

Evolutionary trend of SARS-CoV-2 inferred by the homopolymeric nucleotide repeats

Changchuan Yin *

Department of Mathematics, Statistics, and Computer Science
University of Illinois at Chicago
Chicago, IL 60607
USA

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the current global COVID-19 pandemic, in which millions of lives have been lost. Understanding the zoonotic evolution of the coronavirus may provide insights for developing effective vaccines, monitoring the transmission trends, and preventing new zoonotic infections. Homopolymeric nucleotide repeats (HP), the most simple tandem repeats, are a ubiquitous feature of eukaryotic genomes. Yet the HP distributions and roles in coronavirus genome evolution are poorly investigated. In this study, we characterize the HP distributions and trends in the genomes of bat and human coronaviruses and SARS-CoV-2 variants. The results show that the SARS-CoV-2 genome is abundant in HPs, and has augmented HP contents during evolution. Especially, the disparity of HP poly-(A/T) and ploy-(C/G) of coronaviruses increases during the evolution in human hosts. The disparity of HP poly-(A/T) and ploy-(C/G) is correlated to host adaptation and the virulence level of the coronaviruses. Therefore, we propose that the HP disparity can be a quantitative measure for the zoonotic evolution levels of coronaviruses. Peculiarly, the HP disparity measure infers that SARS-CoV-2 Omicron variants have a high disparity of HP poly-(A/T) and ploy-(C/G), suggesting a high adaption to the human hosts.

keywords: SARS-CoV-2, 2019-nCoV, homopolymeric nucleotides, zoonotic evolution, genome

Highlights

- SARS-CoV-2 variants contain an overabundance of C>T mutations.
- The C>T mutations in SARS-CoV-2 variants imprint the SARS-CoV-2 variant genomes by APOBEC cytosine deaminases in immune response.
- The disparity of homopolymeric nucleotide repeats poly-(A/T) and ploy-(C/G) is the result of C>T mutations.
- The disparity of homopolymeric nucleotide repeats poly-(A/T) and ploy-(C/G) may infer the trend of virus evolution.

1 Introduction

The novel coronavirus SARS-CoV-2, the causative agent for Coronavirus disease-2019 (COVID-19) pandemic, has infected 276.436 million individuals and caused 5.374 million deaths in the globe as

*Correspondence author, cyin1@uic.edu

of Dec. 23, 2021 (WHO, 2021). Understanding the evolutionary origin and trend of SARS-CoV-2 is of importance for controlling the current COVID-19 pandemic, designing effective vaccines and antiviral drugs, and preventing the future epidemic of coronavirus diseases.

To gain a comprehensive zoonotic evolution of SARS-CoV-2, we contextualize SARS-CoV-2 in bat and human coronaviruses. Coronaviruses are enveloped positive-strand RNA viruses with crown-like spikes on their surface. Coronaviruses belong to the family *Coronaviridae* and the order *Nidovirales*. Coronaviruses can widely spread in humans, other mammals, and birds. Human coronaviruses (HCoVs) were first identified in the mid-1960s. Seven common HCoVs are CoV-229E (alpha coronavirus), CoV-NL63 (alpha coronavirus), CoV-OC43 (beta coronavirus), CoV-HKU1 (beta coronavirus), Severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and current SARS-CoV-2. CoV-229E and CoV-OC43 are the cause of the common cold in adults during the mid-1960s. CoV-HKU1 and CoV-NL63 cause disease manifestations include the common cold and chronic pneumonia. CoV-HKU1 has been predominantly reported in children in the United States but less common among adults. Three highly pathogenic human coronaviruses, SARS-CoV, MERS-CoV, and SARS-CoV-2, which emerged in 2002, 2012, and 2019, respectively, have caused severe respiratory diseases (Chen, 2020). The estimated fatality rate in the confirmed cases is 6.6% in SARS-CoV-2, which is lower than those of SARS-CoV and MERS-CoV, 9.6% and 34.3%, respectively (Wang et al., 2020a).

Science consensus admits that SARS-CoV-2 coronavirus originated in bats, specifically horseshoe bats. A few closely related to SARS-CoV-2 have been identified. Bat coronavirus RATG13 was isolated from *Rhinolophus affinis* (a horseshoe bat) in Yunnan China in 2013. RATG13 genome is 96% identical to that of SARS-CoV-2 Zhou et al. (2020b). *R. malayanus* BANAL-52 is the closest known relative of SARS-CoV-2 found in Laos (Temmam et al., 2021). BANAL-52 bat coronaviruses exhibit a high nucleotide identity with SARS-CoV-2 throughout the whole genome. The next-closest coronavirus is RmYN02, found in Malayan horseshoe bats (*Rhinolophus malayanus*), which shares 93% of its genetic sequence with SARS-CoV-2 (Zhou et al., 2020a). RmYN02 possesses high sequence similarity to SARS-CoV-2 of known *Sarbecoviruses* for most of its genome. Malayan pangolins (*Manis javanica*) share up to 92% of their genomes with the coronavirus SARS-CoV-2. The studies suggest that pangolins can host coronaviruses that share a common ancestor with SARS-CoV-2, but do not confirm that pangolins are the immediate host of SARS-CoV-2. The originated source and intermediate host animals of SARS-CoV-2 remain unconfirmed. The intermediate hosts of the coronaviruses can be bats, rodents, rabbits, and hedgehogs (Monchatre-Leroy et al., 2017). Since SARS-CoV was founded in 2003, a few researches have identified closely related bat-CoVs, including SLCoV/ZXC21 and SLCoV/ZC45 (Hu et al., 2017). Despite numerous phylogenetic analyses on these closely related CoVs, the zoonotic origin and timing of SARS-CoV-2 are still not confirmed. Though similarities of the genomes of these CoVs provide some clues on the zoonotic evolution, the evolutionary orders of these CoV are not fully established.

When a virus infects an animal or human host, the host immune system recognizes the foreign infection and strives to eliminate the virus. Immune monitoring and abolishing the viral infectivity infection can be achieved at both protein and nucleic acid levels. As the innate immune antiviral response at nucleic acid level, the interferon-stimulated APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) RNA editing system recognizes the virus genomes and catalyzes Cytosine deamination to Uracils (C>U) in the virus genomes, leading to inhibit viral infectivity (Bishop et al., 2004). Recent studies suggested that the high-frequency C>U mutation mediated by APOBECs RNA editing is important in the rapid evolution and adaptation of the SARS-CoV-2 coronavirus in hosts (Wang et al., 2020b; Di Giorgio et al., 2020; Matyášek and Kovařík, 2020). The host adaptation of the virus infection results in the accumulation of homopolymeric repeats of Uracils (poly-Us, equally, poly-Ts in sequence notation) in the virus genomes. Therefore, the homopolymeric repeat poly-Ts are the fighting hallmarks of the immune system on the virus genomes.

Denver et al. (2004) pioneered the study on homopolymeric repeat distribution in *Caenorhabditis elegans* genome. The study showed that the frequencies and length distributions of A and T homopolymers vastly outnumber G and C homopolymers. The homopolymeric repeats signal the evolution of genomes Denver et al. (2004). The other extensive studies revealed that Simple Sequence Repeats (SSRs), including homopolymeric nucleotide repeats, have been associated with antigenic variation and other adaptation strategies in Gram-negative organisms (Janulczyk et al., 2010).

