The quiescent X, the replicative Y and the Autosomes

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Abstract

From the analysis of the mutation spectrum in the 2,504 sequenced human genomes from the 1000 genomes project (phase 3), we show that gonosomes (X and Y) exhibit a different proportion of indel mutations than autosomes (A), ranking them X>A>Y. We further show that X chromosomes have a higher ratio of deletions/insertions when compared to autosomes. This simple pattern shows that the recent report that non-dividing quiescent yeast cells accumulate relatively more indels (and particularly deletions) than replicating ones also apply to metazoan cells such as humans. Indeed, the X chromosomes have a stronger mutational imprint of the quiescent oocytes than autosomes and the Y chromosomes are solely imprinted by the replicating spermatocytes. From the proportion of indels, we have inferred that *de novo* mutations arising in the maternal lineage are two times more likely to be indels than mutations from the paternal lineage. Our observation, consistent with recent trio analyzes of the spectrum of mutations inherited from the maternal lineage, is likely a major component in our understanding for the origin of anisogamy.

Introduction

In humans, male and female germ cells are produced before birth and remain quiescent until puberty. At this point, oocytes are released monthly, whereas spermatocytes are continuously dividing to produce spermatozoids (1). Haldane proposed the existence of sex-specific mutation rates, arguing that the frequency of new hemophilic males from non-carrier mothers (hemophilia is X-linked) was very low compared to the expectation of the population frequency, under a mutation-selection equilibrium (2). This deficit of *de novo* mutations in the oocytes was taken as a proof that most mutations occurred in the male lineage. This observation was later connected to the higher evolutionary point mutation rates reported for Y than for X (3–5) and more recently to direct counting of neo-mutations in humans (6,7) and in chimpanzees (8).

However, several lines of evidence both theoretical (9) and from observations on CpG sites (6) have suggested that replication-independent mutations accumulate linearly with *absolute* time, regardless of cell division. Because autosome lineages are equally split in both sexes, X chromosome lineages are 2/3 of the time in oocytes and Y lineages are exclusively in spermatozoids, we reasoned that if a quiescence-specific mutational signature exists in humans, it should be revealed by a different mutational spectrum of the X, Y and autosomes.

We have recently shown (10) that the quiescent haploid yeast *Schizosaccharomyces pombe* cells exhibit a distinctive mutational landscape called Chronos: particularly, (i) they accumulate as many indels (insertions or deletions) as SNVs (Single Nucleotide Variants) whereas replicating cells accumulate more SNVs and (ii) they accumulate more deletions than insertions whereas the opposite is observed for dividing cells. Since errors during DNA replication are believed to be responsible for most

mutations in many species, we questioned to what extent the replication-independent mutational landscape observed during quiescence in fission yeast also applies to humans.

Results

To test this idea, we evaluated the distribution of SNVs and indels in human chromosomes. Since indel alleles with less than 3 counts are poorly genotyped in autosomes (Supp Fig. 1), we considered the 39.5 million variable sites that host two or more alleles of 3 counts or more each (MAC≥3) in the 2,504 sequenced human genomes from the 1000 genomes project phase 3 (11). For each variable site, we estimated the number of mutations as the number of alleles minus one, leading to a total of 39.8 million mutations for all chromosomes. We also computed the "accessible size" that was sequenced using variants density. To assess the impact of the mutational bias on a longer evolutionary time, we next compared human and chimpanzee genomes, by counting the number of SNVs and indels in a pairwise alignment between both reference genomes. All counts are reported in Supp Table 1.

As population genetics would predict from an effective population size that is 3/4 and 1/4 that of Autosomes for X and Y, respectively, we observed that the density of mutations per base among the variants segregating in humans is ranked Autosomes>X>Y both for indels and SNVs (Fig. 1). Interestingly, at the divergence level, the degenerating Y chromosome accumulates more indels and SNVs than any other chromosome (Fig. 1), a pattern that was reported previously (12,13). All differences are highly significant (see methods) since counts are typically on the order of millions.

