

Genome Size and Chromosome Number Relationship Contradicts the Principle of Darwinian Evolution from Common Ancestor

Ahmed M El-Shehawi^{1,2*} and Mona M Elseehy²

¹Department of Biotechnology, Taif University, Taif, Saudi Arabia

²Department of Genetics, University of Alexandria, Alexandria, Egypt

*Corresponding author: El-Shehawi AM, Department of Biotechnology, Faculty of Science, Taif University, Taif 26571, Saudi Arabia, Tel: 966554904625; E-mail: elshehawi@hotmail.com

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Abstract

Genome is the nucleotide sequence of a haploid genome distributed among certain number of chromosomes. It controls the reproduction of unique features of a species. The relationship between Genome Size (GS) and Chromosome Number (CN) has been a paradox in biology partially because of the shortage in genome size records with detected chromosome number. Genome size databases provided a valuable source for this type of data allowing the study of this relationship in details. In this study, a number of 6052 genome size records with detected chromosome number were used to investigate the relationship between GS and CN and detect the location of human genome. Results showed that there is no correlation between GS and CN, yet CN is fluctuated with increasing GS values. Also, the relationship between Chromosome Number Sets (CNSs) and Average Genome Size (XGS), average Genome Size per Chromosome (GS/Ch), and the Upper Limit of Genome Size per Chromosome (ULGS/Ch) revealed lack of correlations with various degrees of fluctuation. These various types of interrelationships indicate the lack of evolutionary trend between genome size estimates and chromosome number. The human genome was located at 4/6 away from the controversial common ancestor genome and 2/6 away from the largest detected genome. Results of this study contradict the principle of Darwinian evolution from common ancestor and support the independent appearance of living organisms on earth. This will open the door for new explanations for the existence of living organisms on earth based on genome size.

Keywords: Genome size; Chromosome number; Genome Size per Chromosome (GS/Ch); Genome evolution; Darwinian evolution

Introduction

Genome is the amount of genetic material, DNA, in the haploid cell of an organism known as the "C value". In eukaryotes, this amount of DNA is distributed among a certain number of chromosomes in each species. Genome Size (GS) and Chromosome Number (CN) are two essential taxonomic criteria. Genome is responsible for reproducing the unique features of a species over generations. If genome changes in size/or the number of chromosome, drastic changes in the species' features could occur. Also, the fine unique organization of a genome is very significant because two distinct species could have the same genome size and different chromosome number with completely different structural phenotypic features. For example, the plant Cumin (*Cuminum cyminum*) has genome size of 3.5 pg distributed on 7 chromosomes (2n=14) [1], while human (*Homo sapiens*) has the same genome size (3.5 pg) [2] distributed among 23 chromosomes (2n=46). They have completely different structural features and development.

Genome Size Variation

Variation in genome size known as "genome size enigma" has been extensively discussed in many studies and was proved to have no correlation with organism structural complexity [3-7]. Also intra-specific variation in genome size have been documented and

correlated with phenotypic variations caused by the percentage of heterochromatin and non-coding repetitive DNA sequences [8].

Mutations, recombination, and transposons are considered the main three mechanisms for genome size change (evolution) and are considered the driving forces for diversification (speciation). Studies regarding these mechanisms have been focused on the underlying molecular mechanisms for genome size change and overlooked how these mechanisms lead to species evolution. This leaves a huge gap between theoretical species evolution and the detailed molecular mechanisms of genome size evolution.

The evolution from common ancestor by Charles Darwin [9] based on series of continuous phenotypic structural similarities have been the legitimate explanations for the appearance and existence of living organisms on earth. Since genome controls the reproduction of species with all of its specific features, therefore the genome must evolve before organism evolution. Therefore, the common ancestor genome, according to Darwinian evolution, had to gain more DNA and arrange it in a new unique format to be able to regenerate new genome and then new species with distinct features. In the same time, in eukaryotes, this new genome should be redistributed on a different number of chromosomes in most cases to distinguish the new species from its ancestor. All of these events should happen with minimum deleterious effects on the organism. Formation of new species (speciation) was mistakenly linked to the formation of larger genomes but several studies reported that diversification was also shown to be linked to smaller genomes [3,10,11]. Also, no correlation between overall genome size and speciation rate was reported [12].

Chromosome number evolution (change)

Chromosome number, karyotype, is the distribution of genome among certain number of chromosomes. It has been considered as an essential taxonomic and evolutionary criterion of living species [13-16]. The minimum interaction hypothesis of karyotype introduced by Imai et al. [17] suggested that the karyotype usually tends to increase the number of acrocentric and reduce the formation of metacentric chromosomes (m-chromosomes) [17,18]. This led to the suggestions that there would be a scaling relationship between karyotype asymmetry and m-chromosomes number (m-CN) [19,20]. This introduced the Karyotype Asymmetry Index (KAI) as a valid parameter of size on chromosome arm length measured by the chromosome size/or the centromere position [21].

