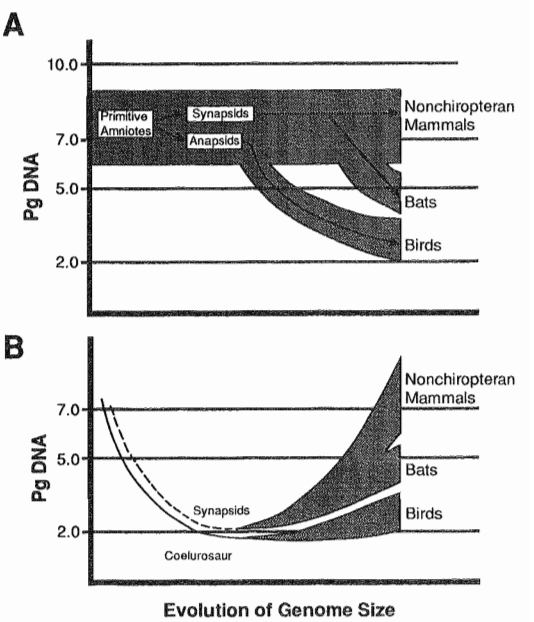

Fig. 1. Two models for evolution of genome size. (A) Conventional model for development of small genome size in bats and birds. (B) Alternative model according to which DNA content in bats and birds arose by retention of a small ancestral genome less than the 5–10 pg suggested by Thomson and Muraszko (1978). A convergent trend towards increased genome size is indicated. With DNA mass severely restricted by the metabolic constraints of flight, bats and birds each characterize an evolutionary snapshot in an ascending gradient of genome sizes among endotherms. The figure does not convey actual time spans or evolutionary distances. From Tiersch and Wachtel (1991); reprinted by permission.

In fact, the earliest fossil bird, *Archaeopteryx lithographica*, was probably a homeothermic endotherm (Houck *et al.*, 1990). And a sparrow-sized bird (*Sinornis santensis*) which appeared soon after *Archaeopteryx* (15 Myr) is known to have possessed many of the traits of modern birds while retaining certain of the traits of *Archaeopteryx* (Sereno and Chenggang, 1992). This indicates that modern flight evolved in small birds soon after *Archaeopteryx*, and supports the idea that the metabolic apparatus able to sustain flight was already well in place prior to the rapid emergence of modern birds. From that perspective, the pronounced mode of 2.5 pg DNA mass which is found in birds (Tiersch and Wachtel, 1991) does not represent the end of an evolutionary reduction in genome size; it is the starting point for avians in general.

REFERENCES

Bachmann K., Harrington B. A. and Craig J. P. (1972) Genome size in birds. *Chromosoma* **37,** 405–416.

Bickham J. W., Tucker P. K. and Legler J. M. (1985) Diploid–triploid mosaicism: an unusual phenomenon in side-necked turtles (*Platemys platycephala*). *Science* **227,** 1591–1593.

Britten R. J. and Davidson E. H. (1971) Repetitive and non-repetitive DNA sequences and a speculation on the origins of evolutionary novelty. *Q. Rev. Biol.* **46,** 111–138.

Burton D. W., Bickham J. W. and Genoways H. H. (1989) Flow-cytometric analyses of nuclear DNA content in four families of neotropical bats. *Evolution* **43,** 756–765.

Carroll R. L. (1988) *Vertebrate Paleontology and Evolution.* W. H. Freeman, New York.

Cavalier-Smith T. (1978) Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. *J. Cell Sci.* **34,** 247–278.

Cavalier-Smith T. (1985a) Evolutionary significance of genome size. In *The Evolution of Genome Size* (Edited by Cavalier-Smith T.), pp. 1–36. Wiley, London.

Cavalier-Smith T. (1985b) Eukaryotic gene numbers, non-coding DNA and genome size. In *The Evolution of Genome Size* (Edited by Cavalier-Smith T.), pp. 69–104. Wiley, London.

Chu E. H. Y., Thuline H. C. and Norby D. E. (1964) Triploid–diploid chimerism in a male tortoiseshell cat. *Cytogenetics* **3,** 1–18.

Dawley R. M. and Goddard K. A. (1988) Diploid–triploid mosaics among unisexual hybrids of the minnows *Phoxinus eos* and *Phoxinus neogaeus*. *Evolution* **42,** 649–659.

De Rocher E. J., Harkins K. R., Galbraith D. W. and Bohnert H. J. (1990) Developmentally regulated systemic endopolyploidy in succulents with small genomes. *Science* **250,** 99–101.