We next computed for each chromosome, the fraction of indel mutations among both types of mutations (SNVs and indels). Results show that the fraction of indel mutations is ranked in a striking simple pattern: X>Autosomes>Y (0.10>0.09>0.06) (Fig. 2a). Using least squares, we estimated the fraction of indels among *de novo* mutations to 0.12 in females and to 0.06 in males. Finally, we observe that among indels, deletions are even more abundant for the X chromosome than for the Autosomes (Fig. 2d). We also noticed the negative correlation between deletions and chromosome size but did not further investigate it yet.

At the inter-species level, we observed a very similar pattern (Fig. 2c, 0.11>0.10>0.07). Interestingly, the recent mutations (segregating within humans) contain relatively fewer indels (0.09 on autosomes) than the older ones that have been fixed and detected between human and chimpanzee (0.10 on autosomes). Similarly, frequent mutations that are more likely to get fixed in humans (Minor Allele Frequency > 0.01) also exhibit a higher fraction of indels (Fig. 2b). This result supports the view arguing that SNVs are more efficiently removed by purifying selection in the long run (14) likely because they contain many "slightly deleterious" alleles (15–17).

Interestingly, the mitochondrial genomes (Supp Table 2) show a low proportion of indels, reminiscent of the Y chromosome (0.010 of segregating mutations within humans and 0.012 for the human-chimpanzee comparison). Although this low proportion of indels could be due to the high density of coding sequences, it is tempting to postulate that the mitochondria keep dividing and replicating their genome in the quiescent oocytes, therefore masking the imprint of quiescence.

Discussion

The difference of mutational spectrum between X, Autosomes and Y is unlikely to result from an experimental bias. For instance, sequencing errors would affect all chromosomes equally and should be absent in mutations detected with a frequency higher than 0.01. Additionally, the fractions of indels for the X chromosome within males or females were analyzed separately and both show a larger value than the autosomal ones (0.10 for females and 0.11 for males), excluding the possibility that the pattern can be due to easier X genotyping in males because of the haploidy.

Our observations infer that, in humans, there is a higher occurrence of indels in the female gamete lineage. Interestingly, in a 2000 review (18), J. Crow intuitively proposed that female gametes may be responsible for indels and male gametes are responsible for SNVs. Here, we show a striking similarity between yeast, humans and chimpanzee suggesting a conserved trend: quiescence accumulates more indels (deletions). The pattern we report here is in complete line with the observed positive correlation between the maternal age and the number of maternally inherited de novo mutations (7) and even more so with the report of the increase of recombination events (19) or double-strand-break related mutations (20) with the mother's age. Although 80% of the mutations (and more particularly SNVs) originates from the paternal lineage (6), we suspect that the recent progress in indels detection with newer generation technologies will undoubtedly reveal many overlooked indels of maternal origin. It now remains to be investigated how the differential contribution of males and females to the genome evolution is advantageous and relates to the origin of anisogamy.

Materials and Methods

Computation of the SNVs and indels in the 1,000 human genomes dataset. We retrieved all VCF files from the 1,000 genome phase 3 from ftp.1000genomes.ebi.ac.uk in the /vol1/ftp/release/20130502 directory. Because indels with less than 3 counts were poorly genotyped in autosomes (Supp. Fig. 1A), only alleles with 3 counts or more were considered. A site is assimilated to a SNVs site if all its alleles have length 1. Complex double mutations (mostly in the mitochondrial genome) and Alu insertions were discarded. Others were assimilated to indels. The number of mutations at a given site was computed as the number of alleles minus one.

Computation of the SNVs and indels in the human-chimpanzee alignment. We retrieved the alignment between the human reference genome (version hg38 that was used in the 1000 genome phase 3) and the reference chimpanzee genome (version panTro4) from http://hgdownload.soe.ucsc.edu/goldenPath/hg38/vsPanTro4/. We considered only the aligned segments where the chromosome number was the same on both species (for chimpanzee, chromosomes annotated 2A and 2B were considered as 2). For the mitochondria genomes, we retrieved the NC_012920.1 (human) and NC_001643.1 (chimpanzee) mitochondrial genomes from NCBI. Both sequences were then globally aligned that resulted in a 16,575 nt alignment. In all pairwise alignments, we simply counted the number of point mutations and indels considering all subsequent gap symbols as the same indel event.

