Evolution of codon usage in 2019-new coronavirus causing human infection

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Abstract

Background: The recent emergence of viral pneumonia was caused by the novel coronavirus (SARS-Cov-2), which has spread to many countries and threatened the global public health.

Results: In the current study, we provide a primary evolutionary analysis based on the codon usage patterns of SARS-Cov-2 genome sequences. We employed bioinformatics technologies to measure the nucleotide compositions, the relative synonymous codon usage (RSCU), the codon adaptation index (CAI), and other indices. Our results reflected that there were some similarities of codon usage bias between SARS-Cov-2 and its natural reservoirs, suggesting that SARS-Cov-2 was tended to evolve its codon usage which was comparable to that of its hosts. Additionally, various degree of adaptation to the SARS-Cov-2 host and vector were estimated, even different genes have different codon adaptation to their reservoirs. We further suggested that SARS-Cov-2 isolates were evolving at a rapid substitution rate under their translation selection pressure of their hosts.

Conclusions: The findings of the present study will provide help for the understanding of the elements leading to viral evolution and adaptation to reservoirs.

Background

SARS-Cov-2, a novel coronavirus, has brought unprecedented panic to people throughout the world since the December of 2019 [1–4]. The SARS-Cov-2 outbreak was a Public Health Emergency of International Concern (PHEIC) that has become a global pandemic [1]. Until now, the virus is spreading in various countries and the healthcare systems in many countries strive. Some reports suggested that SARS-Cov-2 was a relative of the severe acute respiratory syndrome (SARS), which was characterized by the flu-like symptoms such as fever, cough and may transmit from animals to humans [3, 5]. The SARS was initially traced to palm civets while was later determined as the bats [6, 7]. Recent study has demonstrated that bats or snakes were the potential natural hosts of SARS-Cov-2 [8]. However, on the basis of the recent researches, the origin of SARS-Cov-2 is still unclear.

The coronavirinae family contains four genera according to their hereditary properties, such as *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus* [5]. The coronavirus RNA genome is the largest one among all RNA viruses, and ranges from 26 to 32 kb [9]. Some studies have shown that the SARS-Cov-2 belongs to *betacoronaviruses* on the basis of sequence analysis, which also includes SARS-CoV, the Middle East respiratory syndrome coronavirus (MERS-CoV), Bat-SARS-like (SL) -ZC45 and Bat-SL ZXC21. According to the phylogenetic analysis, SARS-Cov-2 is more closely with bat-SL-CoV ZC45 and bat-SL-CoV ZXC2, while is more distantly with SARS-CoV [5]. This new CoV can infect the lower respiratory tract in humans, but it shows apparently that the symptoms of SARS-Cov-2 are milder than that of SARS and MERS [5, 9]. The virus has a nucleocapsid consisted of genomic RNA and four major structural genes, which encode the spike glycoprotein (S), the membrane protein (M), the envelope protein (E) and the phosphorylated nucleocapsid protein (N). N protein is embedded in lipid bilayers and covered with various types of S proteins, including the spike glycoprotein trimmer and the hemagglutinin-esterase (HE). Besides, M proteins and E proteins are found to locate among S proteins in the viral envelope [9].

Synonymous codon usage patterns have been widely reported in organisms, especially in viruses [10–13]. In general, 61 codons can encode 20 different amino acids, so most of them are synonymous codons. Many studies have suggested that each codon was applied in different rates within different individuals and even within various genes of one genome [14–21]. This phenomenon was regarded as codon usage bias. Many researches demonstrated that several factors can force codon usage patterns, such as mutational pressure, protein translation, replication and transcriptional factors [22–24]. However, natural selection pressure and compositional constraint are considered as the dominant roles that may cause the variation of codon usage patterns among various organisms [10, 25].

At present, there is no evidence indicating a clear host to be SARS-Cov-2 reservoir. Studies of codon usage patterns between virus and its reservoirs showed viruses tended to evolve their codon usage patterns that were parallel to its reservoirs [26, 27]. To investigate the potential virus reservoirs and their influences on viral codon usage patterns, we have implemented a comprehensive analysis and comparison. Our results suggest the codon usage patterns of bat are more close to that of SARS-Cov-2, and the viral codon usage patterns are mainly shaped by the translational selection. More interestingly, we explored the adaptation of SARS-Cov-2 to its reservoirs and found the different genes have different adaptation to their hosts, which may explain their different efficiency of protein synthesis within their hosts. We also identified that SARS-Cov-2 isolates were evolving at a rapid substitution rate under their translation selection pressure of their hosts.

Results

Phylogenetic classification

Phylogenetic analysis of 41 SARS-Cov-2 whole genomes indicated that all SARS-Cov-2 strains were clustered into separate clades (Fig. 1). In the tree, SARS-Cov-2 of China (Wuhan) formed a distinct clade, and most of SARS-Cov-2 of USA clustered together in another clade. SARS-Cov-2 of China (Yunnan and Shenzhen), Australia and South Korea were more closely related to that of USA, and the virus of China (Hangzhou) was more closely related to that of China (Wuhan). Besides, SARS-Cov-2 of Japan were distributed in different clades.