It was reported in woody plants that there is a trade-off between chromosome size and chromosome number because it was observed that a 3/4 scaling pattern between Total Chromosomes Number (TCN) and metacentric chromosomes (m-chromosomes) within a nucleus. This indicated that the TCN may have evolved from more to less in woody plants because deciduous species had more m-chromosomes while evergreens had more total chromosomes number in their nuclei. The increase in TCN will trigger an increase in m-chromosomes at a 1.5-fold faster rate [13]. Increasing the number of m-chromosomes preserves karyotype symmetry which opposes the minimum interaction theory mentioned above. Many primary chromosome rearrangements can change the shape and size of chromosomes including position of the centromere, Nucleolus Organizing Region (NOS) position, inversion, reciprocal translocation, insertion by transposition or unequal sister chromatid exchange (mitosis) unequal crossover (meiosis), and deletion [18].

Genome Size per Chromosome (GS/Ch) is another overlooked factor that has never been investigated using large number of genome size records. It should be considered when talking about genome evolution because genome size in each organism is distributed among certain number of chromosomes. When changes to this distribution occur they cause complex genetic problems and sometimes death, therefore, it should be considered as a genome evolution factor. So, the question regarding genome evolution is not only a matter of genome size increase but also genome distribution on specific number of chromosomes (GS/Ch).

Chromosome capacity of DNA is limited because of replication complexity and the size of metaphase chromosomes vary among species. In plants, it was suggested that when the chromosome arm is longer than half of the spindle axis it can cause problems during telophase of mitosis because of the breakage of the non-separated chromatid arms during cell wall formation [22,23]. On the other side, chromosomes that contain less than 1% of the genome do not segregate properly during meiosis [24,25].

The relationship between genome size and chromosome number has not been studied in details in large number of genome records. In this study, the correlation between genome size and chromosome number, the location of human genome among others, the impact of this relationship on the principle of evolution from common ancestor were investigated. Also, the relationship between Chromosome Number Sets (CNSs) with average genome size, average genome size per chromosome, and the upper limit of genome size per chromosome was investigated.

Materials and Methods

Genome size data

The establishment of genome size databases has been important step in studying genomes in living organisms. The data available in various genome size databases provided a powerful source of genome size records from different taxa for studying GS, CN, and their relationship in unprecedented way. Data of genome size and chromosome number were obtained from the four main databases; animal genome size database (<http://www.genomesize.com>), plant genome size database (<http://data.kew.org/cvalues>), fungal genome size database (<http://www.zbi.ee/fungal-genomesize>), prokaryotic genome size database (<http://www.genomesize.com/prokaryotes>). Combined they contain 18103 genome size records from various living organisms. This number of genome size records was used for gathering any information about genome size like the largest and smallest genome size, genome size averages, the genome size range (folds) as general or within every living group. In genome size databases, genome size is expressed by picogram of DNA (pg), but not all genome size records have a detected chromosome number. To make our estimations regarding chromosome number more accurate, we excluded polyploid genomes and any genome records without detected chromosome number. This reduced the number of genome size records with detected chromosome number to 6052 (Supplementary Material 1). This group was used for any estimation about chromosome number in different living groups. Also, data of this group (6052) were used to investigate the relationship between genome size and chromosome number, to locate the human genome among other genomes, and to evaluate other genome size estimates. The formula ($\text{Number bps} = \text{mass in pg} \times 0.978 \times 10^9$) [26] was used to convert genome size in bp into genome size in pg when needed.

Chromosome Number Sets (CNSs) and genome size estimates

A Chromosome Number Set (CNS) is a group of genomes that have the same chromosome number but they do not necessarily have the same genome size. Genome Size (GS) was divided by Chromosome Number (CN) to give the average Genome Size per Chromosome (GS/Ch). The highest GS/Ch in each CNS was considered as the upper limit of genome size per chromosome (ULGS/Ch) in this CNS. Average GS, average GS/Ch, and the ULGS/Ch were separately plotted against CNSs to investigate their relationship (Supplementary Material 2).

Results

Genome size

It has been documented that there is great variation in genome size, but it has not been discussed in deep details using large number of genome size data. Comparing the genome size in 18103 genome records some features can be pointed out. Generally, genome size ranged from 0.0005 pg (*Buchnera* sp.) to 132.83 pg (*Protopterus aethiopicus*) (265660 folds) (Table 1). In animals it ranged from 132.83 pg to 0.02 pg (6661 folds), whereas in plants, it ranged from 86.05 pg to 0.01 pg (8605 folds). In microbes, it ranged from 0.47 pg to 0.0005 pg (940 folds). The general average of genome size ranged from 64.9 in lobe-finned fish to 0.0033 in bacteria (19666 folds).