Statistical significance. The difference in the observed fractions of indel mutations (X: 0.10 > Autosomes: 0.09 > Y: 0.06) is highly significant. We performed a χ^2 test on the counts in Supp Table 1: $\log_{10}(P\chi^2) = -1018$, df=2, pooling all variants from autosomes. The difference in the observed fractions of indel mutations in the human-chimpanzee comparison is also highly significant. We performed a χ^2 test on the counts in Supp Table 1: $\log_{10}(P\chi^2) = -2096$; df=2, pooling all mutations from autosomes. The difference in the relative abundance of deletions and insertions (deletions/insertions is 1.9 for X and 1.6 for autosomes) is also highly significant. We performed a χ^2 test on the counts in Supp Table 2: $\log_{10}(P\chi^2) = -114$, df=1, pooling all autosomes.

Computation of the chromosome "accessible" sizes. We estimated the genotyped part of each chromosome by summing all regions that include variants that are spaced by less than 1 kb. As reported in the 1,000 genome project (11), this represents on average 90% of the chromosome size, with the exception of the Y where it is 18% of the chromosome. On average, variants are spaced by 35 bp and only ~0.01% of them that are spaced by more than 1 kb. Therefore, counting regions with variants spaced by less than 1 kb is a conservative estimate.

Least-squares estimations of the sex-specific indel fractions. For Autosomes, X and Y, the observed fraction of indels is stored in the vector **I** and the mean time spent in male and female lineages in a matrix **T**:

$$I = \begin{pmatrix} 0.088 \\ 0.103 \\ 0.060 \end{pmatrix} \qquad T = \begin{pmatrix} 0.5 & 0.5 \\ 0.33 & 0.66 \\ 1 & 0 \end{pmatrix}$$

Using standard least squares, we can estimate the female and male fraction of indels as $(\mathbf{T}\,\mathbf{T}^t)^{-1}\,\mathbf{T}^t\,\mathbf{I}$, giving rise to 0.06 for males and 0.12 for females. As a goodness of fit, these values predict that the expected indel fractions in Autosomes, X and Y should be (0.091, 0.101, 0.060), that are close to the observed **I** vector.

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Figure Legends

Figure 1 - Density of SNVs and indels in X, Y and Autosomes. *The density was computed as the number of mutations per bp of the "accessible chromosome" (for segregating polymorphisms) or of the "aligned" chromosome (for human-chimpanzee divergence), which is estimated from the data (see Supplementary Material). Autosomes are ranked by number.*

Figure 2 – Comparison of Indels among X, Y and Autosomes. **a** *We report the fraction of indel mutations segregating in a sample of 2,504 human diploid genomes for the X, the Y and the Autosome ranked by chromosome number. Only alleles with at least 3 counts (Minor Allele Counts ≥ 3) were considered here because lower frequency indels are poorly genotyped in Autosomes.* **b** *Variants were further filtered by their frequency (Minor Allele Frequency > 0.01).* **c** *We provide the same results for the human-chimpanzee comparison.* **d** *We report the ratio deletions/ insertions in all indels that were oriented using the inferred Ancestral Allele. No oriented indel is reported for the Y chromosome or the mitochondria.*