Dressler L. G., Seamer L. C., Owens M. A., Clark G. M. and McGuire W. L. (1988) DNA flow cytometry and prognostic factors in 1331 frozen breast cancer specimens. *Cancer* **61,** 420–427.

Elias S., Chandler R. W. and Wachtel S. (1988) Relative DNA content of interphase leukocytes by flow cytometry: a method for indirect diagnosis of chromosomal abnormalities with potential for prenatal diagnosis. *Am. J. Obstet. Gynecol.* **158,** 808–818.

Ellis J. R., Marshall R., Normand I. C. S. and Penrose L. S. (1963) A girl with triploid cells. *Nature* **198,** 411.

Fecheimer N. S. and Jaap R. G. (1978) The parental source of heteroploidy in chick embryos determined with chromosomally marked gametes. *J. Reprod. Fert.* **52,** 141–146.

Feduccia A. (1980) *The Age of Birds.* Harvard University Press, Cambridge, MA.

Gold J. R., Ragland C. J. and Schliesing L. J. (1990) Genome size variation and evolution in North American cyprinid fishes. *Genet. Sel. Evol.* **22,** 11–29.

Gold J. R. and Amemiya C. T. (1987) Genome size variation in North America minnows (Cyprinidae). II. Variation among 20 species, *Genome* **29,** 481–489.

Harris P., Boyd E., Young B. D. and Ferguson-Smith M. A. (1986) Determination of the DNA content of human chromosomes by flow cytometry. *Cytogenet. Cell Genet.* **41,** 14–21.

Hinegardner R. (1976) Evolution of genome size. In *Molecular Evolution* (Edited by Ayala F. J.), pp. 179–199. Sinauer, Sunderland, Massachusetts.

Hinegardner R. and Rosen D. E. (1972) Cellular DNA content and the evolution of teleostean fishes. *Am. Nat.* **106,** 621–644.

Houck M. A., Gauthier J. A. and Strauss R. E. (1990) Allometric scaling in the earliest fossil bird, *Archeopterix lithographica. Science* **247,** 195–198.

Johnson O. W., Utter F. M. and Rabinovitch P. S. (1987) Interspecies differences in salmonid cellular DNA identified by flow cytometry. *Copeia* **1987,** 1001–1009.

Kent M., Chandler R. W. and Wachtel S. (1988) DNA analysis by flow cytometry. *Cytogenet. Cell Genet.* **47,** 88–89.

Lockwood S. F. and Derr J. N. (1992) Intra- and interspecific genome size variation in the salmonidae. *Cytogenet. Cell Genet.* **59,** 303–306.

Lockwood S. F., Holland B. S., Bickham J. W., Hanks B. G. and Bull J. J. (1991) Intraspecific genome size variation in a turtle (*Trachemys scripta*) exhibiting temperature-dependent sex determination. *Can. J. Zool.* **69,** 2306–2310.

Manfredi-Romanini M. G. (1985) The nuclear content of desoxyribonucleic acid and some problems of mammalian phylogenesis. *Mammalia* **49,** 369–385.

McConnell T. S., Cram L. S., Baczek N., Fawcett J. J., Luedemann M. and Bartholdi M. F. (1989) The clinical usefulness of chromosome analysis by flow cytometry. *Semin. Diagnost. Pathol.* **6,** 91–107.

Michaelson M. J., Price H. J., Ellison J. R. and Johnston J. S. (1991) Comparison of plant DNA contents determined by Feulgen microspectrophotometry and laser flow cytometry. *Am. J. Botany* **78,** 183–188.

Mirsky A. E. and Ris H. (1951) The desoxyribonucleic acid content of animal cells and its evolutionary significance. *J. gen. Physiol.* **34,** 451–463.

Murphy W. M., Emerson L. D., Chandler R. W., Moinuddin S. M. and Soloway M. S. (1986) Flow cytometry versus urinary cytology in the evaluation of patients with bladder cancer. *J. Urology* **136,** 815–819.

Nakamura D., Tiersch T. R., Douglass M. and Chandler R. W. (1990) Rapid identification of sex in birds by flow cytometry. *Cytogenet. Cell Genet.* **53,** 201–205.

Ohno S., Kittrell W. A., Christian L. C., Stenius C. and Witt G. A. (1963) An adult triploid chicken (*Gallus domesticus*) with a left ovotestis. *Cytogenet.* **2,** 42–49.

