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# Latent periodicity-2 in coronavirus SARS-CoV-2 genome: Evolutionary implications

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## ABSTRACT

The ongoing global pandemic of infection disease COVID-19 caused by the 2019 novel coronavirus (SARS-CoV-2, formerly 2019-nCoV) presents critical threats to public health and the economy. The genome of SARS-CoV-2 had been sequenced and structurally annotated, yet little is known of the intrinsic organization and evolution of the genome. To this end, we present a mathematical method for the genomic spectrum, a kind of barcode, of SARS-CoV-2 and common human coronaviruses. The genomic spectrum is constructed according to the periodic distributions of nucleotides and therefore reflects the unique characteristics of the genome. The results demonstrate that coronavirus SARS-CoV-2 exhibits predominant latent periodicity-2 regions of non-structural proteins 3, 4, 5, and 6. Further analysis of the latent periodicity-2 regions suggests that the dinucleotide imbalances are increased during evolution and may confer the evolutionary fitness of the virus. Especially, SARS-CoV-2 isolates have increased latent periodicity-2 and periodicity-3 during COVID-19 pandemic. The special strong periodicity-2 regions and the intensity of periodicity-2 in the SARS-CoV-2 whole genome may become diagnostic and pharmaceutical targets in monitoring and curing the COVID-19 disease.

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## 1. Introduction

The current global pandemic of COVID-19 caused by the novel coronavirus SARS-CoV-2, formerly 2019-nCoV, has been a severe threat to public health and the economy since it emerged in Wuhan, China, in December 2019. As of May 29, 2020, 5.7 million COVID-19 cases in the globe have been confirmed, and 357,688 deaths have occurred from COVID-19 disease (WHO, 2020). SARS-CoV-2 is the aetiological agent and responsible for a large-scale outbreak of fatal disease. Understanding the notable features of the SARS-CoV-2 genome in zoonotic origin and the evolutionary trend is of importance for revealing the intervention targets, and disease control and prevention.

Coronaviruses (CoVs) are the largest group of enveloped, positive-sense, single-stranded RNA viruses. Taxonomically, coronavirus SARS-CoV-2 belongs to Nidovirales order, Coronaviridae

family, Coronavirinae subfamily, and beta-CoV genus (Fehr and Perlman, 2015). SARS-CoV-2 is highly pathogenic with similar or lower pathogenicity as severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) in 2002–2003, and lower pathogenicity than Middle-East respiratory syndrome coronavirus (MERS-CoV) in 2012. Nevertheless, SARS-CoV-2 is highly transmissible in humans. To date, seven human CoVs (HCoVs) have been identified. Among them are alpha-CoVs, HCoV-229E, and HCoV-NL63. The remaining five beta-CoVs include HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2. Four common human CoVs, HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 usually cause mild symptoms, like the common cold and/or diarrhea (Su et al., 2016). In contrast, SARS-CoV/MERS-CoV, and current SARS-CoV-2 are particularly pathogenic, causing SARS/MERS and COVID-19, respectively.

Because of the resemblance of SARS-CoV-2 to SARS-like coronaviruses, bats are likely to act as natural reservoir hosts of the progenitor of SARS-CoV-2 (Andersen et al., 2020). It is established that SARS-CoV was transmitted from palm civet to humans (Wang et al., 2006) and MERS-CoV from dromedary camels to humans, yet the zoonotic origin of SARS-CoV-2 is still unclear. The genome sequence analysis shows that SARS-CoV-2 has the closest relative SARS-like SLCoV/RaTG13, found in horseshoe bat (*Rhinolophus affi-*

*Abbreviations:* ACE2, angiotensin converting enzyme 2; APOBEC, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like3; COVID-19, coronavirus disease 2019; CD, a congruence derivative; DMV, double-membrane vesicle; MERS, Middle-East respiratory syndrome; NDU, the normalized distribution uniformity; PAMPs, pathogen-associated molecular patterns; RIG-I, retinoic acid-inducible gene 1; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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nis) from Yunnan, China (Zhou et al., 2020). SARS-CoV-2 has 96.2% overall genome sequence identity to SARS-like coronavirus SLCoV/RaTG13 (Zhou et al., 2020). Therefore, the natural reservoir of SARS-CoV-2 could be the horseshoe bat. Recent evidence suggests pangolins as host candidates (Lam et al., 2020), however, the host pangolins are not firmly determined. Not all bat SARS-like CoV can infect humans. For example, bat coronavirus, swine acute diarrhoea syndrome coronavirus (SADS-CoV) found in 2017 (Zhou et al., 2018), caused millions of piglet deaths, but no human cases. We may postulate that SARS-CoV-2 evolved from its progenitor through mutations and evolutionary fitness in the host-shift and adapting. SARS-CoV-2 are fast-evolving pathogens that continuously undertake mutations in the generations of infection of the host (Yin, 2020; Wang et al., 2020; Korber et al., 2020). This fact suggests that SARS-CoV-2 had evolved mutations in critical proteins and genome structures prior to establishing human infection. To understand how SARS-CoV-2 jumps from animals to adaptively infect humans is important to the surveillance of virus evolution and diversity, therefore ultimately controlling the COVID-19 pandemic and preventing future SARS-like outbreaks.