Supporting Information

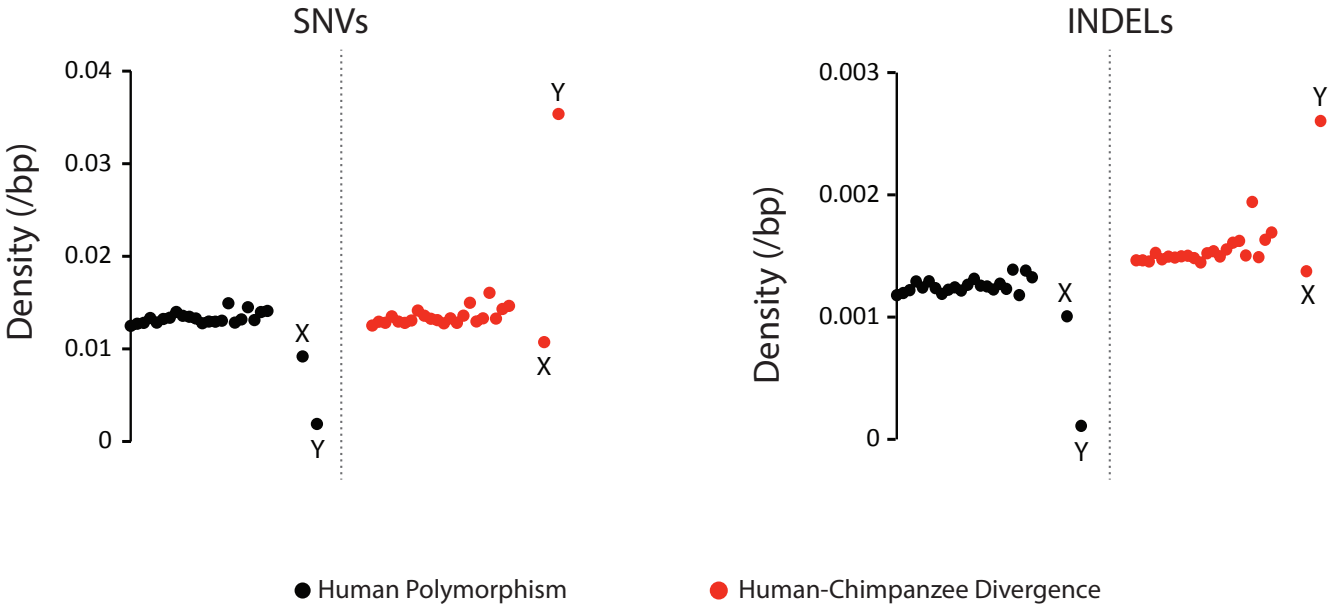
Supplementary Figure 1 – Allele Frequency Spectra (AFS) for SNVs and indels. *We report here the distribution of allele frequency (here given by the Minor Allele Count) for bi-allelic variants. A) Normalized AFS for all indels of human chromosomes zoomed on the lowest frequency. B-D) Normalized AFS for SNVs and indels, excluding variants where the minor allele has less than 3 counts. Results are given for the Y chromosome (B), the X chromosome (C) and all autosomes pooled together (D).*

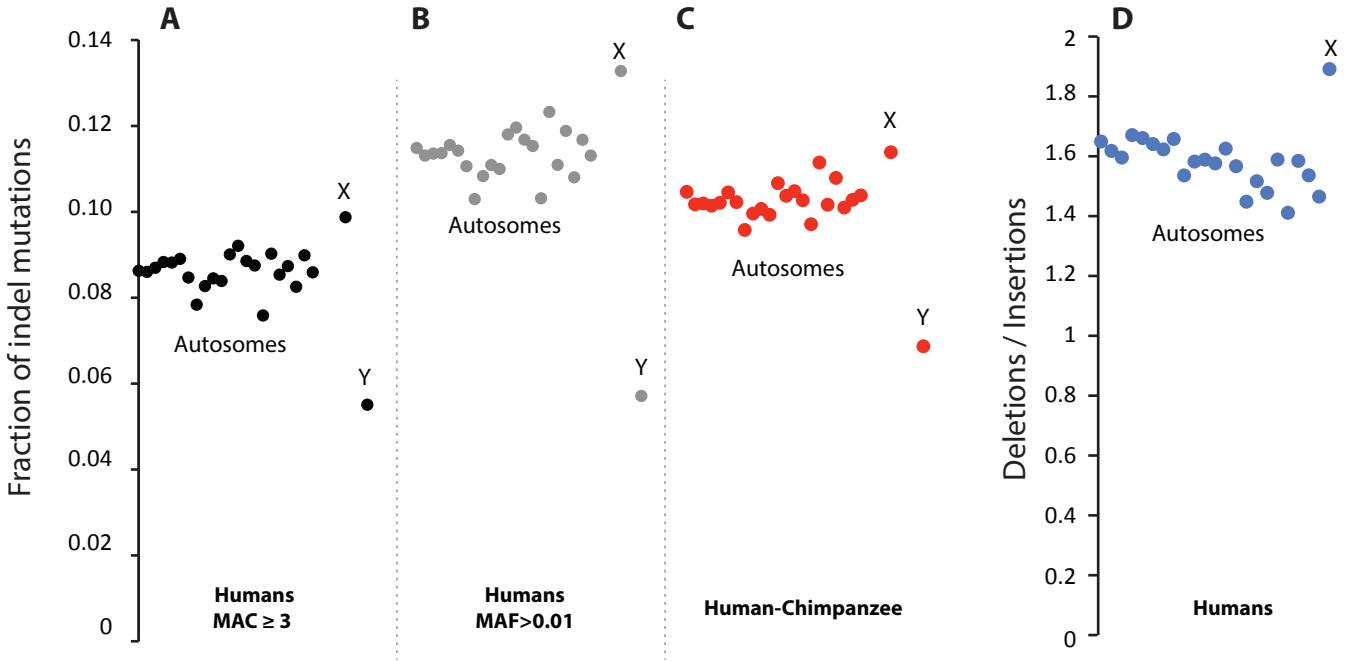
Supplementary Table 1: total number of SNV and indel mutational events for each human chromosome of the 1,000 human genome project phase 3 (white columns) and in the human-chimpanzee genome alignment (grey columns).