A/U nucleotides are more frequent than G/C in SARS-Cov-2 coding sequences

We analyzed 41 whole genomes of SARS-Cov-2, which originates from humans infected in different countries during 2019 to 2020. Our results suggested that the four nucleotides were used at unequal frequencies, the genome compositions were significantly enriched for AU ($62.00\% \pm 0.02$) over GC ($38.00\% \pm 0.01$)

(Table 1, Fig. S1 (A), wilcox.test, $P < 0.01$). Additionally, the mean count of nucleotides A ($29.90\% \pm 0.03$) and U ($32.11\% \pm 0.01$) were significantly higher than G ($19.62\% \pm 0.01$) and C ($18.38\% \pm 0.01$) (Table 1, Fig. S1 (C), wilcox.test, $P < 0.01$). These results were consistent with our prior study of Crimean-Congo hemorrhagic fever virus, which was also indicated the frequencies of A and U were higher than that of C and G [17].

Table 1
Nucleotide composition analysis of SARS-Cov-2 complete sequences (%).

Accession ID	A	U	G	C	AU	GC	A3	U3	G3	C3	AU3	GC3	GC1	GC2	GC12
MN988669.1	29.89	32.11	19.62	18.38	62.00	38.00	38.18	45.84	21.33	19.87	68.10	31.90	34.90	24.10	29.50
MN988668.1	29.89	32.11	19.62	18.38	62.00	38.00	38.18	45.84	21.33	19.87	68.10	31.90	34.90	24.10	29.50
LC522975.1	29.89	32.11	19.62	18.37	62.00	37.99	39.61	43.08	17.76	26.80	65.10	34.90	34.90	24.10	29.50
LC522974.1	29.89	32.11	19.62	18.37	62.00	37.99	39.60	43.07	17.77	26.80	65.10	34.90	34.90	24.10	29.50
LC522973.1	29.89	32.11	19.62	18.37	62.00	37.99	39.60	43.09	17.77	26.78	65.10	34.90	34.90	24.10	29.50
LC522972.1	29.89	32.11	19.62	18.37	62.00	37.99	39.60	43.08	17.79	26.78	65.10	34.90	34.90	24.10	29.50
MT039888.1	29.89	32.12	19.62	18.37	62.01	37.99	39.58	43.09	17.79	26.77	65.10	34.90	34.90	24.10	29.50
LC521925.1	29.88	32.13	19.61	18.38	62.01	37.99	39.58	43.07	17.82	26.81	65.00	35.00	34.90	24.10	29.50
MT020881.1	29.89	32.11	19.62	18.38	62.00	38.00	39.59	43.09	17.79	26.78	65.10	34.90	34.90	24.10	29.50
MT020880.1	29.89	32.11	19.62	18.38	62.00	38.00	39.59	43.09	17.79	26.78	65.10	34.90	34.90	24.10	29.50
MN985325.1	29.89	32.11	19.62	18.38	62.00	38.00	39.59	43.09	17.79	26.78	65.10	34.90	34.90	24.10	29.50
MT044258.1	29.90	32.10	19.62	18.39	62.00	38.01	39.60	43.05	17.80	26.79	65.00	35.00	34.90	24.10	29.50
MT044257.1	29.90	32.11	19.62	18.38	62.01	38.00	39.57	43.09	17.77	26.79	65.10	34.90	33.70	24.10	28.90
MT039887.1	29.89	32.11	19.62	18.38	62.00	38.00	39.58	43.09	17.79	26.78	65.10	34.90	34.90	24.10	29.50
MT027064.1	29.89	32.11	19.62	18.37	62.00	37.99	39.59	43.09	17.79	26.78	65.10	34.90	34.90	24.10	29.50
MT027063.1	29.89	32.11	19.62	18.38	62.00	38.00	39.59	43.09	17.79	26.78	65.10	34.90	34.90	24.10	29.50
MT027062.1	29.89	32.11	19.62	18.38	62.00	38.00	39.59	43.09	17.79	26.78	65.10	34.90	34.90	24.10	29.50
MN997409.1	29.89	32.11	19.62	18.38	62.00	38.00	39.60	43.08	17.77	26.79	65.10	34.90	34.90	24.10	29.50
MN994468.1	29.90	32.11	19.62	18.37	62.01	37.99	39.60	43.07	17.79	26.79	65.10	34.90	34.90	24.10	29.50
MN994467.1	29.89	32.11	19.61	18.38	62.00	37.99	39.57	43.08	17.78	26.81	65.00	35.00	34.90	24.10	29.50
MN988713.1	29.89	32.10	19.62	18.37	61.99	37.99	39.59	43.11	17.77	26.76	65.10	34.90	34.10	24.40	29.25
MN975262.1	29.92	32.10	19.61	18.37	62.02	37.98	39.63	43.09	17.76	26.78	65.10	34.90	34.90	24.10	29.50
MN938384.1	29.86	32.13	19.64	18.38	61.99	38.02	38.56	33.95	29.98	23.92	58.60	41.40	34.90	24.10	29.50
MT007544.1	29.95	32.08	19.60	18.37	62.03	37.97	39.79	43.01	17.73	26.79	65.10	34.90	34.90	24.10	29.50
MT039873.1	29.87	32.11	19.63	18.39	61.98	38.02	39.57	43.07	17.80	26.81	65.00	35.00	34.90	24.10	29.50
MT019533.1	29.90	32.11	19.62	18.38	62.01	38.00	39.60	43.09	17.77	26.79	65.10	34.90	34.90	24.10	29.50
MT019532.1	29.91	32.10	19.62	18.37	62.01	37.99	39.62	43.07	17.78	26.79	65.10	34.90	34.90	24.10	29.50
MT019531.1	29.93	32.08	19.61	18.37	62.01	37.98	39.65	43.06	17.77	26.80	65.10	34.90	34.90	24.10	29.50
MT019530.1	29.92	32.09	19.62	18.38	62.01	38.00	39.61	43.05	17.79	26.82	65.00	35.00	34.90	24.10	29.50
MT019529.1	29.93	32.08	19.62	18.37	62.01	37.99	39.64	43.07	17.79	26.79	65.10	34.90	34.90	24.10	29.50
MT066176.1	29.86	32.12	19.63	18.38	61.98	38.01	39.55	43.08	17.80	26.79	65.00	35.00	34.90	24.10	29.50
MT066175.1	29.87	32.12	19.63	18.39	61.99	38.02	39.55	43.08	17.80	26.79	65.00	35.00	34.90	24.10	29.50
MT049951.1	29.95	32.08	19.60	18.37	62.03	37.97	39.67	43.09	17.76	26.78	65.10	34.90	34.90	24.10	29.50
MT039890.1	29.95	32.10	19.60	18.36	62.05	37.96	39.67	43.06	17.79	26.79	65.10	34.90	34.90	22.90	28.90
MN996531.1	29.86	32.12	19.63	18.39	61.98	38.02	38.14	45.84	21.34	19.88	68.10	31.90	34.90	24.10	29.50
MN996530.1	29.86	32.12	19.63	18.39	61.98	38.02	38.56	33.94	29.98	23.92	58.60	41.40	34.90	24.10	29.50
MN996529.1	29.86	32.12	19.63	18.39	61.98	38.02	39.53	43.08	17.81	26.81	65.00	35.00	34.90	24.10	29.50