In this study, from sequence analysis, we show that the SARS-CoV-2 genome is abundant in homopolymeric nucleotide repeats. We here explore the evolutionary implications and significance of these repeats in the zoonotic shift. We propose to employ the homopolymeric nucleotides as the genome signature for measuring the zoonotic trend and orders of coronaviruses.

2 Methods

2.1 Measurement of the disparity of homopolymeric nucleotide repeats

The changes of genomic structures and compositions of a virus are the result of the evolutionary adaption of the virus to hosts. To estimate the evolutionary trends and orders of coronaviruses from the genomic HP composition, we propose a measure of the HP disparity or HP compositions as follows. Let $HP(\alpha_i)$, $\alpha \in \{A, T, C, G\}$, $i = 3, 4, 5$ denote the HP repeat frequencies of a genome, the HP disparity, $R_{HP(AT/CG)}$ is defined as the frequencies of $HP(A_{3,4,5})$ and $HP(T_{3,4,5})$ over the frequencies of $HP(C_{3,4,5})$ and $HP(G_{3,4,5})$ (Equation 2.1). The homopolymeric nucleotides examined are only from 3-mers to 5-mers. It is noted that the n-mers within the m-mer ($m > n$) are not included when counting in the frequency of n-mers in Equation 2.1. For example, in DNA sequence, T_5 contains 3 T_3 and 2 T_4 , when counting frequencies in Equation 2.1, the frequency T_5 is 1, and the frequencies of T_3 and T_4 are zero.

$$R_{HP(AT/CG)} = \frac{f(HP(A_{3,4,5}) + HP(T_{3,4,5}))}{f(HP(C_{3,4,5}) + HP(G_{3,4,5}))} \quad (2.1)$$

, where $f(\cdot)$ is the frequency of a homopolymeric nucleotide repeat.

Another genomic composition measure is the ratio of A+T and C+G compositions, which is defined as A+T composition over C+G compositions (Equation 2.2).

$$R_{(AT/CG)} = \frac{f(A) + f(T)}{f(C) + f(G)} \quad (2.2)$$

2.2 Genome data

The complete genomes of coronaviruses, including SARS-CoV-2 (Wu et al., 2020), Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), SARS-like coronaviruses (SL-CoVs), Middle East Respiratory Syndrome coronavirus (MERS-CoV), coronaviruses found in bats (bat-CoVs) or pangolins (Pangolin-SLCoV), and human coronaviruses (Human-CoVs), were retrieved from NCBI GenBank or GISAID database (Shu and McCauley, 2017). The complete genomes of SARS-CoV-2 isolates from the infected individuals were retrieved from the GISAID database between Jan.1, 2020 and Dec.4, 2021. Only the complete genome sequences without uncertain nucleotide 'N' of high-coverage were included in the test datasets. The NCBI GenBank and GISAID access numbers of the complete genomes are provided in the result tables.

3 Results

3.1 SARS-CoV-2 genome has abundant HPs

We assessed the HP distributions and contents in the genomes of SARS-CoV-2, SARS-CoV coronaviruses. The HP distribution results demonstrate that the SARS-CoV genomes have an abundance of HPs (Fig.1), compared with human endogenous retroviruses (HERV) (Nelson et al., 2003) (Fig.2). The reason for using HERV as HP reference is that human endogenous retroviruses have been proposed to establish a protective effect against exogenous viral infections (Nelson et al., 2003). The polynucleotide mer length in SARS-CoV-2 genome can reach $poly - T_5$ and $poly - G_4$. Especially, in CoV genomes, the contents of poly- and poly-A are much richer than poly-C and poly-G. but this disparity of poly-(A/T) and poly-(C/G) is not found or insignificant in other virus genomes, for example, HERV (Fig. 2), human immunodeficiency virus (HIV) and the hepatitis C virus (HCV), and Ebola (data not shown). In addition, SARS-CoV-2 genome has increased poly-(A/T) tracts, compared with SARS-CoV. According to these observations, we propose the definition of HP disparity score (Equation 2.1) to measure the biased content of poly-(A/T) vs poly-(C/G).

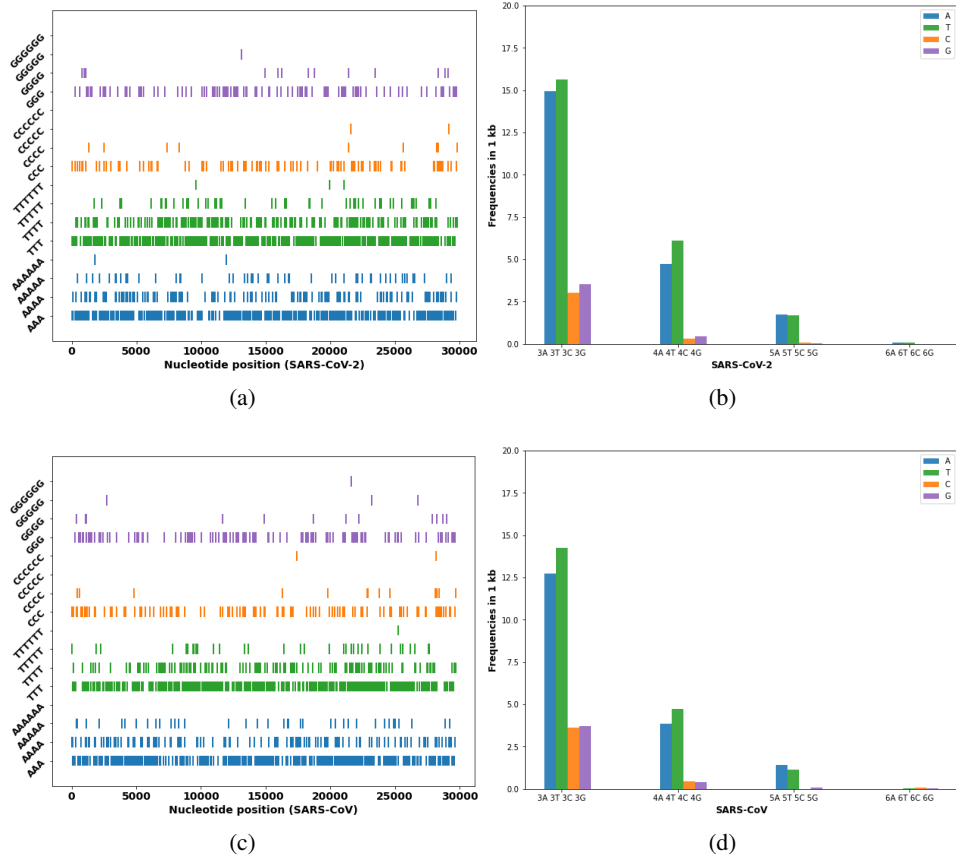


Figure 1: Distributions of HPs in SARS-CoV genomes. (a) HP distribution in SARS-CoV-2 genome (GenBank: NC045512), (b) HP frequencies in SARS-CoV-2 genome, (c) HP distribution in SARS-CoV genome (GenBank: AY274119), (d) HP frequencies in SARS-CoV genome.

3.2 HPs in a CoV genome are the imprint of zoonotic evolution

To investigate the correlation of HPs in the coronavirus genomes and the zoonotic evolution of coronaviruses, we measure the HP repeat disparity scores $R_{HP(AT/CG)}$ and AT/CG composition ratios $R_{(AT/CG)}$ (Equations 2.1 and 2.2) of the coronavirus genomes (Table 1). These two measures estimate the abundance and distribution bias of homopolymeric nucleotides. We also review the association of the HP disparity scores with zoonotic and pathogenicity characteristics.