Group	No of GS Records	Genome size, pg				Folds	XGS* (pg)
		Largest		Smallest			
Animals							
Amphibians	927	<i>Necturus lewisi</i>	120.6	<i>Limnodynastes ornatus</i>	0.95	127	18.9
Annelids	145	<i>Spirosperma ferox</i>	7.64	<i>Dinophilus gyrocolliatus</i>	0.06	127	1.3
Arachnids	137	<i>Boophilus microplus</i>	7.5	<i>Tetranychus urticae</i>	0.08	93.7	2.8
Birds	896	<i>Struthio camelus Ostrich</i>	2.16	<i>Archilochus alexandri</i>	0.91	2.4	1.36
Chordates	5	<i>Branchiostoma lanceolatum</i>	0.59	<i>Phallusia mammillata</i>	0.06	9.8	0.2
Cnidarians	11	<i>Hydra attenuate</i>	1.85	<i>Nematostella vectensis</i>	0.23	8	1
Crustaceans	330	<i>Ampelisca macrocephala</i>	64.62	<i>Cyclops kolensis</i>	0.14	461	5.1
Ctenophores	2	<i>Haeckelia rubra</i>	3.16	<i>Mnemiopsis leidyi</i>	0.31	10	1.7
Echinoderms	48	<i>Thyonella gemmata</i>	4.4	<i>Paracentrotus lividus</i>	0.54	8	1.3
Cartilaginous Fish	183	<i>Oxynotus centrina</i>	17.05	<i>Hydrolagus colliei</i>	1.51	11.3	5.6
Jawless Fish	26	<i>Myxine garmani</i>	4.59	<i>Lampetra fluviatilis</i>	1.29	3.6	2.3
Lobe-Finned Fish	17	<i>Protopterus aethiopicus</i>	132.83	<i>Latimeria chalumnae</i>	2.8	47	64.9
Ray-Finned Fishes	1819	<i>Acipenser brevirostrum</i>	9.32	<i>Tetraodon fluviatilis</i>	0.35	26.6	1.3
Flatworms	69	<i>Otomesostoma auditivum</i>	20.52	<i>Stenostomum brevipharyngium</i>	0.06	342	2.1
Insects	930	<i>Podisma pedestris</i>	16.93	<i>Mayetiola destructor</i>	0.09	188	1.2
Mammals	778	<i>Tympanoctomys barrerae</i>	8.4	<i>Lophostoma carrikeri</i>	1.63	5	3.2
Molluscs	263	<i>Diplommatina kiiensis</i>	7.85	<i>Lottia gigantean</i>	0.43	18	2.1
Myriapods	15	<i>Scutigera coleoptrata</i>	2.14	<i>Strigamia maritima</i>	0.3	7	0.7
Nematodes	66	<i>Parascaris univalens</i>	2.5	<i>Hemicyclophora conida</i>	0.02	125	0.15
Reptiles	418	<i>Testudo graeca</i>	5.44	<i>Chalcides mionecton</i>	1.05	5	2.3
Rotifers	9	<i>Philodina roseola</i>	1.22	<i>Adineta vaga</i>	0.25	4.9	0.68
Sponges	101	<i>Geodia cydonium</i>	1.8	<i>Tethya actinia</i>	0.04	45	0.26
Tardigrades	21	<i>Amphibolus volubilis</i>	0.82	<i>Diphyscon nobilei</i>	0.08	10	0.37
Misc Inverts	69	<i>Eoperipatus sp.</i>	19.87	<i>Trichoplax adhaerens</i>	0.04	496	2.12
All Animals	7285		19.33		0.55	35	5.12
Plants							
Alge	253	<i>Chara contraria</i>	19.6	<i>Cyanidium caldarium</i>	0.01	196	0.88
Bryophyte	232	<i>Mylia taylorii</i>	7.97	<i>Holomitrium arboreum</i>	0.17	46.9	0.6
Pteridophyte	128	<i>Psilotum nudum</i>	72.86	<i>Selaginella sp. (apoda)</i>	0.09	809.6	10.63
Gymnosperm	355	<i>Pinus ayacahuite</i>	36	<i>Gnetum ula</i>	2.25	16	18.08
Angiosperm	7541	<i>Fritillaria platyptera</i>	86.05	<i>Genlisea margaretae</i>	0.06	1434	5.8
All Plants	8509		44.5		0.52	85.6	7.2
Fungi	1628	<i>Rhizopus oryzae</i>	0.047	<i>Ascosphaera apis</i>	0.025	1.9	0.046
Bacteria	681	<i>Sorangium cellulosum</i>	0.0125	<i>Buchnera sp</i>	0.0005	25	0.0033
Total	18103		15.97		0.27	59	5.3

Table 1: Genome size (GS) profile among living groups including species of highest and lowest genome size in each group and average genome size (XGS).