SARS-CoV-2 is an enveloped positive-strand RNA virus, having an exceptionally long (29.9 kb) genome (Zhou et al., 2020). The genome consists of 5' leader cap sequence along with a 3' poly (A) tail, genes encoding non-structural proteins (nsps), and structural proteins, as well as several accessory proteins. Approximate two-thirds of the genome comprises two large overlapping open reading frames (ORF1a and ORF1ab), encoding polyproteins that are subsequently cleaved by viral proteases to generate 16 non-structural proteins (nsp1 to nsp16). Non-structural proteins are essential for RNA replication, transcriptions, and immune evasion. Nsp3, a large multi-domain and multi-functional protein, plays essential roles in virus replication. The papain-like protease (PLpro) activity of nsp3 is responsible for the initial processing of ORF1a protein. In addition, nsp3, together with nsp4 and nsp6, recruits intracellular membranes to form double-membrane vesicles (DMVs) to support viral RNA replication. Nsp5 is a second viral protease (3C-like protease, 3CLpro) that splits both ORF1a and ORF1ab proteins. The downstream regions of the genome encode structural proteins, the spike (S) protein, the nucleocapsid (N) protein, the envelope (E) protein, and the membrane (M) protein. The four structural proteins are all required to produce a structurally complete viral particle. The S protein mediates viral attachment to the ACE2 receptor host, and the subsequent fusion between the viral and host cell membranes enables the virus to enter host cells. The nucleocapsid (N) protein, one of the most abundant viral proteins, can bind to the RNA genome and participate in processes of replication, assembly, host cell response during viral infection (McBride et al., 2014).

The SARS-CoV-2 genome has been sequenced and annotated, but little is known for the complex structure of the genome in light of evolutionary fitness and host infectivity. Conventionally, RNA viruses use encoded proteins to interact with the components in cellular response. However, numerous discoveries show that the virus RNA structures, determined by genome composition may also play central roles in maximizing virus replication and evolutionary fitness (Jensen and Thomsen, 2012). For example, recent studies show that increased CG and TA dinucleotides in both coding and non-coding regions of echovirus 7 inhibit replication initiation during post-entry in several cell lines (Fros et al., 2017). Therefore, RNA viruses simulate host mRNA composition, for example, the dinucleotide frequencies (Fros et al., 2017). Animal genomes have a bias in their dinucleotide composition, and the heavy under-representation of CG and TA dinucleotides is especially well known. Most animal RNA and small DNA viruses suppress genomic CG and TA dinucleotide frequencies, apparently mimicking host mRNA composition (Di Giallonardo et al., 2017).

If a virus RNA composition or structure is very different from host mRNA, RIG-I (retinoic acid-inducible gene 1)-like receptors may detect RNA molecules that are absent from the uninfected host (Goubau et al., 2013). Detecting evolutionary microbial structures known as pathogen-associated molecular patterns (PAMPs) is an important feature of the innate immune system. Host-cells possess intrinsic defense pathways that prevent replication of viruses with increased CG and TA frequencies in mechanisms independent of codon usage (Belalov and Lukashev, 2013).

The genomic spectrum demonstrates dinucleotide, trinucleotide, and multi-nucleotide distributions. The dinucleotide distributions are often considered as the signature of a genome (Karlin and Burge, 1995) and the periodicity-3 patterns are distinguishing characteristics for the protein-coding regions (Tsonis et al., 1991). The strengths of the periodicity-2 and periodicity-3 in a genome are determined by the perfect levels and copy numbers of dinucleotide and trinucleotide repeats, respectively. Because latent periodicity-2 and periodicity-3 are the essential characteristics of a genome, in this study, we only examine these two periodicities from the genomic spectrum when analyzing the genome. Note that this study is to investigate the latent periodicity-2 in SARS-CoV genomes, rather than dinucleotide repeats. Especially, the long enough stretches with tandem dinucleotide repeats are absent in viral genomes. The generation of latent periodicity-2 does not necessarily need the occurrence of perfect dinucleotide repeats.

In this study, we present the genomic spectrum of SARS-CoV-2 and identify predominated latent periodicity-2 in ORF1a (nsps 3-6) that have been instrumental in interacting host immune systems and pathogenicity and host infectivity. These latent periodicity-2 regions are essential to the survival and infectivity of the microbe and can be considered as one of PAMPs in SARS-CoV-2. These genomic elements are evidence of the evolutionary fitness of SARS-CoV-2 in host-shift and adaptation. Tracking the evolution of these elements may provide insights into the zoonotic origin of SARS-CoV-2 and the control of COVID-19 disease. In addition, the critical regions of ORF1a (nsps 3-6) that have elevated periodicity-2 can be key pharmaceutical targets and possibly the attenuating virus structure that is for vaccine development.

## 2. Concepts and methods

To inspect the insightful traits of the SARS-CoV-2 genome, we utilize our periodicity analysis method to survey the nucleotide periodic distributions and the rendered periodicities in the genome. We previously proposed the periodicity analysis method to quantitatively detect the nucleotide repeats and periodicities in a genome (Yin, 2017). The method employs nucleotide distributions on periodic positions in a genome and identifies approximate repeat structures as the signatures of the genome. Because we have included more functionalities, such as smoothing the periodicity profile, from the original method, here we describe the method in detail though the technical algorithms had been delineated previously (Yin, 2017). Our computer programs of the periodicity analysis of a genome are available to the public at GitHub repository <https://github.com/cyinbox/DNADU>.