ENC represents the effective number of codons.

GC12 represents the G + C content at the first and second positions of codons.

GC3 represents the G + C content at the third positions of codons.

AU3 represents the A + U content at the third positions of codons.

Accession ID	A	U	G	C	AU	GC	A3	U3	G3	C3	AU3	GC3	GC1	GC2	GC12
MN996528.1	29.92	32.10	19.61	18.37	62.02	37.98	39.62	43.07	17.78	26.79	65.10	34.90	34.90	24.10	29.50
MN996527.1	29.85	32.13	19.63	18.38	61.98	38.01	39.56	43.09	17.80	26.79	65.00	35.00	34.90	24.10	29.50
MN908947.3	29.94	32.08	19.61	18.37	62.02	37.98	39.66	43.07	17.77	26.79	65.10	34.90	34.90	24.10	29.50
NC_045512.2	29.94	32.08	19.61	18.37	62.02	37.98	39.66	43.07	17.77	26.79	65.10	34.90	34.90	24.10	29.50
Mean ± SD	29.90 ± 0.03	32.11 ± 0.01	19.62 ± 0.01	18.38 ± 0.01	62.00 ± 0.02	38.00 ± 0.01	39.45 ± 0.43	42.83 ± 2.16	18.64 ± 2.76	26.14 ± 1.89	64.98 ± 1.67	35.02 ± 1.67	34.85 ± 0.22	24.08 ± 0.19	29.46 ± 0.14
ENC represents the effective number of codons.															
GC12 represents the G + C content at the first and second positions of codons.															
GC3 represents the G + C content at the third positions of codons.															
AU3 represents the A + U content at the third positions of codons.															

To investigate the magnitude of codon usage bias in SARS-Cov-2, we calculated the mean values of nucleotide contents for all triplets during the research. At the third codon position, the nucleotide percentage of A3 ($39.45\% \pm 0.43$) and U3 ($42.83\% \pm 2.16$) were significantly higher than G3 ($18.64\% \pm 2.76$) and C3 ($26.14\% \pm 1.89$) (Table 1, Fig. S1 (D), wilcox.test, $P < 0.01$). These values were similar with the total nucleotide composition, showing that A3, U3, G3 and C3 may be influenced by the total nucleotide composition. GC nucleotide composition at each codon position was also considered to reflect the base composition bias. The ranges of GC content were as follows: 33.70–34.90% (mean = 34.85, SD = 0.22) at the first codon position; 22.90–24.40% (mean = 24.08, SD = 0.19) at the second codon position; and 28.90–29.50% (mean = 29.46, SD = 0.14) at the first and second codon positions. In addition, the mean values of AU3 and GC3 compositions were $64.98\% \pm 1.67$ and $35.02\% \pm 1.67$, respectively, suggesting that A and U nucleotides may be more enriched in the end of codons (Table 1, Fig. S1 (B), wilcox.test, $P < 0.01$).