Group	No of CN records	Chromosome number (CN)			Folds	XCN [*]	
		Highest CN	Lowest CN				
Animals							
Amphibians	324	<i>Xenopus ruwenzoriensis</i>	108	<i>Bufo regularis</i>	20	5.5	28.4
Annelids	32	<i>Octolasion cyaneum</i>	190	<i>Ophryotrocha costlowi</i>	6	31.7	24.6
Arachnids	14	<i>Ixodes scapularis</i>	28	<i>Amblyomma americanum</i>	21	1.3	23.4
Birds	99	<i>Alcedo atthis</i>	138	<i>Falco peregrines</i>	50	2.8	77.3
Chordates	2	<i>Ciona intestinalis</i>	28	<i>Branchiostoma lanceolatum</i>	24	1.2	26
Crustaceans	15	<i>Monoporeia affinis</i>	52	<i>Protohyale schmidtii</i>	22	2.4	43.3
Cartilaginous fish	52	<i>Notorynchus cepedianus</i>	104	<i>Narcine brasiliensis</i>	28	3.7	73.4
Jawless fish	13	<i>Geotria australis</i>	178	<i>Myxine garmani</i>	14	12.7	88
Lobe-Finned fish	5	<i>Neoceratodus forsteri</i>	54	<i>Protopterus annectens</i>	34	1.6	48
Ray-Finned fish	667	<i>Acipenser gueldenstaedtii</i>	250	<i>Galaxias maculatus</i>	22	11.4	56.2
Insects	276	<i>Sphaeroderma testaceum</i>	52	<i>Aedes albopictus</i>	6	8.7	21.6
Mammals	385	<i>Tympanoctomys barrerae</i>	102	<i>Muntiacus muntjak</i>	6	17	46.4
Molluscs	3	<i>Goniobasis livescens</i>	40	<i>Goniobasis proxima</i>	34	1.2	36
Reptiles	170	<i>Apalone ferox</i>	66	<i>Sceloporus occidentalis</i>	22	3	38.3
Sponges	2	<i>Ephydatia fluviatilis</i>	46	<i>Ephydatia fluviatilis</i>	46	1	46
All Animals	2059		95.73		23.67	4	44.6
Plants							
Bryophytes	1	<i>Pellia borealis</i>	18	<i>Pellia borealis</i>	18	1	18
Pteridophyte	59	<i>Equisetum variegatum</i>	216	<i>Selaginella apoda</i>	18	12	94.7
Gymnosperm	220	<i>Pinus ayacahuite</i>	24	<i>Gnetum ula</i>	22	1	23.1
Angiosperm	3713	<i>Carex nubigera</i>	112	<i>Brachycome dichromosomatica</i>	4	28	21.6
All Plants	3993		92.5		15.5	6	22.8
Total	6052		94		19.5	4.8	33.7

Table 2: Chromosome number (CN) profile among living groups including highest and lowest chromosome number species and average chromosome number (XCN).

In animals, it ranged from 64.9 pg to 0.15 pg (433 folds), while in plants it ranged from 18.08 pg to 0.6 pg (30 folds), and from 0.046 pg to 0.0033 pg (14 folds) in microbes. The highest limit of genome size ranged from 132.83 pg in the lobe-finned fish to 0.0125 pg in the *Sorangium cellulosum* bacteria (10626 folds). In animals, it ranged from 132.83 pg in the lobe-finned fish *P. aethiopicus* to 0.82 pg in the tardigrade *Amphibolus volubilis* (162 fold). In plants it was less variant ranging from 86.05 pg in the angiosperm *Fritillaria platyptera* to 7.97 pg in bryophyte *Mylia taylorii* (11 folds) (Table 1). The lower limit of

genome size also showed great variations. Generally, it ranged from 2.8 pg in the lobe-finned fish *Latimeria chalumnae* to 0.0005 pg in the *Buchnera* sp. bacteria (4600 folds). In animals, it ranged from 2.8 pg in the lobe-finned fish *L. chalumnae* to 0.02 pg in the nematode *Hemicyclophora conida* (140 folds). In plants, it ranged from 2.25 pg in the angiosperm *Gnetum ula* to 0.01 pg in the alga *Cyanidium caldarium* (225 folds).