### 2.1. Periodic nucleotide frequencies in a DNA sequence

The approximate tandem repeats and the rendered periodicity- $p$  in a genome can be identified by counting the nucleotides of the same types over the positions corresponding to some integer period  $p$ . To formulate the strength of a periodicity, we may construct four numerical vectors for four nucleotides. The elements in each vector are the counts of the corresponding nucleotide on the peri-

odic positions. The four numerical vectors of nucleotides are named a congruence derivative (CD) vectors (Yin and Wang, 2016; Yin, 2017). The CD vector of a nucleotide for a specific periodicity is constructed by the cumulative frequencies of the nucleotide at these periodic positions (Definition 2.1).

**Definition 2.1.** For a DNA sequence of length  $n$ , let  $u_\alpha(k) = 1$  when the nucleotide  $\alpha$  appears at position  $k$ , otherwise,  $u_\alpha(k) = 0$ , where  $\alpha \in \{A, T, C, G\}$  and  $k = 1, \dots, n$ . The congruence derivative vector of the nucleotide  $\alpha$  of the DNA sequence for periodicity  $p$ , is defined as

$$f_{\alpha,j} = \sum_{\text{mod}(k,p)=j} u_\alpha(k) \quad (1)$$

$$j = 1, \dots, p, k = 1, \dots, n,$$

where  $\text{mod}(k, p)$  is the modulo operation and returns the remainder after division of  $k$  by  $p$ , and  $f_\alpha = (f_\alpha(1), f_\alpha(2), \dots, f_\alpha(p))$ .

Four congruence derivative vectors  $f_\alpha$  of periodicity  $p$  for nucleotides A, T, C and G form a congruence derivative (CD) matrix of size  $4 \times p$ . The columns of the CD matrix indicate nucleotide frequencies at the periodic positions  $k = pt - q$ , where  $k$  is the position index of a DNA sequence,  $t = 1, 2, \dots$ , and  $q = p - 1, \dots, 2, 1, 0$ . For example, consider the CD matrix of periodicity 5 for DNA sequence, the first column of the CD matrix shows the nucleotide frequencies at periodic positions  $k = 1, 6, 11, \dots, 5t - 4$ ; the second column of the matrix shows the nucleotide frequencies at periodic positions  $k = 2, 7, 12, \dots, 5t - 3$ ; the third column of the matrix shows the nucleotide distributions at periodic positions  $k = 3, 8, 13, \dots, 5t - 2$ , and so on. The CD matrix of a DNA sequence describes nucleotide frequencies at all periodic positions and can be used to efficiently compute the Fourier power spectrum and determine periodicities in the DNA sequence (Yin and Wang, 2016). Therefore, the CD vector reflects the arrangement of repetitive sequence elements and intrinsic periodicities in the DNA sequence.

## 2.2. The normalized distribution uniformity (NDU) of a DNA sequence

From the nucleotide frequencies on periodic positions, the periodicity strength can then be calculated from the statistical properties of the nucleotide frequencies over the periodic positions. Since the CD matrix contains the nucleotide frequencies on periodic positions, the variance of the matrix elements can measure the nucleotide distribution. For the CD matrix of periodicity  $p$ , the summation of  $4p$  elements of the matrix is equal to the length  $n$  of the DNA sequence, and the mean of the elements of the CD matrix is  $\frac{n}{4p}$ . Therefore, to quantify the nucleotide distribution, we define the normalized distribution uniformity (NDU) of a DNA sequence using the CD matrix (Definition 3.2).

**Definition 2.2.** For a DNA sequence of length  $n$ , let  $f_{ij}$  be an element of the CD matrix of periodicity  $p$ , the normalized distribution uniformity of periodicity  $p$  of the DNA sequence is defined as

$$NDU(p) = \frac{1}{n} \sum_{i=1}^4 \sum_{j=1}^p \left( f_{ij} - \frac{n}{4p} \right)^2 \quad (2)$$

From Definition 2.2., we notice that the normalized distribution uniformity at periodicity  $p$  is an intuitive description for the level of unbalance of nucleotide frequencies on periodic positions. It depends on the quadratic function of the nucleotide frequencies, sequence, and periodicity length.  $NDU(p)$  can be used to indicate

the presence and intensity of the periodicity  $p$  in a DNA sequence. This method offers an elaboration of the repetitive elements such as the repeat consensus, copy number, and the perfect level (Yin, 2017).

When using a sliding window along a genome, the periodicities of 2 to 10 are calculated in each window segment. The sliding window length is 250 bp with one base step size. Therefore, a two-dimension periodicity spectrum is formed for the genome. The two-dimension spectrum of a genome can be considered as the signature or the barcode of the genome.

The nucleotide frequencies over the phased positions are impacted by indels because indels change the phases. To mitigate the impact of indels on the periodicity- $p$  magnitude, we insert  $0, 1, \dots, p - 1$  zeros into even divided points of a DNA sequence and compute the periodicity- $p$  from these sequences. The periodicity magnitude is the maximum of the obtained periodicity magnitudes from all the sequence with insertions. Because of the computational complexity for long periodicities, this indels mitigation approach is only used when computing the periodicity-2 and periodicity-3 in the SARS-CoV-2 isolates from the COVID-19 pandemic.