The HP computation result shows that if a coronavirus can infect the human host with high pathogenicity, its HP disparity score is low, vice versa, if a coronavirus adapts to human host and has low virulence, its HP score is high. For example, two high virulent CoVs, SARS-CoV (2003) (Marra et al., 2003) has low HP score 4.5139. The SARS-CoV related CoVs (SLCoV/RsSHC014, SLCoV/Rs3367, SLCoV/MA15, SLCoV/WIV1, SLCoV/Shaanxi2011, civets-SCoV/SZ3) have similar low HP scores as SARS-CoV. The highly lethal MERS-CoV (2012) (van Boheemen et al., 2012) has very low HP score 3.2432. *Rousettus* bat coronavirus bat-CoV/HKU9-1 (2011) (Lau et al., 2010), closely related to MERS-CoV, has HP score 3.0114. Note that highly pathogenic Swine acute diarrhea syndrome coronavirus (SADS-CoV) (2017) (Zhou et al., 2018) with potential human infection (Edwards et al., 2020) has low HP score 5.0192. The SAD-CoV is closely related and shares 95% sequence identity to SLCoV/HKU2 (2004) (Zhou et al., 2018), which has low HP score 4.9346. Additionally, we measure the HP disparity of non-coronavirus Ebola virus, which is very virulent with a mortality rate of 51% kucharski2014case,di2020viral. The HP score of Ebola virus is very low 2.5927. In brief, the low HP scores of the CoV genomes can be considered as the native state of the viruses in the original wild world.

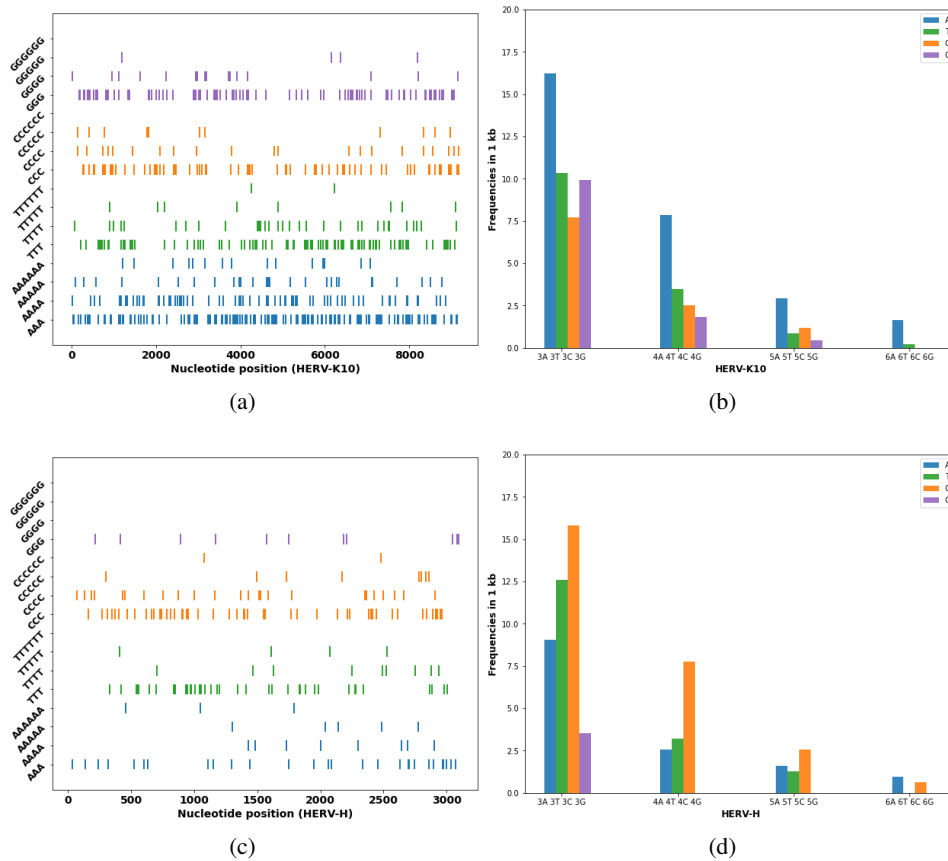


Figure 2: Distributions of HPs in HERV genomes. (a) HP distribution in HERV-K10 genome, (b) HP frequencies in HERV-K10 genome (GenBank: M14123), (c) HP distribution in HERV-H genome (GenBank: AH007766), (d) HP frequencies in HERV-H genome.

Since the 1960s when the first human coronavirus (HCoV) strain (HCoV-B814, 1965) was identified from a patient's nasal discharge, over 30 different HCoV strains have been isolated, the most notable of which include HCoV-229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43 (Ye et al., 2020). Human CoVs (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1) are found worldwide and cause common cold predominantly in winter and spring (Poutanen, 2018). The HP computational result shows that Human-CoVs (229E (1963), NL63 (2004), and HKU-1 (2004)) have high HP scores (6.6788, 9.4172, and 10.4088, respectively). Note that HCoV-OC43 (1963) has low HP score 5.7846. The fact that HCoV-OC43 is actually virulent CoV to human may account for the low HP score. The colds due to HCoV-OC43 are more severe and are indistinguishable from colds due to rhinoviruses (Poutanen, 2018). The clinical symptoms of HCoV-OC43 outbreak in France include severe vomiting, diarrhea, abdominal pain, pneumonia, and even infection in the brain (Vabret et al., 2003). Previously studies underscored the virulence of human-CoV/OC43 in elderly populations (Patrick et al., 2006) and Human-CoV/OC43 has serological cross-reactivity with SARS-CoV. Molecular clocks of Human-CoV/OC43 speculated that Human-CoV/OC43 caused the 1889–1890 flu pandemic (Vijgen et al., 2005). Hence the low $R_{HP(AT/CG)}$ correlates the virulence of human-CoV/OC43. The high HP score of the CoV genome may be due to zoonotic adaptive infection and evolution, and indicates relative light virulence of the virus.

From Dec. 2019 and ongoing, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, 2019) is a highly transmissible and pathogenic coronavirus of COVID-19, and the morbidity and mortality of the SARS-CoV-2 are less than SARS-CoV or MERS-CoV. The two coronaviruses SARS-CoV/Tor2 and MERS-CoV are very virulent for human host, and originated from bat host (Hemida et al., 2014). Accordingly, SARS-CoV-2 has a higher HP score 6.0860 than SARS-CoV and MERS-CoV's. The three SARS-CoV-2 close related CoVs, SLCoV/RaTG13 (Zhou et al., 2020b), SLCoV/BANAL-52

(Temmam et al., 2021) and bat-CoV/RmYN02 (Zhou et al., 2021) have a slightly large HP scores 6.2465, 6.3412, and 6.1050, respectively. Once Pangolin was considered as an intermediate animal host for SARS-CoV-2, Pangolin-SLCoV (2020) (Lam et al., 2020) has HP score 6.8144, the value is much higher than the HP score of SARS-CoV-2.