Chromosome Number (CN)

Chromosome number showed less variations among living groups compared to GS. Generally, it ranged from 250 in the ray-finned fish *Acipenser gueldenstaedtii* [27] to 4 in the angiosperm plant *Brachycome dichromosomatica* [28] (62 folds). In animals, CN ranged from 250 in the ray-finned fish *A. gueldenstaedtii* to 6 in the insect *Aedes albopictus* (41 folds), whereas in plants it ranged from 216 in the pteridophyte *Equisetum variegatum* to 4 in the angiosperm plant *B. dichromosomatica* (54 folds). The general average of CN ranged from 88 to 18 (4.9 folds), whereas it ranged from 88 to 21.6 (4 folds) in animals and from 94.7 to 18 (5.2 folds) in plants. The upper limit of CN ranged from 250 in the ray-finned fish *A. gueldenstaedtii* to 18 in the bryophytes *Pellia borealis* (13.9 folds). In animals, it ranged from 250 in the ray-finned fish *A. gueldenstaedtii* to 28 (8.9 folds) in the arachnid *Ixodes scapularis*. In plants, it ranged from 216 in the pteridophyte *Equisetum variegatum* to 18 (12 folds) in the bryophytes *Pellia borealis*. The minimum CN ranged from 50 in the bird *Falco peregrines* to 4 (8.3 folds) in the angiosperm plant *Brachycome dichromosomatica*, where in animals it ranged from 50 in the bird *Falco peregrines* to 6 (8.3 folds) in the insect *A. albopictus* and in plants it ranged from 22 in the gymnosperm *G. ula* to 4 (5.5 folds) in the angiosperm plant *Brachycome dichromosomatica* (Table 2).

Genome size and chromosome number

Genome size and chromosome number are two main unique criteria of different species. To investigate the relationship between genome size and chromosome number, 6052 diploid genomes with detected chromosome number were used. Figure 1 shows the relationship between Genome Size (GS), Chromosome Number (CN), and the ratio of GS to CN (GS/CN) which reflects the amount of genome per chromosome. It is evident that over the gradual increase of genome size there was a random fluctuation in chromosome number and consequently genome size per chromosome (Figure 1) indicating the independence of chromosome number genome size through living organisms.

Due to the lack of correlation between GS and CN, it was important to determine the location of human genome among other various genomes from different taxa. This is very critical and controversial issue. Results showed that out of 6052 genomes there were 3943 smaller and 2108 larger genomes than human genome including plants and animals in both groups. Therefore, human genome is located 4/6 away from the assumed common ancestral genome and 2/6 away from the largest detected genome (Figure 1).

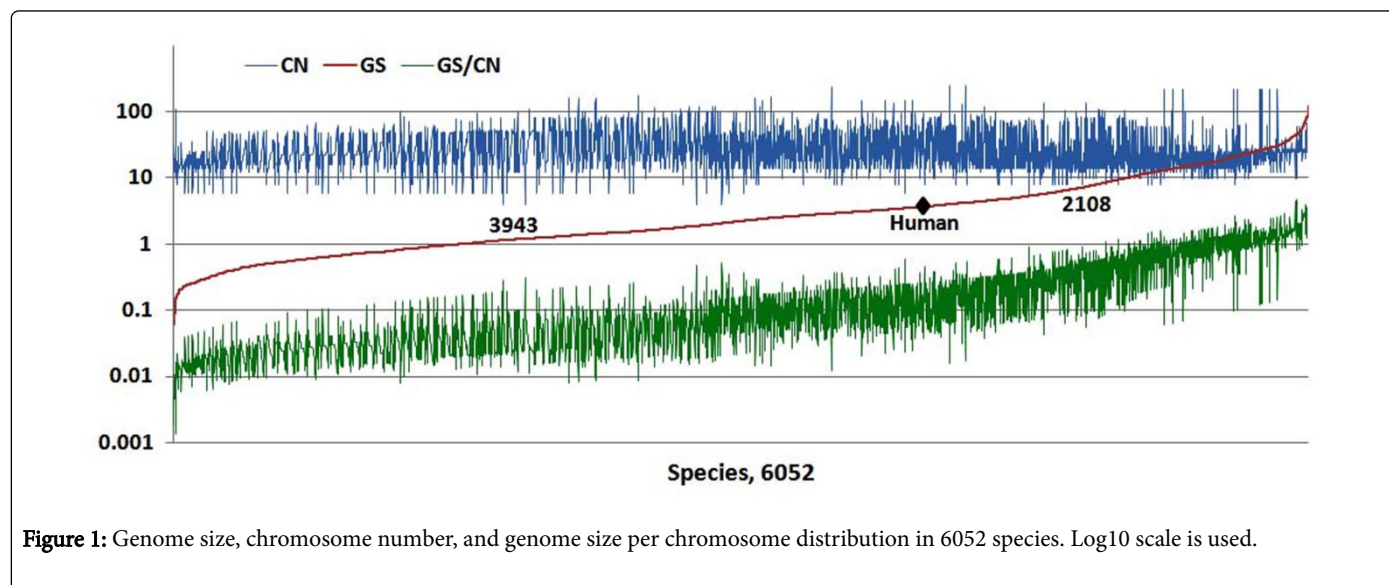


Figure 1: Genome size, chromosome number, and genome size per chromosome distribution in 6052 species. Log10 scale is used.