## 2.3. Noise filtering and peaks detection

To locate the positions of a repeat region in a genome, we smooth and filter the corresponding sliding-window periodicity by moving average convolution (De Jong, 1989). Then the peaks in the periodicity profile are detected using the Z-score algorithm (Brakel, 2020). The peak positions are used to demarcate repeats in a genome. Note that in addition to the proposed NDU method, the exact locations and the sequence patterns of repeats in a genome can be further depicted using RepeatMasker (Tarailo-Graovac and Chen, 2009) or Tandem Repeats Finder (Tarailo-Graovac and Chen, 2009).

In a nutshell, to compute distribution uniformities of different periodicities of a DNA sequence, we first scan the sequence in different periodicity sizes, construct the congruence derivative matrix of each periodicity, and compute the  $NDU(p)$  of these periodicities  $p$ . The periodicity with the maximum distribution uniformity reflects the predominant pattern of repetitive elements. The  $NDU$  values of periodicities indicate the perfect levels, and copy numbers of corresponding repeat regions.

## 2.4. Genome data

This study depends on the complete genomes of coronaviruses, including SARS-CoV-2 (Wu et al., 2020), Severe Acute Respiratory Syndrome (SARS) related coronavirus, Middle East Respiratory Syndrome coronavirus (MERS-CoV), and human infection coronaviruses (human-CoVs). These genome data are retrieved from the National Center for Biotechnology Information (NCBI) Gene Bank. The bat SLCoV/RaTG13 complete sequence was reported by (Zhou et al., 2020), and retrieved from the GISAID repository (<http://www.GISAID.org>) (Shu and McCauley, 2017). 3479 complete genomes of the SARS-CoV-2 isolates in the globe from Jan. to Dec. 2020 are randomly downloaded. The complete SARS-CoV-2 genomes satisfy the following conditions: The genome sequences have no uncertain nucleotides N, and the genome lengths are full according to the reference genome. The genome data in this study are listed and acknowledged in the [supplementary material](#).

### 3. Results and analysis

#### 3.1. Genomic spectrum coronavirus SARS-CoV-2 reveals rich periodicity-2 pattern in *nsp3*

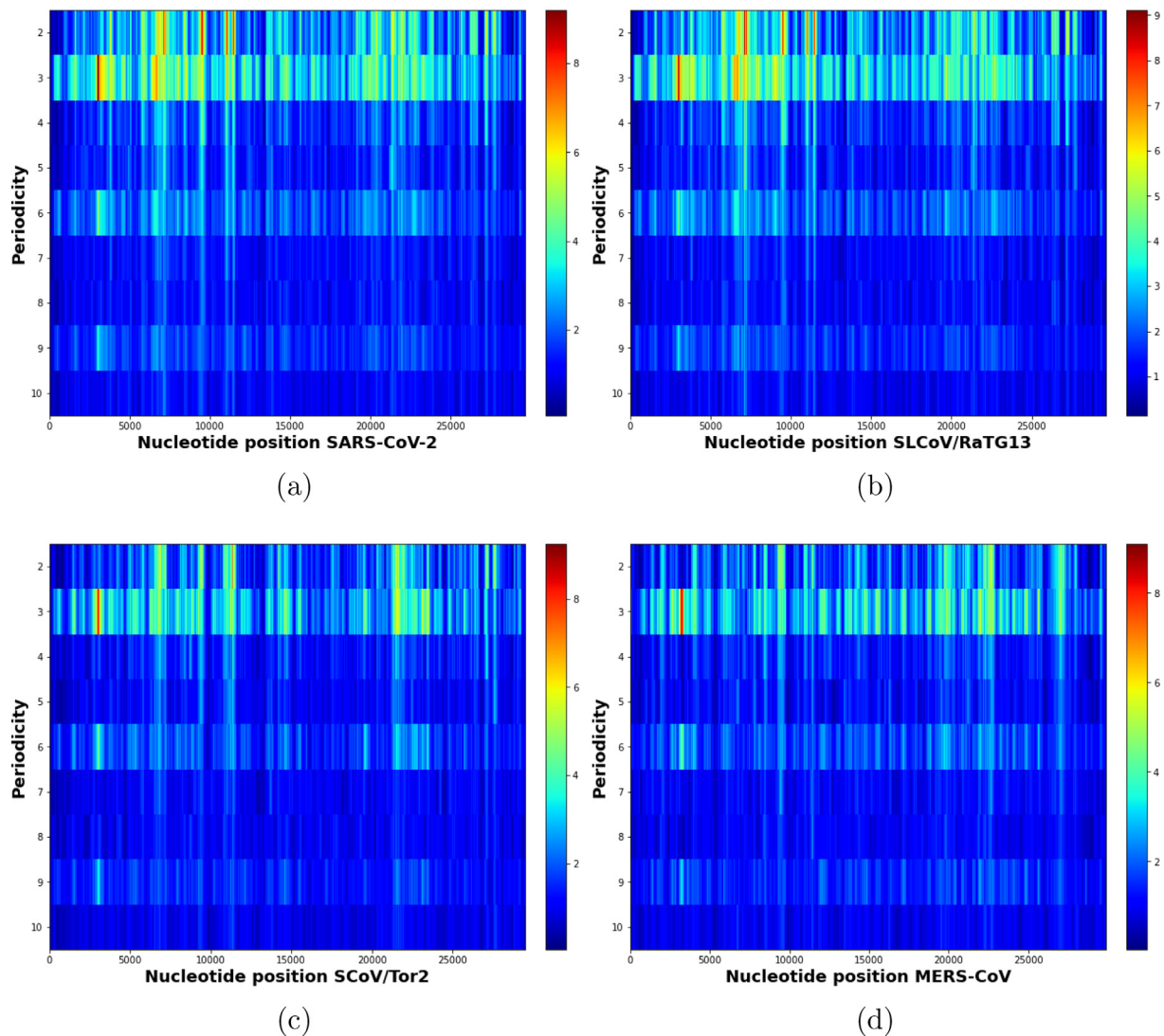
To identify the signature features of the coronavirus SARS-CoV-2 genome, we employ the periodicity spectrum analysis to identify the characteristic periodicities in the genome. We create the genomic spectrum (barcode) of SARS-CoV-2 (Fig. 1(a)) using the sliding window NDU method and compare it with the counterparts of SLCoV/RaTG13 (Fig. 1(b)), SARS-CoV/Tor2 (Fig. 1(c)), and MERS-CoV (Fig. 1(d)). From the spectrum comparison, we observe that SARS-CoV-2 and SLCoV/RaTG13 both have pronounced periodicity-2 in four regions while both SARS-CoV/Tor2 and MERS-CoV only have an extremely low level of periodicity-2 in the corresponding regions. The strong periodicity-2 in SARS-CoV-2 encouraged us to investigate the causes in detail.