The zoonotic evolution of a coronavirus is the period of its infecting humans or closely related hosts from the wild state. In the wild state when the virus is with the native bat, we speculate that the HP score of the virus genome can be stable and relatively small. When the coronavirus infects human or mammalian hosts, under the host immune response and natural selection, the virus genome may undergo mutations, therefore resulting in an increased HP score for survival in the host. We speculate that the HP scores in CoV genomes are increasing during human host-interaction evolution. Additionally, Denver et al. (2004) previous study showed that the frequencies and run-length distributions of HP AT greatly outnumber HP GC in eukaryotic genome of *Caenorhabditis elegans*. Yet the implication of the Homopolymeric nucleotides in virus genome evolution is little known. The HP distributions of the genomes of SARS-related CoVs in this study agree with the observations found in the eukaryotic genomes.

In summary, we observe the correlation between the HP disparity values $R_{HP(AT/CG)}$ and the virulence of coronaviruses. Higher $R_{HP(AT/CG)}$ values indicate lower virulence. This can be explained that when a bat coronavirus of wild status, because the virus is new to human host, then human immune system may have a strong reaction as cytokine storm on the infection of the wild virus, causing high pathogenicity (Mehta et al., 2020). If the viruses already infected human for some periods, the virus genomes undergo multiple mutations, especially C>T mutations, as the result of the human APOBECs RNA editing. The resulting virus genomes will have more homopolymeric nucleotide T repeats, indicated as higher $R_{HP(AT/CG)}$. After some periods of virus infection and genome mutations, the virus adapts to the human immune system, and the human immune system reactions become less severe on the virus infection, therefore, the virulence of the virus is reduced.

Table 1: The HP scores of SARS-Like coronaviruses and Ebola virus.

Virus	GenBank	$(A + T)/(C + G)$	$R_{HP(AT/CG)}$
SARS-CoV-2	NC_045512	1.6335	6.0860
SLCoV/RaTG13	MN996532	1.6295	6.2465
SLCoV/BANAL-52	EPI_ISL_4302644	1.6338	6.3412
bat-CoV/RmYN02	EPI_ISL_412977	1.6151	6.1050
Pangolin-SLCoV/GD/1	MT084071	1.6109	6.8144
SARS-CoV/BJ01	AY278488	1.4513	4.5139
SARS-CoV/Tor2	AY274119	1.4533	4.5400
MERS-CoV	NC_019843	1.4250	3.2432
bat-CoV/HKU9-1	EF065513	1.4362	3.0114
SLCoV/ZXC21	MG772934	1.5758	5.3249
SLCoV/ZC45	MG772933	1.5705	5.3898
SLCoV/HKU2	NC_009988	1.5457	4.9346
SLCoV/RsSHC014	KC881005	1.4445	4.5484
SLCoV/Rs3367	KC881006	1.4512	4.7344
SLCoV/MA15	JF292916	1.4495	4.5422
SLCoV/WIV1	KF367457	1.4530	4.7085
SLCoV/Shaanxi2011	JX993987	1.4043	4.0818
civets-SCoV/SZ3	AY304486	1.4492	4.5400
swine-SADS-CoV	MG557844	1.5403	5.0192
Human-CoV/229E	NC_002645	1.6136	6.6788
Human-CoV/NL63	NC_005831	1.9018	9.4172
Human-CoV/OC43	AY585229	1.7188	5.7846
Human-CoV/HKU1	AY597011	2.1192	10.4088
Ebola virus	KP120616	1.4272	2.5927

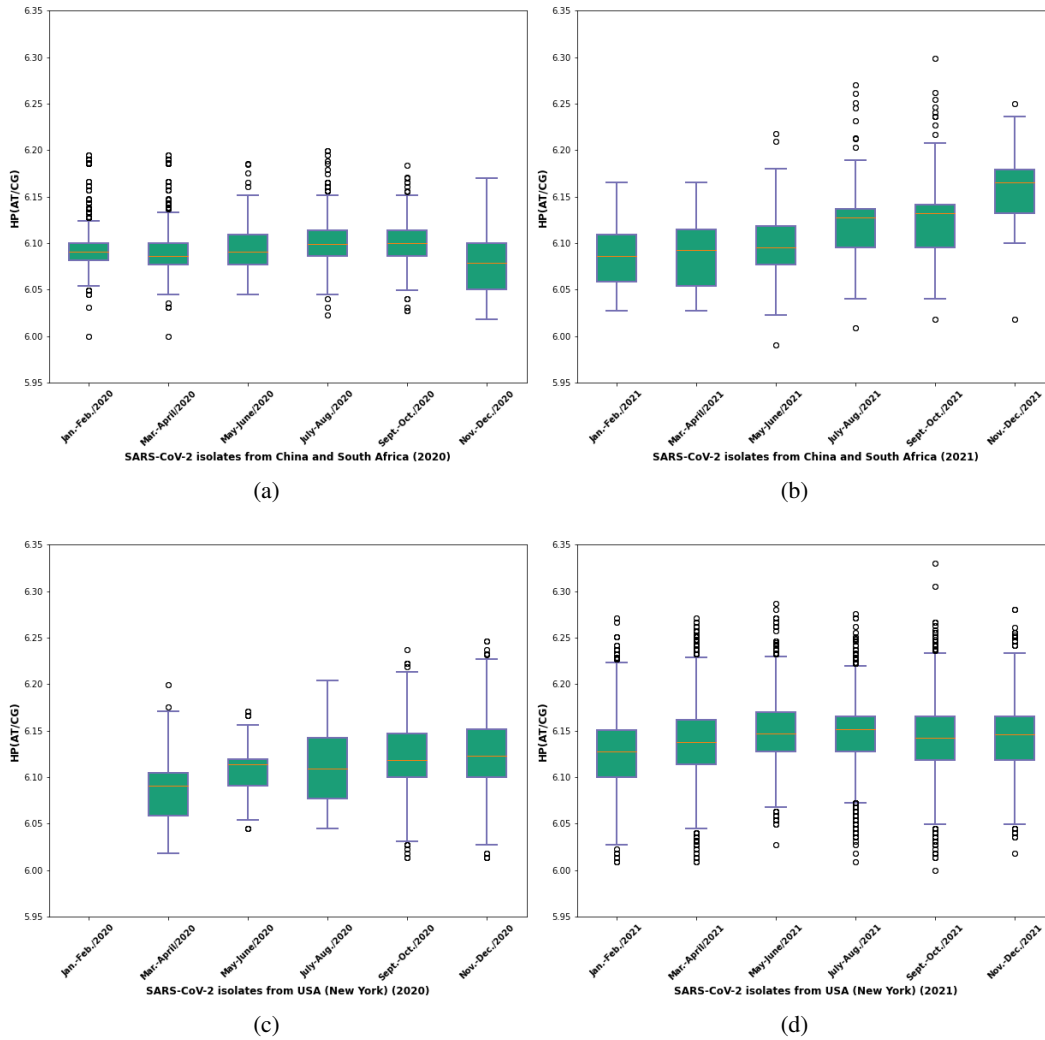


Figure 3: Boxplot of $R_{HP(AT/CG)}$ of SARS-CoV-2 isolates from different periods. (a) China and South Africa (2020), (b) China and South Africa (2021, as of Dec. 3, 2021), (c) USA (New York, 2020), (d) USA (New York, 2021, as of Dec. 3, 2021).