Chromosome Number Sets (CNSs) and Genome Size (GS)

There are many genomes with the same chromosome number (CNS); therefore, we tested the relationship between average genome sizes in each CNS of genomes with their chromosome number. In eukaryotes, genome size is distributed among specific number of chromosomes. Investigation of the relationship between average genome size and CNS revealed that genomes which are distributed among 24 chromosomes showed the highest average of genome size with 13.162 pg, whereas genomes distributed among 6 chromosomes showed the minimum genome size average of 1.059 pg (Figure 2). Also, there was no clear trend for the relationship between average GS and CNSs. This indicates the independence of genome size and chromosome number sets through various living taxa, but the fluctuation in GS average with CNSs is less because genome size averages are used (Figure 2).

Chromosome Number Sets (CNSs) and Genome Size per Chromosome (GS/Ch)

Average Genome Size per Chromosome (GS/Ch) is a measurement of how a genome is quantitatively distributed on a number of chromosomes in various species. We estimated the GS/Ch in various genomes within the different CNSs. Genomes with 10 chromosomes exhibited the highest average of GS/Ch followed by 24 chromosome genomes, whereas genomes distributed among 100 chromosomes exhibited the lowest average GS/Ch. The data showed that the average of GS/Ch also does not correlate with chromosome number sets, but there was random fluctuation in average GS/Ch with increasing chromosome number sets (Figure 3), yet the fluctuation is less than that of the relationship between average GS or CNSs (Figure 2).

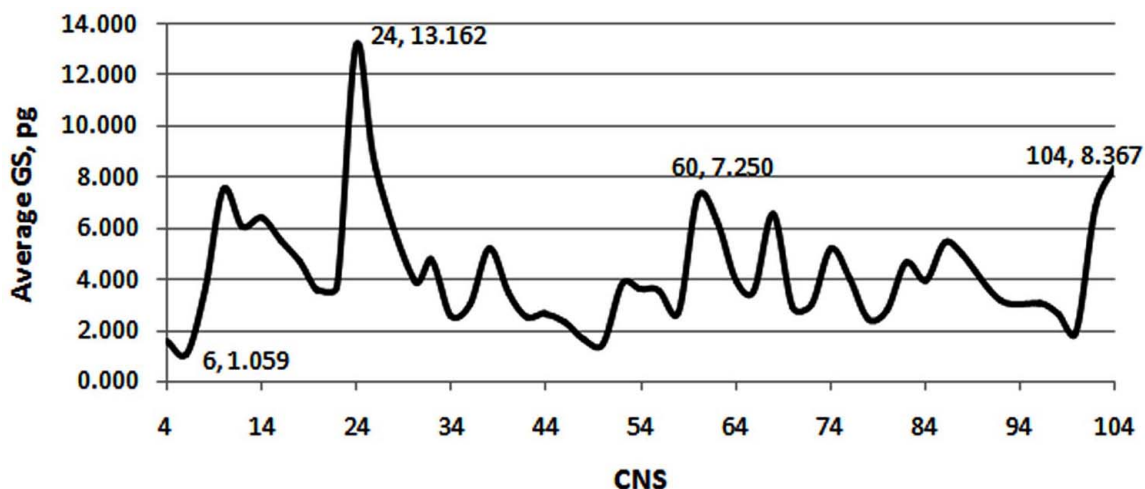


Figure 2: Relationship between average GS and CNSs.

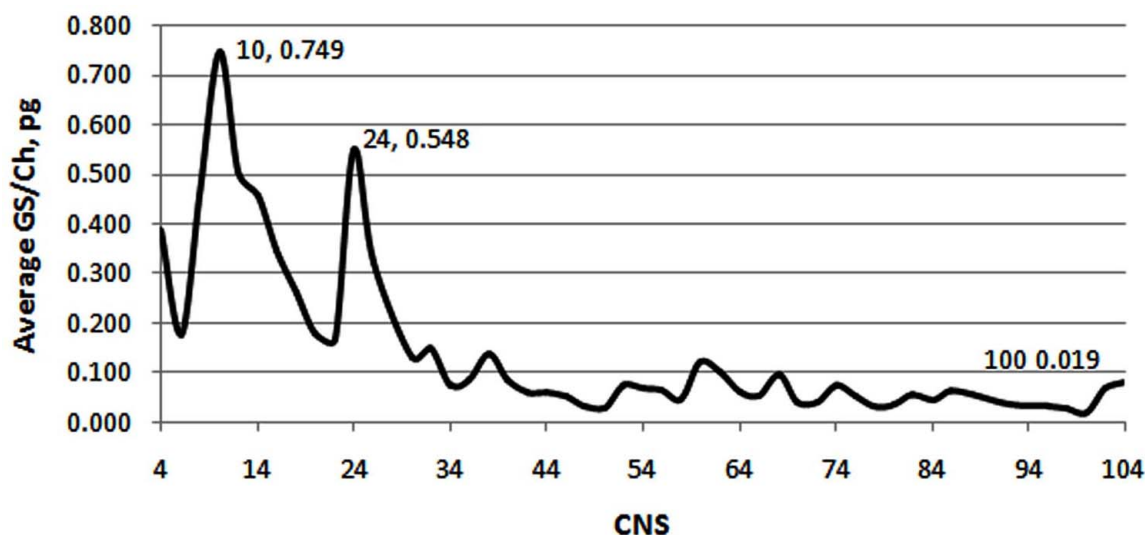


Figure 3: Relationship between average GS/Ch and CNSs.