To locate the regions of rich periodicity-2 motifs, we verify that periodicity-2 and periodicity-3 are strong signals among all genomic periodicities (Fig. 2(a, d)), and detect the peaks of the sliding-window periodicity profiles (Fig. 2(b, c)). The peak positions are used to demarcate the dinucleotide motif regions in the genome. The dinucleotide motif regions (dinucleotide TN islands, N is A,T,

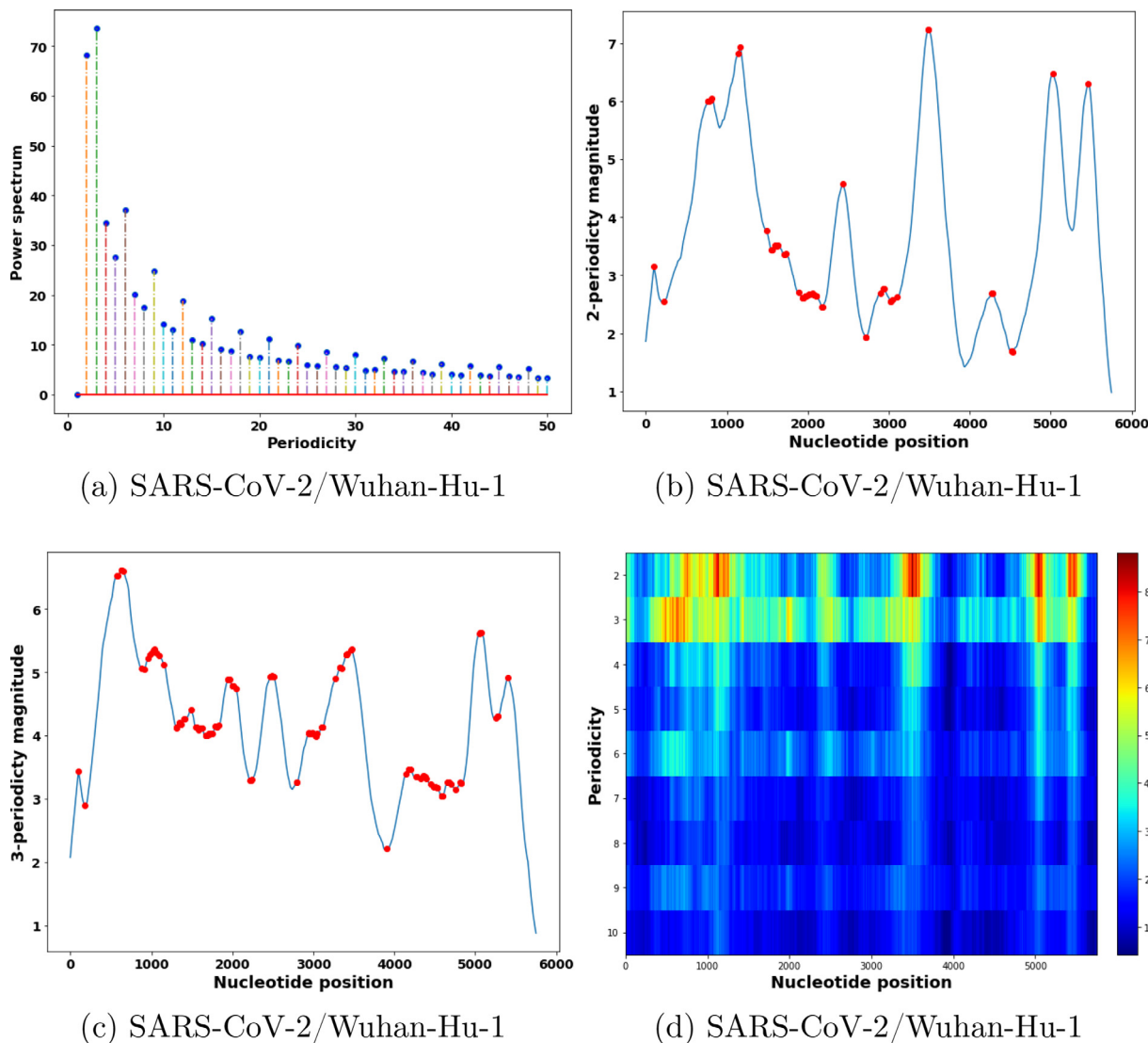
C or G) are in ORF1a and the corresponding genes are listed in Table 1.