3.3 SARS-CoV-2 genomes have increasing HPs during zoonotic evolution

The HP distributions in the genomes of bat and human CoVs suggest that the genome HPs of a CoV may indicate the trend of zoonotic evolution of the CoV. Therefore, we expect that the HP scores in SARS-CoV-2 isolates increase during the infection and evolution period from 2020 to 2021. To verify this expectation, we measure and compare the $R_{HP(AT/CG)}$ scores of SARS-CoV-2 isolates in global territories from different periods. The results show that the SARS-CoV-2 isolates in all test countries have increased HP scores over time. Fig.3 is the HP score trends of SARS-CoV-2 isolates in China, South Africa, and USA in 2020 and 2021 (as of 12/3/2021). Fig.3 indicates that SARS-CoV-2 isolates in China, South Africa, and USA (New York) keep increasing HPs during infection and evolution. For instance, the average HP score SARS-CoV-2 isolates in China and South Africa is 6.0937 in Jan./Feb. 2020, and is increased to 6.1586 in Nov./Dec., 2021 (Fig.3 (a) (b) and Table 2). A similar HP trend is shown in the SARS-CoV-2 isolates in USA (New York) (Fig.3(c)(d) and Table 3), the HP score is increased from 6.0868 in Mar./April, 2020 to 6.1455 in Nov./Dec., 2021. Additionally, similar HP score trends of SARS-CoV-2 isolates are found in India, Sweden, and Israel (in Supplementary Materials).

Table 2: $R_{HP(AT/CG)}$ scores of SARS-CoV-2 isolates in China and South Africa (2020-2021).

period	sample size	max	min	mean	std
Jan.-Feb./2020	587	6.1944	6.0000	6.0937	0.0239
Mar.-April/2020	822	6.1944	6.0000	6.0909	0.0239
May-June/2020	134	6.1852	6.0450	6.0939	0.028
July-Aug./2020	753	6.1991	6.0224	6.0989	0.0252
Sept.-Oct./2020	336	6.1835	6.0269	6.1007	0.0256
Nov.-Dec./2020	130	6.1697	6.0179	6.0791	0.0329
Jan.-Feb./2021	33	6.1651	6.0269	6.0885	0.0328
Mar.-April/2021	76	6.1651	6.0269	6.0904	0.0363
May-June/2021	207	6.2176	5.9909	6.0980	0.0344
July-Aug./2021	375	6.2698	6.0089	6.1206	0.0337
Sept.-Oct./2021	340	6.2991	6.0179	6.1269	0.0411
Nov.-Dec./2021	21	6.2500	6.0179	6.1586	0.0528

Table 3: $R_{HP(AT/CG)}$ scores of SARS-CoV-2 isolates in USA (New York) (2020-2021).

period	sample size	max	min	mean	std
Mar.-April/2020	2378	6.1991	6.0179	6.0868	0.0271
May-June/2020	181	6.1705	6.0450	6.1098	0.0256
July-Aug./2020	264	6.2037	6.0450	6.1092	0.0392
Sept.-Oct./2020	1541	6.2372	6.0135	6.1216	0.0347
Nov.-Dec./2020	2567	6.2465	6.0135	6.1248	0.0344
Jan.-Feb./2021	6988	6.2710	6.0089	6.1267	0.0353
Mar.-April/2021	16682	6.2710	6.0089	6.1381	0.0356
May-June/2021	2337	6.2864	6.0269	6.1473	0.0332
July-Aug./2021	8022	6.2757	6.0089	6.1455	0.0318
Sept.-Oct./2021	9647	6.3301	6.0000	6.1412	0.0354
Nov.-Dec./2021	2227	6.2804	6.0179	6.1455	0.0349

During infection and zoonotic evolution, SARS-CoV-2 can mutate to some Variants of Concern (VOC). On November 24, 2021, the Omicron variant of SARS-CoV-2 (B.1.1.529) was detected in mid of November 2021, South Africa (Pulliam et al., 2021), and will spread more easily than the original SARS-CoV-2 virus. The Omicron variant and the initial SARS-CoV-2 isolates in Jan.-Feb/2020 are compared for HP distributions (Fig. 4(a)). The result shows that the HPs in Omicron rise much higher than the initial SARS-CoV-2 isolates. The mean HPs of Omicron variants is 6.2498, and the initial SARS-CoV-2 is 6.0860. The Omicron variant has higher HP than SLCoV/RaTG13 (Table 1). For example, the Omicron strain (EPI_ISL_7509826|2021 – 11 – 25, England) has HP as 6.3019. We hypothesis that the Omicron variant with high HPs may not be very virulent. Furthermore, we measure the HPs in the genomes of SARS-CoV and MERS-CoV strains that were collected in various sources during 2003-2004, and 2012-2013, respectively (Fig. 4(b)) (The GenBank access numbers of the SARS-CoVs and MERS-CoVs, and the HP statistics are in Supplementary Materials). The results further confirm that HP disparity scores are increased in SARS-CoVs and MERS-CoVs during evolution over time.

To further analyze the correlation of polynucleotide compositions with the evolution of coronaviruses in human hosts, we plot the HPs (4-mer) along the genomes of SARS-like coronaviruses (SARS-CoVs) and typical beta-coronavirus human-CoV/OC43 (Fig.5). The results show that poly-T in human-CoV/OC43 is the highest along the CoV genomes (Fig.5(a)). This result is due to the fact human-CoV/43 has been infecting human hosts for long periods, while SARS-related coronaviruses are relatively new to human hosts. When infecting intermediate animals and human hosts, the host APOBEC immune system mutates C>T in the virus genome as a defense mechanism, therefore, more poly-T can occur and accumulate in the virus genomes over time. Among the tested SARS-CoVs, SARS-CoV-2 has the most poly-T tracts, indicating SARS-CoV-2 undergoes the highest C>T

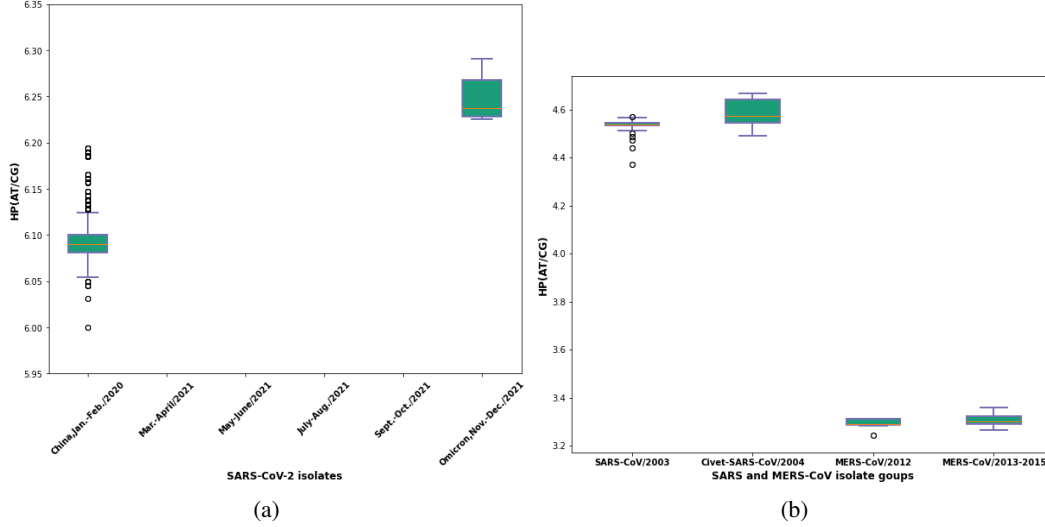


Figure 4: Boxplot of $R_{HP(AT/CG)}$ of SARS-CoV-2, SARS-CoV and MERS-CoV isolates in the globe from different periods. (a) SARS-CoV-2 (Jan.-Feb.,2020 and Omicron variants), (b) SARS-CoV and MERS-CoV.