Chromosome Number Sets (CNSs) and the Upper Limit of Genome Size per Chromosome (ULGS/Ch)

The ULGS/Ch expresses the maximum DNA capacity of a chromosome in a CNS. This feature has not been studied quantitatively in large number of genomes with detected chromosome number. The ULGS/Ch varied enormously among different CNSs (Figure 3). It was found 4.6 pg for genomes of 10 chromosomes, whereas it was the

minimum (0.038 pg) for genomes of 96 chromosomes. Also, the upper limit of GS/Ch in various CNSs showed random distribution indicating that the ULGS/Ch is independent of genome size or chromosome number across taxa. It is important to notice that the fluctuation of the ULGS/Ch exhibited more fluctuation than the average of GS/Ch and less than the fluctuation in the average GS among different CNSs (Figure 4).

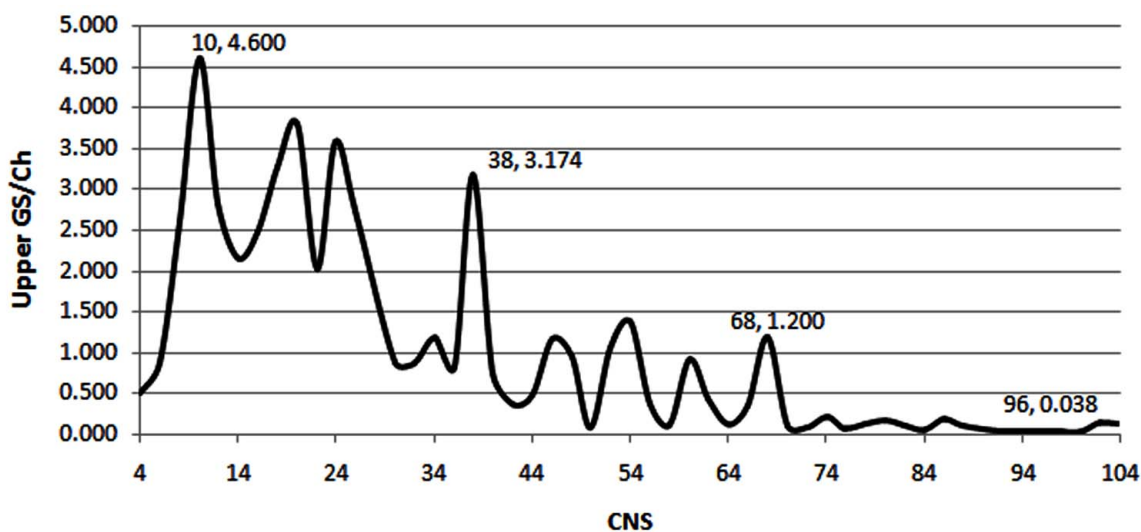


Figure 4: Relationship between ULGS/Ch (pg) and CNSs.

Discussion

Although genome size showed vast variations among living groups, plant genome size showed more variations compared to animals. Variations in genome size were as high as 8605 folds in plants compared to 6661 folds in animals. The range of the higher limit of genome size varied about 162 folds in plants, while it was 11 folds in animals. On the other hand, the average of genome size showed more variations in animals compared to plants. Genome size average in animals varied about 433 folds variation, while in plants variation was about 30 folds. This could partially due to the high number of plant genome size records (8509) compared to the animal genome size records (7285). In this study, the average of genome size is based on the total number of genome size records in each group. Also, some animal groups are represented by quite low number of genome size records. For example, the lobe-finned fish group is represented by 17 records which increased the average genome size in this group to 64.9 pg (the highest average in all living groups in this study) and the following genome size average is 18.9 pg in amphibians. Higher variations in genome size among plants were discussed in several previous studies [5-7]. Chromosome number showed less variations among living groups compared to genome size because chromosome number is not a continuous variable and is represented in even numbers. Generally, it showed 62 folds variation which represent the maximum range in living groups and variations within any group will be at less magnitude.

Genome size and chromosome number

The lack of correlation between GS and CN as well as the location of human genome among other genomes provide evidence against the Darwinian evolution theory. Results indicate that human which is considered the most developed and complicated species does not have the largest genome or chromosome number among living organisms. The 3943 genomes smaller than human genome and the 2108 genomes larger than human genome have a mix of plant and animal genomes. In addition, some genomes have the same genome size, but form and reproduce completely different organisms (Supplementary Material 3).