From the genomic spectrum analysis, the relative abundance of the dinucleotide motifs, particularly dinucleotide motif TN regions are mapped to the genes of ORF1a (*nsp3*, *nsp4*, and *nsp6*) (Fig. 3 and Table 1.). However, these dinucleotide motifs are weak or imperceptible in the corresponding regions in the SARS-CoV and MERS-CoV genomes.

In the coronavirus SARS-CoV-2 RNA genome, the gene for replicase of 20 kb encodes two overlapping polyproteins, ORF1a (replicase 1a) and ORF1ab (replicase 1ab). The genome structure and the corresponding dinucleotide regions identified are illustrated in Fig. 3. The two polyproteins are responsible for viral replication and transcription (Chen et al., 2020). The expression of the C-proximal portion of pp1ab requires (-1) ribosomal frame-shifting. The first dinucleotide motifs are in the coding region of Papain-like proteinase (PL proteinase, non-structural protein 3, *nsp3*). *Nsp3* is the largest essential component of the replication and transcription complex. The PL proteinase in *nsp3* cleaves *nsp3* 1-3 and blocks host innate immune response, promoting cytokine expression (Lei et al., 2018; Serrano et al., 2009). The second dinucleotide repeat is in the coding region of non-structural protein 4 (*nsp4*). *Nsp4* is responsible for forming double-membrane vesicles



**Fig. 1.** Genomic spectra of SARS-CoV-2 and SARS-related CoVs (SLoVs). (a) SARS-CoV-2. (b) SLCoV/RaTG13. (c) SARS-CoV/Tor2. (d) MERS-CoV. The sliding window is 250 bp.



**Fig. 2.** Genomic spectra of the region 6 kb–12 kb of the SARS-CoV-2 genome (GenBank:NC\_0455127). (a) The periodicity magnitudes. (b) The periodicity-2 spectrum of 250 bp sliding windows. (c) The periodicity-3 spectrum of 250 bp sliding windows. (d) The genomic spectra of 250 bp sliding windows.

**Table 1**  
The dinucleotide motifs TN in SARS-CoV-2/Wuhan–Hu-1 genome (GenBank:NC\_045512).

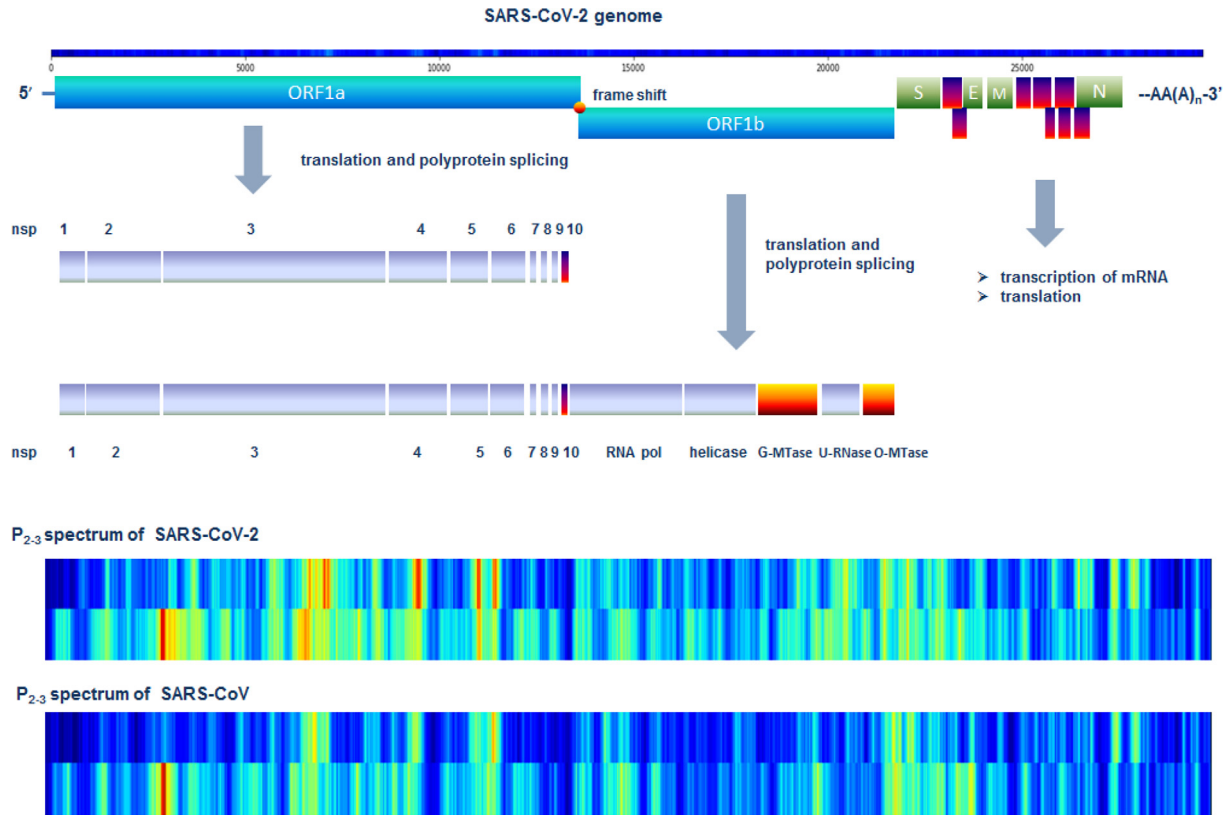
Region	Location	Periodicity-2	Perfection level	Protein
sub-region 1	6227:7886	6.9320	0.3599	nsp3
sub-region 2	9101:10273	7.2362	0.3696	nsp4
sub-region 3	10850:12000	6.4728	0.3783	3CLPro and nsp6