Codon usage patterns of SARS-Cov-2 and its hosts

The RSCU analysis was used to estimate the codon usage patterns of SARS-Cov-2 complete genomes and their major gene sequences. Among the eighteen most abundantly used codons in SARS-Cov-2 complete genomes, twelve codons [UUU (Phe), UUA (Leu), AUU (Ile), GUU (Val), AGU (Ser), CCU (Pro), ACA (Thr), GCU (Ala), UAU (Tyr), UGU (Cys), AGA (Arg) and GGU (Gly)] had A/U at the end (three A-ended; nine U-ended) and the remaining six [CAC (His), CAG (Gln), AAC (Asn), AAG (Lys) and GAC (Asp)] had G/C at the end (three C-ended; three G-ended), when the SARS-Cov-2 coding sequences were similar according to their gene groups. This indicates that A- and U-ended codons are preferred in the SARS-Cov-2 coding sequences. An analysis of overall RSCU values showed that 3 of the 18 preferred codons [ACA (Thr), AGA (Trg) and GGU (Gly)] had RSCU values > 1.6 , while RSCU values for the remaining preferred codons were > 0.6 and < 1.6 . The evaluation of overall RSCU values may potentially hide gene-specific patterns, thus we also estimated the RSCU values of SARS-Cov-2 coding sequences based on the gene groups. We found that the preferred codons were differentiated among the gene groups. The ratios of consistent/inconsistent preferred codons between the SARS-Cov-2: E gene, M gene, 2 N gene and S gene were 4:14, 6:12, 4:14 and 8:10, respectively (Table 2, Fig. S2). Patterns of gene-specific over-represented codons were also observed in the SARS-Cov-2 isolates, 11 of the 18 preferred codons were over-represented in the E gene, 10 of the 18 were over-represented in the M gene, 6 of 18 were over-represented in the N gene and 9 of 18 were over-represented in the S gene. The gene-specific RSCU patterns indicated the independent evolution dynamics of the SARS-Cov-2 isolates. In addition, to estimate the potential effects of the host and vector on the viral codon usage pattern, the RSCU patterns were considered and matched with various potential hosts such as human and bat (Table 2, Fig. S2). Among these 18 preferred codons, we found that the ratio of common/uncommon preferred codons was 6:12 between SARS-Cov-2 and human and 13:5 between SARS-Cov-2 and bat (Table 2, Fig. S2).

Table 2

The relative synonymous codon usage frequency (RSCU) of SARS-Cov-2 complete genomes, major viral genes, its natural hosts and transmission vectors.

A.A	Codons	E gene	M gene	N gene	S gene	SARS-Cov-2	Human	Bat
Phe	UUU	0.8	0.91	0.48	1.53	1.33	0.97	1.31
	UUC	1.20	1.09	1.52	0.47	0.67	1.03	0.69
Leu	UUA	0.43	0.69	0.46	1.56	1.40	0.5	1.76
	UUG	0.86	0.69	1.99	1.12	1.30	0.85	0.64
	CUU	2.99	2.06	1.77	2.00	1.19	0.81	0.76
	CUC	0.00	1.03	0.44	0.67	0.46	1.07	0.70
	CUA	0.86	0.86	0.66	0.50	0.88	0.46	1.51
	CUG	0.86	0.69	0.66	0.17	0.77	2.33	0.63
Ile	AUU	1.00	1.65	1.93	1.74	1.46	1.13	1.29
	AUC	1.00	0.90	0.86	0.55	0.64	1.37	0.69
	AUA	1.00	0.45	0.21	0.71	0.90	0.5	1.02
Val	GUU	2.15	1.00	1.00	1.98	1.45	0.79	0.91
	GUC	0.31	0.00	1.50	0.87	0.55	0.90	0.85
	GUA	0.92	2.00	0.50	0.62	0.93	0.52	1.78
	GUG	0.62	1.00	1.00	0.54	1.06	1.79	0.46
Ser	UCU	3.00	0.80	1.30	2.24	1.50	1.15	1.33
	UCC	0.00	1.20	0.49	0.73	0.55	1.17	1.33
	UCA	0.75	1.20	1.45	1.58	1.48	0.93	1.93
	UCG	0.75	0.40	0.32	0.12	0.30	0.36	0.33
	AGU	0.75	1.60	1.46	1.03	1.57	0.98	0.93
	AGC	0.75	0.80	0.97	0.30	0.51	1.42	1.23
Pro	CCU	4.00	0.80	1.14	2.00	1.60	1.20	1.40
	CCC	0.00	0.00	1.00	0.28	0.32	1.22	0.44
	CCA	0.00	2.40	1.57	1.72	1.37	1.14	1.23
	CCG	0.00	0.80	0.29	0.00	0.73	0.45	1.12
Thr	ACU	1.00	1.54	2.00	1.81	1.59	1.03	1.32
	ACC	0.00	0.92	0.75	0.41	0.31	1.32	0.32
	ACA	2.00	0.92	1.00	1.65	1.82	1.19	0.94
	ACG	1.00	0.62	0.25	0.12	0.63	0.46	1.28
Ala	GCU	1.00	2.51	2.06	2.13	1.26	1.08	1.66
	GCC	1.00	0.43	0.76	0.41	0.29	1.51	0.12
	GCA	0.00	0.84	0.86	1.37	1.03	0.95	1.31
	GCG	2.00	0.21	0.32	0.10	0.97	0.46	0.69
Tyr	UAU	0.00	0.89	0.36	1.48	1.22	0.93	1.08
	UAC	2.00	1.11	1.64	0.52	0.72	1.07	0.82
His	CAU	2.00	1.60	1.50	1.53	0.76	0.85	0.81
	CAC	0.00	0.40	0.50	0.47	1.13	1.15	1.05
Gln	CAA	0.00	1.00	1.54	1.48	0.87	0.49	0.95
	CAG	0.00	1.00	0.46	0.52	1.23	1.51	1.52