mutations. It is noted that three SARS-related coronaviruses SARS-CoV-2, RaTG13, and RmYN02 have almost the same poly-T tracts from position 1 to 11000 (the end position of nsp5), but different poly-T from 11000 to the end. In addition, RaTG13 and RmYN02 have very similar poly-T along the genomes. The poly-A pattern is similar to poly-T. The three coronaviruses, SARS-CoV-2, RaTG13, and RmYN02 have high poly-A tracts (Fig.5(b)). These results suggest that SARS-CoV-2's direct ancestry is RmYN02, followed by RaTG13 (Fig.5(a)). The longer a coronavirus stays in and adapts to human hosts, the higher poly-T may occur in the virus, and therefore lower virulence, and vice versa. For the poly-C and poly-G tracts, MERS-CoV has the highest poly-C and poly-G contents (Fig.5(c) and Fig.5(d)). Because MERS-CoV is the severe virulent coronavirus, the high poly-C is an indicator for the native status of the virus because few C>T mutations occurred in MERS-CoV during a very short zoonotic period. Hence, we may postulate that MERS-CoV is the youngest zoonotic coronavirus among SARS-CoVs. Moreover, Fig. 5(d) shows that more poly-Gs are in the S-protein gene regions, indicating the hot-spots of immune response action, or recombinant events in the S protein genes.

Because poly-T may be the consequence of C>T mutations of the APOBEC Cytidine-to-Uracil deamination during interacting with human hosts, the result suggests that SARS-CoV-2 has a longer evolution period in human hosts than SARS-CoV/Tor2. Equally, SARS-CoV-2 has been lived long in human hosts than SARS-CoV/Tor2, i.e., SARS-CoV-2 is older in the zoonotic age than SARS-CoV/Tor2. Therefore, we may consider HP disparity of poly-(A/T) and poly-(C/G) as a measure for zoonotic age of the virus since its zoonotic infection.

In summary, we see that the results from SARS-CoV-2 isolates, SARS-CoV and MERS-CoV are consistent with the observations that coronaviruses have been increasing $R_{HP(AT/CG)}$ values during zoonotic evolution time. These results can be possibly explained as follows. During SARS-CoV-2 infection, human immune system fights SARS-CoV-2 virus and more C>T mutations then generate homopolymeric nucleotide repeat Ts. Consequently, we may use the HP distributions to measure the zoonotic age or evolution trend of coronaviruses.

4 Discussions

The COVID-19 pandemic has caused substantial health emergencies and economic stress in the world. Since there is no treatment efficacy for curing COVID-19, it is urgent to identify the zoonotic transmission of the causative agent SARS-CoV-2 so we may control and prevent future SARS-CoV-2 outbreaks. It is established that SARS-CoV, MERS-CoV, and SARS-CoV-2, cross the species barrier and move from bats to infect humans. Yet the necessity and mechanism that SARS-CoV-2 jumping

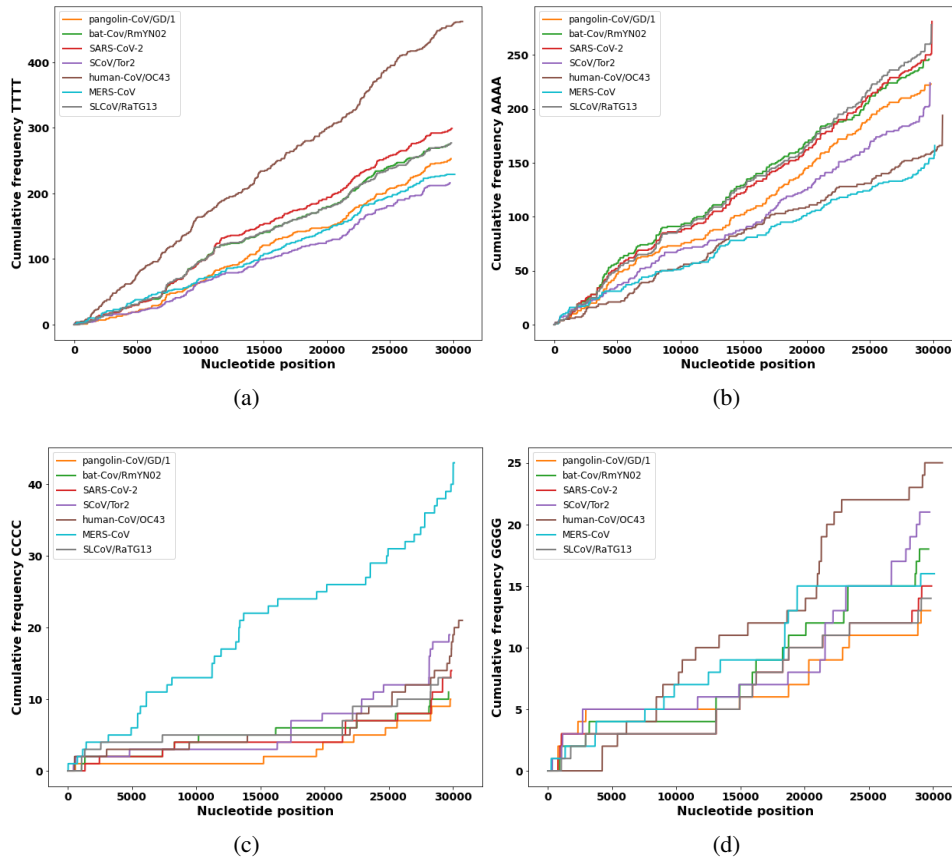


Figure 5: The cumulative frequencies of HPs along the SARS-CoV genomes. (a) The cumulative frequency of HP-T4. (b) The cumulative frequency of HP-A4. (c) The cumulative frequency of HP-C4. (d) The cumulative frequency of HP-G4.

from the intermediate animals to humans are unknown. One may argue that the bat coronaviruses can directly infect humans without being through the intermediate animals. If that is the case, the polynucleotide repeat analysis suggests that SARS-CoV-2 may have a similar or the same level of polynucleotide repeats as wild bat coronavirus. In fact, our results show that SARS-CoV-2 has a similar level polynucleotide repeat level as bat-CoV/RmYN02, suggesting that SARS-CoV-2 may originate from bats and jump to human host directly, or, having long periods of adapting to human host before COVID-19 outbreak in Dec. 2019.