(DMV). The third dinucleotide motif is in the coding region of the C-terminal 3CLPro protease (3 chymotrypsin-like proteinase, 3CLpro) and nsp6. 3CLPro protease is essential for RNA replication. The 3CLPro proteinase is responsible for processing the C-terminus of nsp4 through nsp16 for all coronaviruses (Anand et al., 2003). Therefore, conserved structure and catalytic sites of 3CLpro may serve as attractive targets for antiviral drugs (Kim et al., 2012). Together, nsp3, nsp4, and nsp6 can induce DMV (Angelini et al., 2013).

In summary, the high latent periodicity-2 islands found in this study are located in the host-interaction regions of the genome

of SARS-CoV-2. These special periodicity-2 regions in ORF1a most likely contribute to the adaptive immune response, therefore, implying evolution fitness.

Coincidentally, previous work on MERS-CoV using co-evolution analysis revealed that nsp3 represents a preferential selection target in adaptive evolution for zoonotic MERS-CoV to a new host (Forni et al., 2016). Our finding that nsp3 is involved in evolution fitness is consistent with the discovery in MERS-CoV. We investigate the correlation of dinucleotides AA and TA contents in SARS-CoV genomes and virulence. We examine the dinucleotide in the genomic regions (6 kb–12 kb). The increased periodicity-2 in the



**Fig. 3.** Paradigm of dinucleotide motifs in the SARS-CoV-2 genome organization. The paradigm is drawn according to the reference genomes of SARS-CoV-2 (NC\_045512) and SARS-CoV/Tor2 (NC\_004718).

genomic regions of SARS-CoV-2 and SARS-like CoVs are the results of the unbalanced distributions of dinucleotides.

**3.2. Dinucleotide repeats in major SARS-like coronaviruses (SLCoVs) in evolution**

To understand the evolutionary tendency of coronavirus genomes, we examine the genomic spectra of four major bats SARS-like coronaviruses (SLCoVs), pangolin-SLCoV, SLCoV/ZXC21, SLCoV/WIV1, and SLCoV/Shaanxi2011, all of which naturally live in bat *Rhinolophidae horseshoe*. Because pangolin-SLCoV was found similar to SARS-CoV-2, pangolin was exploratorily postulated as an intermediate animal host of SARS-CoV-2. SLCoV/ZXC21 is the second similar strain to SARS-CoV-2, with 82% similarity (Hu et al., 2018). SLCoV/WIV1 was closely related to SARS-CoV/Tor2 in terms of genome identity and ACE2 binding in human cells (Ge et al., 2013). SLCoV/Shaanxi2011 was found in 2011 (Yang et al., 2013).

The results show that pangolin-SLCoV displays three major latent periodicity-2 regions in nsp3 and nsp4, but lacks the corresponding dinucleotide repeats in 3CLPro and nsp6 as found in SARS-CoV-2 (Fig. 4(a)). So SARS-CoV-2 is mostly closed to SLCoV/RaTG13 (Fig. 1(b)) and SLCoV/ZXC21 (Fig. 4(b)), not pangolin-SLCoV. If pangolins are the intermediary hosts of SARS-CoV-2, and SARS-CoV-2 was indeed evolved from pangolin-SLCoV, we may infer that pangolin-SLCoV would need to evolve dinucleotide repeats in 3CLPro and nsp6 during evolution fitness before infecting human hosts.