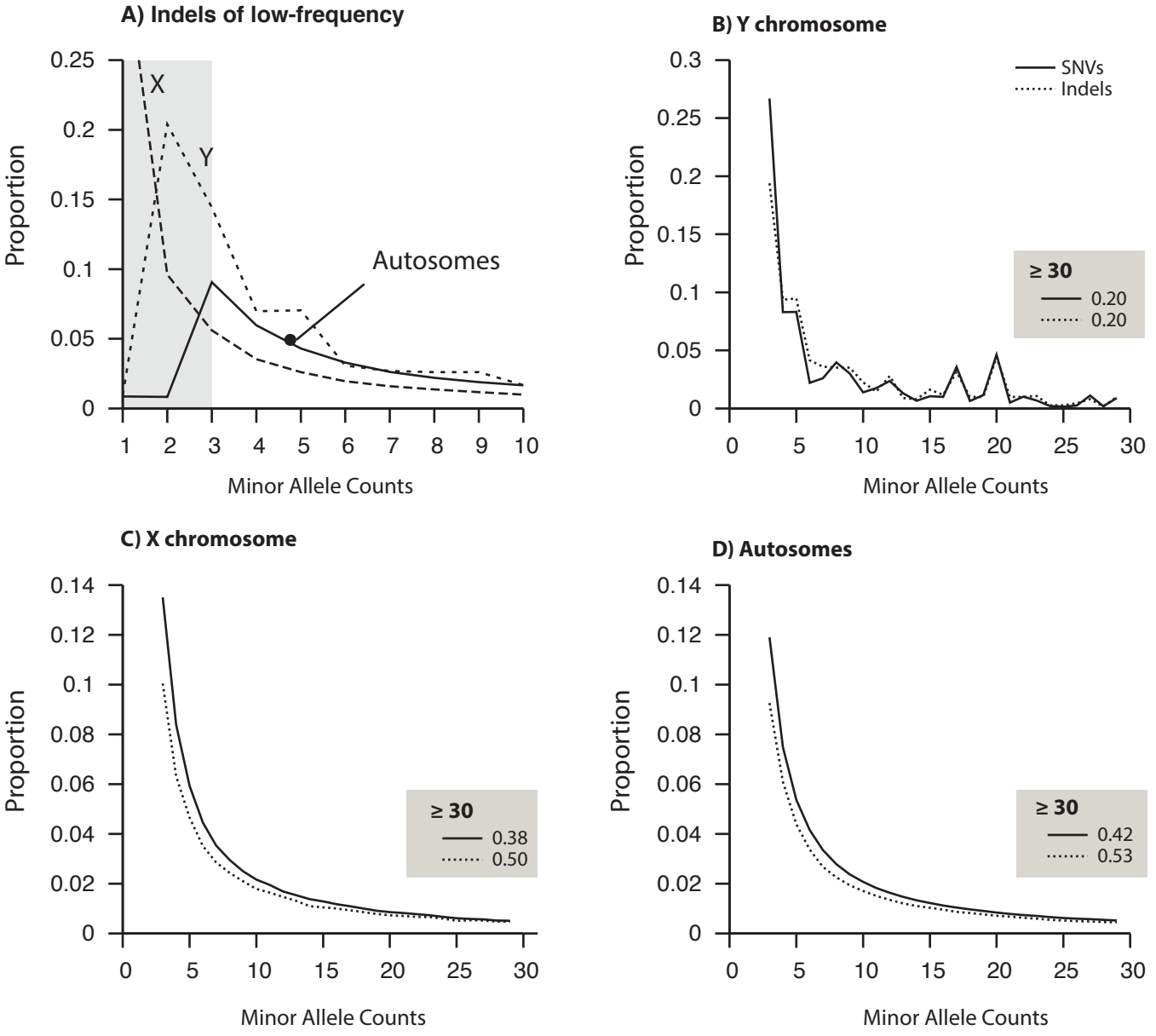
CHR	Accessible Size	f	#SNV mutations	#indel mutations	Size in the alignments	#SNV mutations	#indel mutations
1	221,736,733	0.89	2768960	260527	214,676,400	2,688,535	314,370
2	236,052,317	0.97	3002009	281477	228,827,157	2,959,684	335,063
3	194,355,142	0.98	2489710	236234	189,206,467	2,425,105	275,297
4	187,072,586	0.98	2494816	240766	180,579,048	2,440,890	275,447
5	175,575,335	0.97	2253782	217139	170,781,708	2,211,653	251,451
6	166,853,196	0.98	2207279	214962	159,651,922	2,044,988	238,650
7	153,483,682	0.10	2051182	189098	148,837,804	1,944,712	221,314
8	141,740,687	0.97	1982718	168012	133,437,499	1,887,351	199,792
9	113,138,271	0.80	1535673	137968	108,925,459	1,479,736	163,590
10	129,224,414	0.95	1740547	160084	122,258,850	1,620,335	181,258
11	130,592,589	0.97	1735297	158322	120,380,559	1,577,676	174,155
12	130,139,809	0.97	1661977	163920	125,126,235	1,596,621	190,471
13	95,439,646	0.83	1236299	124901	85,593,830	1,138,577	131,714
14	87,931,610	0.82	1137684	110137	84,068,261	1,077,028	125,701
15	79,583,776	0.78	1038025	99186	76,611,935	1,041,023	119,018
16	77,059,256	0.85	1150388	94213	73,152,921	1,095,981	117,689
17	76,927,197	0.95	987529	97678	72,619,467	941,287	117,893
18	74,552,378	0.95	982968	91414	72,246,386	960,886	108,675
19	55,368,119	0.94	802833	76706	49,629,257	796,857	96,378
20	59,328,211	0.94	777839	69758	56,442,295	749,135	84,111
21	34,724,000	0.72	485412	47754	33,280,999	476,305	54,331
22	34,258,169	0.67	483044	45299	31,793,980	465,627	53,803
X	147,824,448	0.95	1357783	148449	125,354,575	1,345,122	172,364
Y	10,048,563	0.18	19022	1109	16,040,479	567,386	41,772
MT	16,545	1.00	1886	20	16,575	1,451	18