AA represents amino acid; the "RSCU" value represents the pattern of relative synonymous codon usage; orange colors represents the codons favored by SARS-Cov-2 and hosts (RSCU > 1); over-represented (RSCU > 1.6), and under-represented (RSCU < 0.6) codons are marked as bold with red and green colors, respectively, the ideal codons for SARS-Cov-2 are marked as underline.

A.A	Codons	E gene	M gene	N gene	S gene	SARS-Cov-2	Human	Bat
Asn	AAU	1.60	0.73	1.45	1.23	0.77	0.98	0.48
	AAC	0.40	1.27	0.55	0.77	1.14	1.02	0.95
Lys	AAA	2.00	1.14	1.35	1.25	0.86	0.88	1.05
	AAG	0.00	0.86	0.65	0.75	1.31	1.12	1.14
Asp	GAU	2.00	0.33	1.17	1.39	0.69	0.99	0.86
	GAC	0.00	1.67	0.83	0.61	1.23	1.01	1.00
Glu	GAA	1.00	1.71	1.33	1.42	0.77	0.85	1.00
	GAG	1.00	0.29	0.67	0.58	1.06	1.15	1.10
Cys	UGU	0.67	2.00	0.00	1.40	1.00	0.95	1.00
	UGC	1.33	0.00	0.00	0.60	0.77	1.05	0.17
Arg	CGU	2.00	2.14	1.24	1.28	0.41	0.54	2.23
	CGC	0.00	0.86	1.03	0.14	0.33	1.11	0.70
	CGA	2.00	0.43	1.03	0.02	1.37	0.76	0.49
	CGG	0.00	0.00	0.41	0.28	0.79	1.31	0.58
	AGA	2.00	1.29	2.07	2.85	2.63	1.18	1.39
	AGG	0.00	1.29	0.21	1.43	1.42	1.10	0.82
Gly	GGU	4.00	1.43	0.93	2.29	1.71	0.71	0.75
	GGC	0.00	0.86	1.49	0.73	0.81	1.35	0.96
	GGA	0.00	1.71	1.21	0.83	1.01	1.01	1.49
	GGG	0.00	0.00	0.37	0.15	0.46	0.93	0.80

AA represents amino acid; the "RSCU" value represents the pattern of relative synonymous codon usage; orange colors represents the codons favored by SARS-Cov-2 and hosts (RSCU > 1); over-represented (RSCU > 1.6), and under-represented (RSCU < 0.6) codons are marked as bold with red and green colors, respectively, the ideal codons for SARS-Cov-2 are marked as underline.

Measuring the similarity influences between the overall codon usage of SARS-Cov-2 and that of hosts

Spearman's correlational distance analysis was used to further estimate similarity of codon usage patterns and to investigate how the overall codon usage patterns of hosts and SARS-Cov-2 participated in evolutionary process. This analysis was performed to determine the similarities of general codon usage patterns between SARS-Cov-2 and hosts. Such RSCU-dependent analysis was applied routinely for the viral hosts, and remain limited to codon usage patterns and similarities [28–31]. Here, we performed this method through the hierarchical clustering analysis of virus and hosts in this study, and estimated their overall codon usage similarities. This optimized method was performed to present a clear sight of codon usage patterns. Two main groups were noted in this analysis. It was shown that one cluster included the virus and the vector (bat) and the other cluster only included the host (human) (Fig. 2). The statistical tests for the distances of RSCU values (each of which was compared with a synonymous shuffling null model) indicated that a significant signature of codon usage patterns existed for vector and SARS-Cov-2 ($P < 0.01$) compared with human and SARS-Cov-2 ($P > 0.05$). This suggested that possible viral transmission in humans may depend on the vector (bat).