From Table 1, we show that the zoonotic ages are correlated to the virulence of viruses because in theory when a virus fits more host immune system, under natural selection, the virulence becomes reduced (Alizon et al., 2009). Here, we postulate that the CoVs in wild bat host have a low $R_{HP(AT/CG)}$ value, and then more virulent to human host. After zoonotic infection to the human hosts, the virus genomes will have more homopolymeric nucleotide T repeats due to human immune system APOBECs RNA editing. We suggest that $R_{HP(AT/CG)}$ can be used as an approximate measure for the zoonotic age of the CoVs in human hosts. The values of $R_{HP(AT/CG)}$ from small to large correspond to the zoonotic evolution age from short to long. From the pAT/CG in Table 1 and C>T mutations as shown in Fig.2(c), we may presumably infer the order of the relative zoonotic ages of the coronaviruses from bat host to humans is as follows: MERS-CoV→SARS-CoV/Tor2→SARS-CoV-2→bat-CoV/RmYN02→SLCoV/RaTG13→Pangolin-SLCoV. Here we see that SARS-CoV-2 is younger in human hosts than Pangolin-SLCoV. Therefore, we may infer the virulence of Pangolin-SLCoV shall be less than SARS-CoV-2. In addition, Table 1 shows bat coronavirus swine-SADS-CoV has low $R_{HP(AT/CG)}$ about 5. Note that this swine-SADS-CoV virus had caused millions of pig deaths in 2017, and low HP disparity score suggests the virus is in a native bat host state and much virulent to a new mammalian host.

When a human host is infected by a virus, two major deamination enzymes in human, ADAR and APOBEC, are responsible for adenosine-to-inosine deamination and cytidine-to-uracil deamination, leading to an observed A>G and C>T changes in the virus genomes, respectively. The consequence of Simple sequence repeats (SSRs) or microsatellites are DNA stretches consisting of short, tandem repeats of tri-, tetra- or penta-nucleotide HP motifs. HPs are very special SSRs, and are ubiquitously dispersed in genomes. The SSR polymorphisms in bacterial pathogens are resulted from interactions with host structures and can be considered as a molecular marker of the genome evolution while infecting hosts (Moxon et al., 2006).

The generation mechanism of the homopolymeric repeat G in SARS-CoV-2 genome is still unclear. One possible reason is that RNA-specific adenosine deaminase 1 (ADAR1) RNA editing adenosine-to-inosine deamination (A>G) may produce HP poly-G tracts (Picardi et al., 2022). It is well established that sequences with G-blocks (adjacent runs of Guanines) can potentially form non-canonical G-quadruplex (G4) structures (Choi and Majima, 2011; Métifiot et al., 2014). For example, HP poly-G repeat is homopolymeric tract of Guanines (Gs) occurring in *wafN* gene of *Campylobacter jejuni* (Linton et al., 2000), and 23 tandem repeats of the tetramer 5'-CAAT-3' present in *lic2A* gene of *Haemophilus influenzae*. The G4 structures are formed by stacking two or more G-tetrads by Hoogsteen hydrogen bonds and often are the sites of genomic instability, serving one or more biological functions (Bochman et al., 2012; Chen and Yang, 2012). G-quadruplexes are also found in SARS-CoV genome. The non-structural protein 3 (nsp3) is one component of the viral replicase complex and contains a domain referred to as SARS unique domain (SUD), which interacts with G4s (Tan et al., 2009; Kusov et al., 2015). The G4 nucleotides have been suggested in SARS-CoV-2 genomes (Frick et al., 2020; Ji et al., 2020; Zhang et al., 2020). Therefore, poly-T and poly-G resulted from C>T and A>G mutations by immune response APOBEC and ADAR system, can be an evolution imprint that record the interaction between coronavirus and host. Here we propose to use the distribution of polynucleotide repeats for measuring the zoonotic evolution of coronaviruses (Hu et al., 2017).

5 Conclusions

In this study, we analyze the SARS-CoV genomes and discover that SARS-CoV-2 genome is significantly enriched HP repeats. We propose the HP distributions of a CoV genome can be an effective indicator for the zoonotic evolution of CoV. We investigate the HP distributions in coronavirus genomes and present a method using the HP disparity as the measure of zoonotic evolution. The low HP disparity $R_{HP(AT/CG)}$ value of a coronavirus indicates that the virus is in native status in a bat and is far from infecting humans, therefore, the virus can be much virulent for humans. After a coronavirus infects humans, interacting with human immune system may produce more T bases, and also change the G4 structure landscape in the genome, therefore, increasing the HP disparity $R_{HP(AT/CG)}$ value.

SARS-CoV-2 emerged in Dec. 2019 as unexpected since SARS-CoV disappeared in 2004. From the outbreak of SARS-CoV-2, which is a recurrence of SARS-like CoV, it is expected that in the future more coronavirus outbreaks may occur, probably by different bat-CoV strains. Therefore, strict epidemic surveillance is indispensable for preventing future coronavirus outbreaks. The proposed polynucleotide repeat measure can be used in surveying the zoonotic evolution age and relative virulence of a given virus, therefore, it is of importance for tracking, virulence assessment, and controlling the outbreak of a coronavirus.

Abbreviations

- ADAR1: RNA-specific adenosine deaminase 1
- APOBEC: apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like
- CoV: coronavirus
- COVID-19: coronavirus disease 2019
- HP: homopolymeric nucleotide repeats
- MERS-CoV: middle east respiratory syndrome coronavirus
- SARS: severe acute respiratory syndrome

- SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
- SADS-CoV: swine acute diarrhoea syndrome coronavirus
- SSR: simple sequence repeat
- WHO: the world health organization

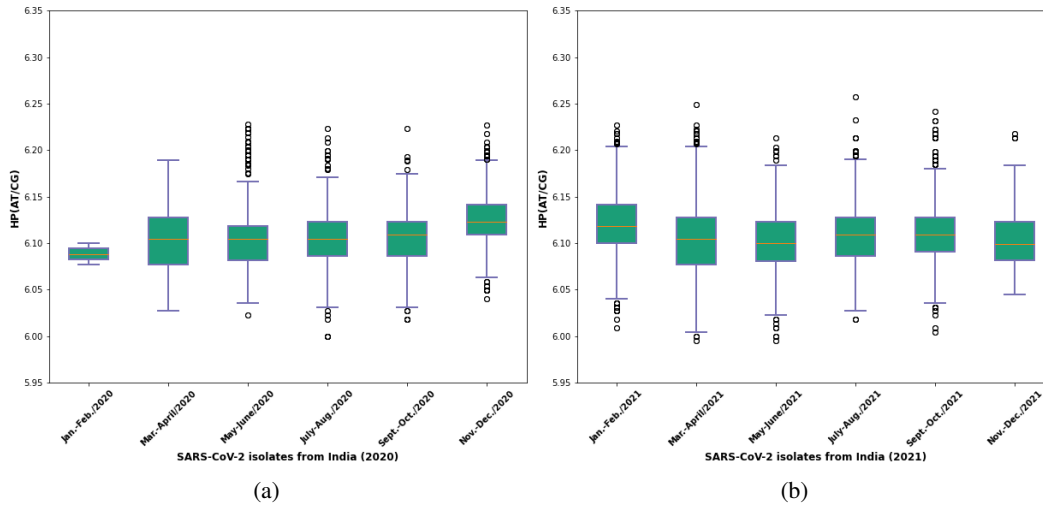


Figure 6: Boxplot of HPs $R_{HP(AT/CG)}$ of SARS-CoV-2 isolates in India.

Supplementary Materials

A The SARS-CoV-2 isolates during infection and evolution have increased ratios of the homopolymeric nucleotide repeats

The HP disparity scores of SARS-CoV-2 isolates from India, Sweden, and Israel since Jan 2020 were computed and listed in the following tables and figures. The results show that SARS-CoV-2 isolates have increased homopolymeric nucleotide (HP) scores during infection and evolution. The HP disparity score is defined in the method section of the main text of the paper.