Supplementary Table 2: Annotated Insertions and Deletions for each human chromosome of the 1,000 human genome project phase 3.

CHR	#insertions	#deletions
1	30,966	50,504
2	34,153	54,599
3	29,605	46,655
4	29,221	48,297
5	26,430	43,421
6	26,022	42,211
7	22,246	35,682
8	20,291	33,269
9	16,954	25,679
10	19,541	30,525
11	19,368	30,382
12	19,380	30,154
13	15,255	24,507
14	13,458	20,810
15	12,292	17,511
16	10,711	16,010
17	11,332	16,486
18	11,337	17,789
19	7,662	10,624
20	8,314	13,008
21	6,087	9,221
22	5,047	7,278
X	28,109	52,822


SNVs
INDELs
Density (/bp)
0.04
0.03
0.02
0.01
0
0.003
0.002
0.001
X
Y
Human Polymorphism
Human-Chimpanzee Divergence


A
B
C
D
Fraction of indel mutations
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
X
Y
Autosomes
Humans
MAC ≥ 3
Humans
MAF>0.01
Human-Chimpanzee
Deletions / Insertions
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
Humans


A) Indels of low-frequency
Proportion
Minor Allele Counts
X
Y
Autosomes
B) Y chromosome
SNVs
Indels
≥ 30
0.20
0.20
C) X chromosome
≥ 30
0.38
0.50
D) Autosomes
≥ 30
0.42
0.53