Codon usage adaptation in SARS-Cov-2

Codon adaptation index (CAI) analysis was performed to investigate the relationship between the codon usage patterns and the expression levels of SARS-Cov-2 coding sequences, which reflected the adaptation of virus to their host cellular machinery. The CAI values are ranged from 0 to 1, and higher CAI values are considered as higher levels of codon usage bias [32]. The CAI values were obtained for each gene of SARS-Cov-2 in relation to human and bat, respectively (Fig. 2 and Table S1). In the SARS-Cov-2 isolates of E genes, the mean CAI value was noted in relation to human (0.617 ± 0.001) and bat (0.602 ± 0.001). In the M genes, the mean CAI value was noted in relation to human (0.674 ± 0.001) and bat (0.670 ± 0.001). In the N genes, the mean CAI value was noted in relation to human (0.731 ± 0.001) and bat (0.710 ± 0.001). In the S genes, the mean CAI value was noted in relation to human (0.710 ± 0.001) and bat (0.755 ± 0.001). The Student's t-test was applied to estimate the significant differences in this study, and it suggested that there were significant differences in the CAI values (Fig. 3 and Table S1).

Evolutionary rates of various genes in SARS-Cov-2

To investigate why the CAI value is not coincident between S genes and the other three genes in relation to human and bat, we estimated the evolutionary rates of SARS-Cov-2 strains whose collection date was known, by the Bayesian coalescent approach according to the sequences of various protein-coding genes. Using the best-fit model, Bayesian estimated the mean substitution rates for these genes were between 2.35×10^{-4} and 4.21×10^{-3} substitutions per site/year (Table 3). Among the structural proteins-encoding genes, the E gene had the fastest evolutionary rate (4.21×10^{-3}) and a 95% highest probability density (HPD) of between 5.40×10^{-8} and 1.3×10^{-2} . The S gene evolved at the slowest rate of 2.35×10^{-4} with the 95% HPD of between 1.29×10^{-8} and 7.21×10^{-4} .

Table 3
Bayesian estimates of evolutionary rate of specific gene segments of SARS-Cov-2.

Gene	Evolutionary rate (nt substitutions per site per year)	95% HPD
E	4.21×10^{-3}	$5.40 \times 10^{-8} \sim 1.3 \times 10^{-2}$
M	1.45×10^{-3}	$1.11 \times 10^{-7} \sim 4.41 \times 10^{-3}$
N	7.96×10^{-4}	$1.57 \times 10^{-7} \sim 2.36 \times 10^{-3}$
S	2.35×10^{-4}	$1.29 \times 10^{-8} \sim 7.21 \times 10^{-4}$
HPD, highest probability density.		

Discussion

In the present study, an evolutionary analysis was performed using 41 whole genome sequences of SARS-Cov-2 obtained from different geographic locations. Our results indicated that the SARS-Cov-2 occurring in various regions mainly formed two groups. Apparently, the SARS-Cov-2 has spread to many countries/regions during 2019–2020, and led to a severe global outbreak [33]. Besides, the phylogenetic tree also indicate that the geographical locations play an important role in SARS-Cov-2 evolution, and such findings may help to trace the viral root of emerging strains in the future. In addition, current results also suggest that some infected countries have more than one genetic lineages.

This survey of the SARS-Cov-2 whole genomes suggests a preference for A and U nucleotide over G and C, indicating this preference influences the codon usage patterns for the viral translation process. This result is similar to our previous study on Crimean-Congo hemorrhagic fever virus (CCHFV), which is also enriched with A/U nucleotide [17]. However, the biological senses of this phenomenon are still unclear, making it crucial to investigate the causes for the significantly increased A/U nucleotide content in the viral genomes [34]. Some previous reports revealed that the amino acids content was also an important factor determining the nucleotide composition at the 1st and 2nd codon locations of viral genomes, while the protein alteration was driven by functional selection. Meanwhile, 69% of the variation at the 3rd codon locations always implied synonymous or silent mutations, without the influences of functional selection [34].