Variations in genome size were mentioned to be independent of changes in chromosome numbers. This makes the process of genome evolution more complicated composed of many different factors including genome size changes, chromosome number shifts, and other factors that contribute to converting the impact of these genome evolution mechanisms into function turning their effect as organism evolution [6]. Some early theories explained variation in genome size by large amounts of non-coding DNA [29], but it was criticized by the fact that evolution does not possess such foresight and the non-coding DNA in eukaryotic genomes mostly consists of repetitive elements of various lengths and does not contribute to the structure of functional genes [6]. This confirms the lack of genome size evolution trend of living groups and that plants and animal genomes appeared simultaneously not in a specific sequence as it has been claimed by the Darwinian evolution theory.

Chromosome Number Sets (SNSs) and other genome size estimates

CNS represents a higher level of individual CN data and is supposed to reveal less variation because every CNS includes various numbers of genomes that have the same CN. The nature of relationships between CNS and other GS estimates such as the average GS, GS/Ch, and the ULGS/Ch in CNS were tested. All three relationships did not reveal any type of correlation between CNS and the three GS estimates, yet they showed independent relationships. This also support the idea that beside the independence of absolute GS and CN data in the 6052 genomes, the lack of correlation also was the case at the higher level of CNS and the aforementioned genome size estimates. The vast variation in GS and CN as well as the lack of correlation between GS and CN or CNSs and GS estimates greatly weakens the evolution from common ancestor.

Location of human genome contradicts evolution from common ancestor

It is certain that a genome controls the organism structure and development therefore; the genome is expected to evolve before the

evolution of the organism. So, based on Darwinian evolution from common ancestor, we expect gradual change (increase) in genome size from the assumed common ancestor (smallest detected genome in this study, *Buchnera* sp.) to the largest detected genome (*P. aethiopicus*). Based on this assumption, human is expected to have the largest genome because it is the most recent and the most developed species on earth [30-32] and consequently is expected to lie at the end of genome size evolution curve. In addition, according to the Darwinian evolution from common ancestor, the gradual increase in genome size must be correlated with gradual increase or decrease in chromosome number (chromosome number evolution) as well as with organism evolution. The location of human genome among other genomes based on genome size and chromosome number (Figure 2) confirms that there is no correlation between genome size of species and their emergence on earth (genome evolution). This rolls out the idea that human genome evolved from smaller pre-existing genome. It is well documented that the genome size of an organism does not reflect its structural complexity which raised the question about what mechanisms led to these huge variations in genome size [33]. This was described as the 'C-value enigma' [6]. In addition, finding diploid plants with larger genome size than human genome raises a cloud of doubt about the sequence of appearance of living organisms on earth.

According to the Darwinian evolution from common ancestor, someone would argue that human genome should have been developed through multistep loss of DNA from a larger genome. This can only be explained if the common ancestor original genome was able to go through uncountable changes in genome size and chromosome number simultaneously in both directions (up and down) to reach the human genome. On the other hand, the estimated age of earth (4.5×10^9 years) is not enough for all of these change events in the absence of detailed mechanisms to explain the high frequent changes in chromosome number in diploid genomes. Moreover, genome evolution time was estimated by mutation rate from the smallest detected genome (*Buchnera* sp.) to the largest detected genome (*P. aethiopicus*) through 8 evolution leaps (8 genomes) to be much higher than the estimated age of earth (4.5×10^9 years). In the same study, the average genome evolution time was estimated at 2.7×10^{12} years per genome (590 folds of the estimated age of earth). Currently, the number of characterized species is 1.2×10^6 and the number of predicted species is 8.7×10^6 [34]. The estimated genome evolution time for the characterized genomes is 3.2×10^{18} years and for the predicted genomes is 2.3×10^{19} years which is equivalent to 7.2 $\times 10^8$ and 5.2 $\times 10^9$ folds of the estimated age of earth respectively [35].

This provides evidence that the estimated age of earth would not have been enough for the evolution of the characterized or predicted number of species (genomes). This beside the independent data of genome size and chromosome number does not support the aforementioned pattern of appearance of human genome, yet it supports the random appearance of living species with different genome size and chromosome numbers that existence of genomes on earth cannot be explained by evolution from common ancestor.

Conclusion

In conclusion, there are great unexplained variations in genome size and chromosome number. Also, GS estimates (average GS, GS/Ch, ULGS/Ch) and CN or CNSs exhibited an independent relationships. The data suggest that there is no clear trend for genome evolution from common ancestor at the genome size and chromosome number levels. In addition, the location of human genome supports the idea of

independent appearance of living organisms on earth. Together, results contradict the evolution from common ancestor and open the door for introducing other explanations for the existence of so many genomes with little difference in size distributed on an independent chromosome numbers.

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