In the tables, the terms min and max are the minimum and maximum HP disparity scores of the samples, respectively. The terms mean and std are average and standard deviation of HP disparity scores of the samples, respectively.

Table 4: $R_{HP(AT/CG)}$ scores of SARS-CoV-2 isolates in India (2020-2021).

period	sample size	max	min	mean	std
Jan.-Feb./2020	2	6.1000	6.0769	6.0884	0.0163
Mar.-April/2020	465	6.1889	6.0269	6.0993	0.0312
May-June/2020	1576	6.2279	6.0224	6.1035	0.0304
July-Aug./2020	802	6.2233	6.0000	6.1051	0.0284
Sept.-Oct./2020	745	6.2233	6.0179	6.1059	0.0262
Nov.-Dec./2020	1123	6.2269	6.0405	6.124	0.0271
Jan.-Feb./2021	1431	6.2269	6.0089	6.1193	0.0328
Mar.-April/2021	5379	6.2488	5.9955	6.105	0.035
May-June/2021	2034	6.213	5.9955	6.1007	0.0307
July-Aug./2021	1031	6.257	6.0179	6.1103	0.0315
Sept.-Oct./2021	1174	6.2419	6.0045	6.1109	0.0327
Nov.-Dec./2021	86	6.2176	6.0448	6.1079	0.0373

Table 5: $R_{HP(AT/CG)}$ scores of SARS-CoV-2 isolates in Sweden (2020-2021).

period	sample size	max	min	mean	std
Jan.-Feb./2020	7	6.0905	6.0631	6.069	0.0108
Mar.-April/2020	573	6.1521	6.0314	6.0746	0.0188
May-June/2020	245	6.156	6.0045	6.0834	0.0225
July-Aug./2020	153	6.1507	6.0495	6.0931	0.0229
Sept.-Oct./2020	193	6.1553	6.0089	6.0886	0.0306
Nov.-Dec./2020	700	6.2279	5.9866	6.0719	0.0408
Jan.-Feb./2021	15520	6.316	5.9911	6.1463	0.0478
Mar.-April/2021	42831	6.316	5.9689	6.1356	0.0382
May-June/2021	9904	6.3052	6.027	6.1336	0.0334
July-Aug./2021	10671	6.2315	6.0359	6.1238	0.0229
Sept.-Oct./2021	5834	6.2372	6.0359	6.1299	0.0255
Nov.-Dec./2021	2562	6.2454	6.0405	6.1267	0.0292

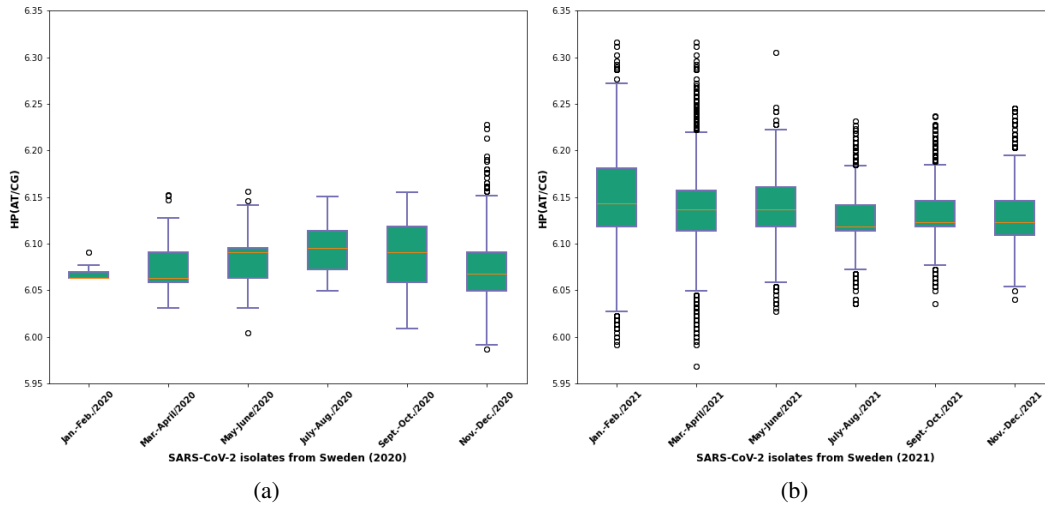


Figure 7: Boxplot of HPs $R_{HP(AT/CG)}$ of SARS-CoV-2 isolates in Sweden.

Table 6: $R_{HP(AT/CG)}$ scores of SARS-CoV-2 isolates in Israel (2020-2021).

period	sample size	max	min	mean	std
Jan.-Feb./2020	2	6.1045	6.0769	6.0907	0.0195
Mar.-April/2020	303	6.2000	6.0226	6.0907	0.027
May-June/2020	33	6.1227	6.0543	6.0769	0.0164
July-Aug./2020	47	6.2037	6.0179	6.0794	0.0339
Sept.-Oct./2020	5	6.1136	6.0545	6.0835	0.0223
Nov.-Dec./2020	1010	6.2279	5.9775	6.0963	0.0342
Jan.-Feb./2021	1214	6.2074	6.0	6.1101	0.0236
Mar.-April/2021	1560	6.2279	6.0000	6.1100	0.0238
May-June/2021	195	6.1705	6.0314	6.1026	0.0216
July-Aug./2021	216	6.2074	6.0541	6.1062	0.0276
Sept.-Oct./2021	350	6.2757	6.0314	6.1209	0.0357
Nov.-Dec./2021	153	6.2757	6.0359	6.129	0.0376

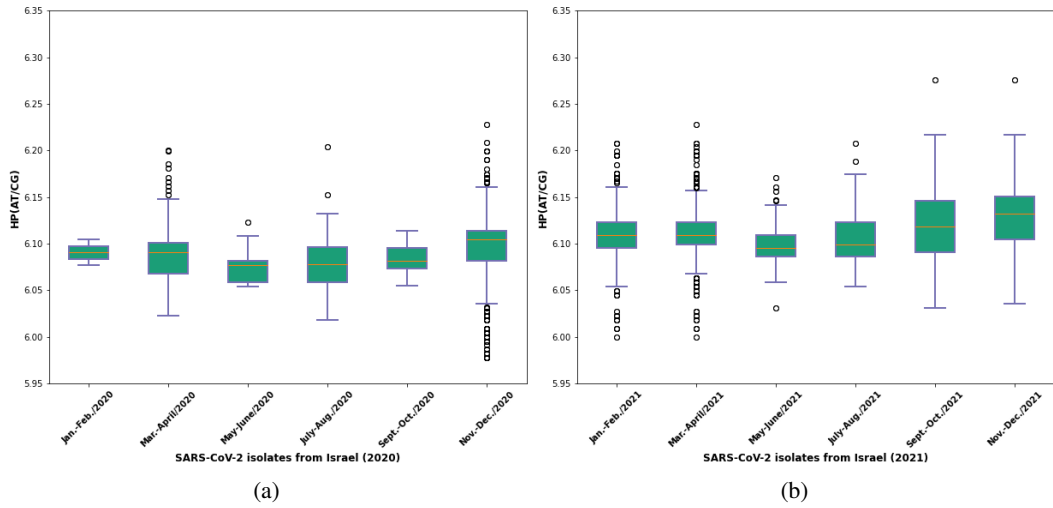


Figure 8: Boxplot of HPs $R_{HP(AT/CG)}$ of SARS-CoV-2 isolates in Israel.

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