RSCU analysis can provide some insights to the influence of natural hosts on SARS-Cov-2 although it may require further validation with experimental researches in animal models. Earlier reports indicated that the codon usage patterns of Ebola virus (EBOV) were not similar with its hosts [35, 36]. Our findings are consistent with the previous studies suggesting that A/U-ended codons are higher enriched in viral genomes than that in the host genomes [37, 38]. Additionally, some earlier researches also suggested that the identical contents of codon usage patterns between virus and their hosts may improve the translation efficiency of the amino acids, while the opposite contents of codon usage patterns may ensure the exact folding of viral proteins [14, 36, 39, 40]. These findings also indicated that the similar codon usage patterns between SARS-Cov-2 and their hosts could improve the ability of viral genome to participate in the translational process. Particularly, the codon usage patterns of SARS-Cov-2 genomes could be largely impacted by the selection pressure of their common hosts, which may promote the adaptation to the cellular environment of their hosts and efficient replication [29, 41]. However, the effect of selection pressure from hosts (human) on building SARS-Cov-2 codon usage patterns is not similar with the vector (bat). Previous reports on EBOV, CCHFV and *Flaviviridae* virus reflected the codon usage patterns are different within their hosts and vectors [35, 36, 42]. Based on RSCU values, Spearman's correlational distance analysis was also performed to estimate the general codon usage similarities between SARS-Cov-2 and hosts. That makes it clear that possible SARS-Cov-2 transmission in humans is based on its vector (bat). Models of the infection of animals have indicated the substantial constraints on the evolution of arbovirus [43–46]. Consistent with the earlier reports, our results have clearly showed the correlation of overall SARS-Cov-2 codon usage patterns with bat and not with human. According to this finding, we demonstrated that translational selection pressure plays an important role in shaping the codon usage patterns of SARS-Cov-2.

Interestingly, the codon usage patterns of different coding sequences were different, even within a single isolate at the same time. The codon usage patterns of S genes and N genes were closer to that of humans, while E genes and M genes were closer to that of bats (Table 2 and Fig. S2). This suggested that the evolution of codon usage patterns of SARS-Cov-2 individual coding sequences is possibly related to the function of different genes in viral pathogenesis. To

further confirm the effect of natural selection, the CAI analysis was performed. CAI was frequently employed to measure the gene expression and the adaptation of SARS-Cov-2 genes to their hosts, which indicated the effect of natural selection. It would suggest that the highly expressed genes reflected a strong bias for specific codons than genes that were less frequently expressed. Therefore, if the CAI value was high, the codon usage bias would be extremely high and the effect of natural selection was prevalent, and vice versa [47]. Based on the CAI values for the SARS-Cov-2 coding sequences, different levels of adaptation of SARS-Cov-2 host and vector were observed. In our results, the greatest adaptation of SARS-Cov-2 S genes were to bat, closely followed by human, indicating that the replication of S genes may be more efficient in the vector cells than in the host cells. However, CAI values for the other three genes (E, M and N) tended to be higher for human, which may be attributable to the possibly higher efficiency of protein synthesis within its host.

To further investigate the inconsistent adaptation of different genes, we deduced the evolutionary rates of SARS-Cov-2 strains. In most cases, it is less significant to consider the collection date than the location of isolated sequences. However, as for fast-evolving organisms like RNA viruses, isolation changes in the date could be used to evaluate the time since virus last shared one common ancestor. So we required the collection date of SARS-Cov-2 to assume the constant evolutionary rates of divergence between sequences. Additionally, according to the assumption of substitution rate constancy, the differences of isolation dates could provide information about the rates of molecular evolution. In other words, the quantity of evolutionary rate has accumulated since the collection date [48]. A Bayesian coalescent approach suggested the mean substitution rates were between 2.35×10^{-4} and 4.21×10^{-3} in different genes, which was comparable with the reports for hepatitis A and B viruses and Newcastle disease virus [49–51]. It has suggested that the lowest substitution rate of the SARS-Cov-2 S genes may contribute to its more important function in viral synthesis and replication, which may be subjected to lesser natural selection pressure and suffered fewer substitution.

Conclusion

In conclusion, this study indicated that the codon usage patterns of SARS-Cov-2 were mainly shaped by the translation selection. Importantly, there were similarities of codon usage patterns between SARS-Cov-2 and its natural hosts. It also demonstrated that different genes have different adaptation to their hosts. And we further suggested that SARS-Cov-2 isolates were evolving at a rapid substitution rate under their translation selection pressure of their hosts. The present study will be required to build SARS-Cov-2 adaptation in different hosts that will contribute to the understanding and control of SARS-Cov-2 infection and transmission.

Methods

Data collection

The newly sequenced 41 SARS-Cov-2 complete genomes have been downloaded

from the GenBank database (<http://www.ncbi.nlm.nih.gov/genbank/>). The comprehensive information of SARS-Cov-2 strains were provided in Supplementary Table S1, including the accession number, collection date, viral host and the geographic origin of isolation. Out of them, different datasets were extracted from the whole genome sequences, containing the E, M, N and S structural genes, and used in the further analysis. The sequences were aligned using the local software MAFFT v7.450 [52], and manually adjusted using BioEdit v7.2.5 [53].

Phylogenetic analysis of SARS-Cov-2

The phylogenetic tree was reconstructed using maximum-likelihood method in PAML v4.9 and was estimated by the bootstrap analysis with 1000 replicates. The tree was designed by the online software named the Interactive Tree Of Life v2 [54]. A total of 41 SARS-Cov-2 strains were used in our research.