Oliveira C., Almeida-Toledo L. F., Mori L. and Toledo-Filho S. A. (1992) Extensive chromosomal rearrangements and nuclear DNA content changes in the evolution of the armoured catfishes genus *Corydoras* (Pisces, Siluriformes, Callichthyidae). *J. Fish Biol.* **40,** 419–431.

Olmo E., Capriglione T. and Odierna G. (1989) Genome size evolution in vertebrates: trends and constants. *Comp. Biochem. Physiol.* **92B,** 447–453.

Pellicciari C., Formenti D., Redi C. A. and Manfredi-Romanini M. G. (1982) DNA content variability in primates. *J. Human Evol.* **11,** 131–141.

Price H. J. (1976) Evolution of DNA content in higher plants. *Botan. Rev.* **42,** 27–52.

Ragland C. J. and Gold J. R. (1989) Genome size variation in the North American sunfish genus *Lepomis* (Pisces: Centrarchidae). *Genet. Res.* **53,** 173–182.

Rasch E. M., Barr H. J. and Rasch R. W. (1971) The DNA content of sperm of *Drosophila melanogaster*. *Chromosoma* **33,** 1–18.

Rasch R. W. and Rasch E. M. (1973) Kinetics of hydrolysis during the Feulgen reaction for deoxyribonucleic acid. *J. Histochem. Cytochem.* **21,** 1053–1065.

Rasch E. M. (1985) DNA "standards" and the range of accurate DNA estimates by Feulgen absorption microspectrophotometry. In *Advances in Microscopy* (Edited by Cowden R. R. and Harrison F. W.), pp. 137–166. Alan R. Liss, New York.

Sereno P. C. and Chenggang R. (1992) Early evolution of avian flight and perching: new evidence from the lower Cretaceous of China. *Science* **255,** 845–848.

Sessions S. K. and Larson A. (1987) Developmental correlates of genome size in plethodontid salamanders and their implications for genome evolution. *Evolution* **41,** 1239–1251.

Sherwood S. W. and Patton J. L. (1982) Genome evolution in pocket gophers (Genus *Thomomys*) II. Variation in cellular DNA content. *Chromosoma* **85,** 163–179.

Swift H. (1950) The constancy of desoxyribose nucleic acid in plant nuclei. *Proc. natn. Acad. Sci. U.S.A.* **36,** 643–654.

Szarski H. (1974) Cell size and nuclear DNA content in vertebrates. *Int. Rev. Cytol.* **44,** 93–111.

Thomson K. S. and Muraszko K. (1978) Estimation of cell size and DNA content in fossil fishes and amphibians. *J. exp. Zool.* **205,** 315–320.

Thorne M. H., Collins R. K. and Sheldon B. L. (1987) Live haploid–diploid and other unusual mosaic chickens (*Gallus domesticus*). *Cytogenet. Cell Genet* **45,** 21–25.

Tiersch T. R. and Mumme R. L. (1993). An evaluation of the use of flow cytometry to identify sex in the Florida Scrub Jay. *J. Field Ornithol.* (in press).

Tiersch T. R. and Wachtel S. S. (1991) On the evolution of genome size of birds. *J. Hered.* **82,** 363–368.

Tiersch T. R., Chandler R. W., Wachtel S. S. and Elias S. (1989) Reference standards for flow cyometry and comparative studies of nuclear DNA content. *Cytometry* **10,** 706–710.

Tiersch T. R., Simco B. A., Davis K. B., Chandler R. W., Wachtel S. S. and Carmichael G. J. (1990) Stability of genome size among stocks of the channel catfish. *Aquaculture* **87,** 15–22.

Trask B., van den Engh G., Mayall B. and Gray J. W. (1989) Chromosome heteromorphism quantified by high-resolution bivariate flow karyotyping. *Am. J. Hum. Genet.* **45,** 739–752.

Vrijenhoek R. C., Dawley R. M., Cole C. J. and Bogart J. P. (1989) In *A List of the Known Unisexual Vertebrates* (Edited by Dawley R. M. and Bogart J. P.), pp. 19–23. Bulletin 466, New York State Museum, Albany, NY.

Wachtel S., Demas S., Tiersch T., Pechan P. and Shapiro D. (1991) Bkm satellite DNA and *ZFY* in the coral reef fish *Anthias squamipinnis*. *Genome* **34,** 612–617.

Wall W. J. and Butler L. J. (1989) Classification of Y chromosome polymorphisms by DNA content and C-banding. *Chromosoma* **97,** 296–300.