Nucleotide contents analysis

The nucleotide content analysis of 41 SARS-Cov-2 whole genomes were calculated using CodonW software. The total nucleotide content of A, U, G and C, the nucleotide content at the 3rd location (A3, U3, G3 and C3), and GC nucleotide content at the 1st (GC1), 2nd (GC2) and 3rd (GC3) locations were measured individually. Additionally, the average frequency of GC at 1st and 2nd positions and the whole AU/GC compositions were also calculated. Nonsynonymous codons (UGG and AUG) and termination codons (UAA, UAG and UGA) were abandoned from this study.

Synonymous Codon Usage Analysis

To evaluate the relative synonymous codon usage (RSCU) bias of SARS-Cov-2 and the potential hosts, coding sequences of SARS-Cov-2 whole genome (408321 codons) and *Vespertilio murinus* genes (29616 codons) were calculated in Codon W1.4.2 [15, 55, 56]. The RSCU of *Homo sapiens* genes (40662582 codons) was obtained from the Codon Usage Database (<http://www.kazusa.or.jp/codon/>). The RSCU values of each structural gene (E, M, N and S) were also considered in this study. The RSCU values are the ratio between one observed codon usage frequency in a gene sequence and its expected usage frequency of the corresponding synonymous codon family, provided that all codons for specific amino acids are used equally. The codons with $RSCU > 1.0$ suggest positive codon usage bias, which are defined as ‘abundant’ codons. While those with $RSCU < 1.0$ suggest negative codon usage bias, which are named as ‘less abundant’ codons. And there is equally or randomly used of synonymous codons for the corresponding amino acids when the RSCU is 1.0 [57]. The $RSCU > 1.6$ and < 0.6 are treated as ‘overrepresented’ codons and ‘underrepresented’ codons, respectively [29]. Besides, a heat map of RSCU values was performed by Heatmap Illustrator v1.0 software [58]. The SARS-Cov-2 and its potential hosts were clustered by the Euclidean distance method.

Identifying the similarity effect of codon usage bias

In this study, the RSCU values of all synonymous codons were used to discern the similarity influence among these organisms. Except for Met (AUG), Trp (UGG) and three stop codons (UAA, UAG, UGA), all codons were arranged in a matrix containing $N \times M$ dimensions. Here, N represents the number of organism species and M represents the number of degenerated synonymous codons. Hierarchical clustering for this matrix was performed by Bioconductor with Ward's method on the basis of Spearman's correlational distance for RSCU values [59]. The resulting dendrogram was drawn with ggplot2 in R program [60]. Additionally, the observed probability was performed as the P-value to display significance.

Codon adaptation index

As a quantitative measure, the codon adaptation index (CAI) was performed to estimate the gene expression level on the basis of its coding sequences, and ranged from 0 to 1. The higher frequent codons suggested the higher relative adaptation to their hosts, and genes with higher CAI values were considered to be fitted over those with lower CAI values [61]. CAI analysis of the SARS-Cov-2 coding sequences was employed with CAIcal server, which implemented an improved method of CAI calculation [47]. The codon usage patterns of *Vespertilio murinus* and *Homo sapiens* were used as references. Except for the three termination codons, nonsynonymous codons (UGG and AUG) were also excluded in our study.

Evolution substitution rates

We analyzed 41 representative whole genome sequences of SARS-Cov-2 isolates during 2019 to 2020 with the MCMC program in BEAST v 2.4.8 software to measure the evolutionary rate of different genes. Under the model that assumed a constant substitution rate, this program provided a maximum-likelihood method to estimate the rate. The jModel Test software v 2.0.1 was performed to choose the best-fitted model based on the Akaike information criterion (AIC) [62]. For model comparison, the Bayes factor could estimate each model test and yield the best results with marginal likelihood estimated on the basis of Newton & Raftery method [63]. As implemented in BEAST package, the best-fitted model was GTR (general time reversible) + Γ 4 (gamma distribute rate variation) + I (proportion of invariant sites) + Lognormal relaxed uncorrelated clock and coalescent constant population models. The MCMC analysis was implemented with 300 million generations and sampled every 2000 generations with 10% burn-in. The results were analyzed by Tracer v 2.4.8 program. Statistical uncertainty was indicated in the 95% HPD values.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are available in the GenBank repository, [<http://www.ncbi.nlm.nih.gov/genbank/>].

Competing interests

The authors declare that they have no competing interests.

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Author contributions

DKC and WTM conceived and designed experiments; XTY and YFX performed all experiments. XTY, XG and SLN collected and analyzed the data. XTY, SUR and CXZ drafted the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Phylogenetic tree based on the whole genome sequences of 41 SARS-CoV-2 strains. The tree was generated by the maximum likelihood (ML) method using PAML v4.9. The tree was designed by using the online tool "iTOL".

Figure 2

Similarity distance analysis of the codon usage using SARS-Cov-2 and its hosts (Spearman correlational distances = $1 - \text{SpearmanRho}$).

Figure 3

CAI of SARS-Cov-2 coding sequences to its hosts. In the plot, the x-axis represents different genes of SARS-Cov-2. The Y-axis represents the CAI value. Different colors represent different host species: blue- bat, and yellow - human. Different asterisks represent statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Supplementary Files

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