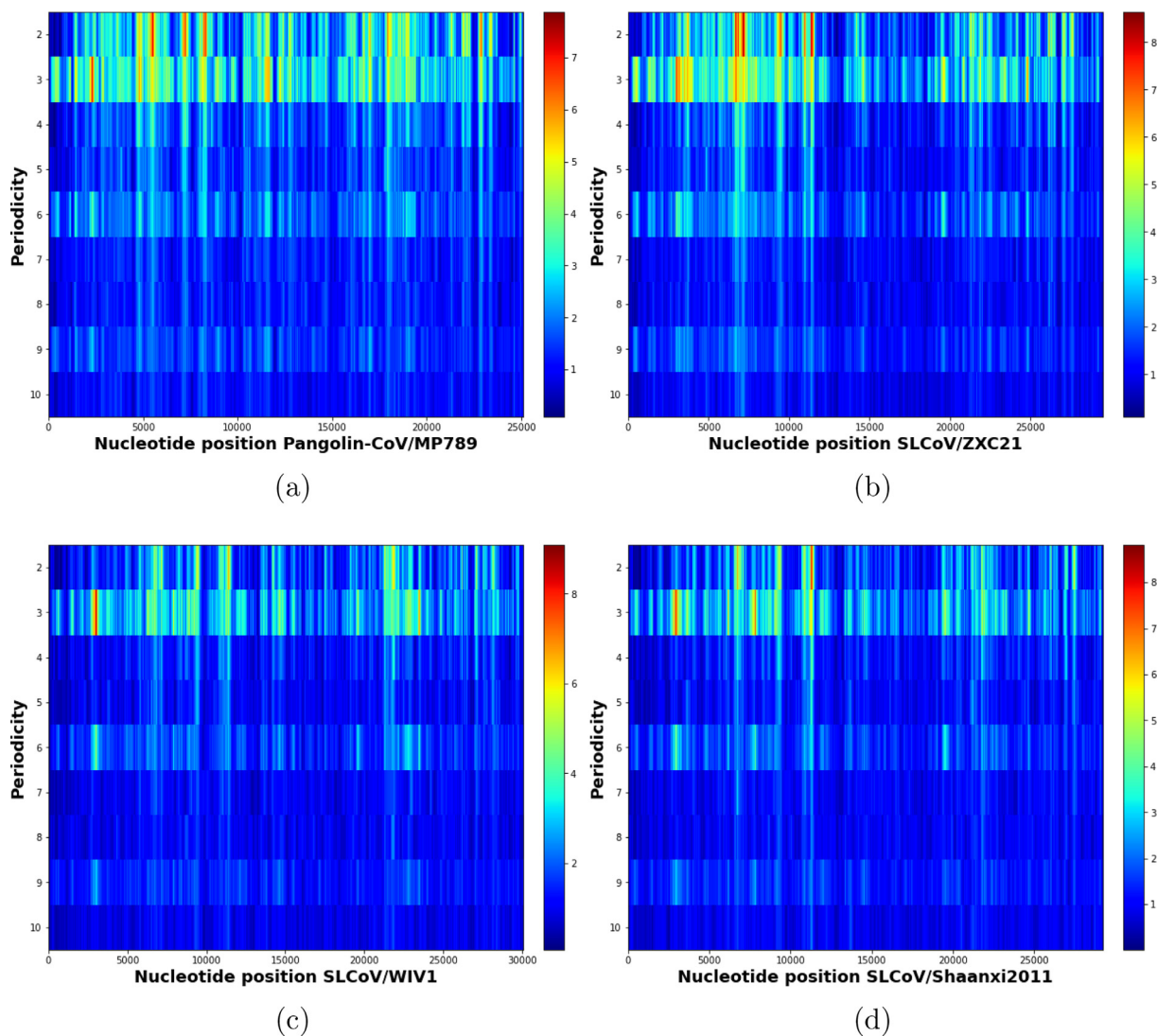
We also observed the evolution trend between SLCoV/WIV1 (Fig. 4(c)) and SLCoV/Shaanxi2011 (Fig. 4(d)). The genomic spectrum of SLCoV/Shaanxi2011 is similar to SLCoV/WIV1, but has additionally increased dinucleotide repeat in 3CLPro and nsp6. This new dinucleotide repeat in the region 3CLPro and nsp6 in SLCoV/Shaanxi2011 is consistent with the regions found in SARS-CoV-2,

SLCoV/RaTG13, and SLCoV/ZXC21. Therefore, the high latent periodicity-2 regions 3CLPro and nsp6 probably play an important role in the evolutionary fitness of SARS-CoV-2 to the human hosts.

The results show that only SARS-CoV and SARS-like CoVs have low latent periodicity-2. The low-dinucleotide contents can be considered as in early evolution fitness when interacting with the human immune system, then low-dinucleotides may render high virus virulence because the virus has not adapted to the host immune system, and the host immune system acts intensely.

**3.3. Trend of latent periodicity-2 in human coronaviruses during evolution**

As we have seen that latent periodicity-2 is the special signal in CoV genomes and is caused by unbalanced dinucleotide motif contents. To investigate the correlation of dinucleotide contents and pathogenicity of coronaviruses, we produce and compare the genomic spectra of four common human coronaviruses (Fig. 5). Classical human coronavirus 229E (HCoV-229E) and human coronavirus OC43 (HCoV-OC43) were identified in 2004. The two viruses are close relatives, and the virus characteristics are similar to human pathogenicity. Both HCoV-229E and HCoV-OC43 can cause young children and the elderly and have a low immune function. Almost 100% of children are infected in early childhood, mainly as self-limiting upper respiratory infections, such as the common cold and intestinal infections Symptoms caused by HCoV-OC43 strain are generally more severe than those of HCoV-229E virus. From the genomic spectrum analysis, we observe higher periodicity-2 in HCoV-229E than in HCoV-OC43 (Fig. 5(a,c)). These periodicity-2 rich regions correspond to the three sub-regions in SARS-CoV-2. High periodicity-2 value may attenuate the virus replication and therefore reduce severe virulence. That is in an agreement



**Fig. 4.** Genomic spectra of SARS-like CoVs (SLCoVs). (a) pangolin-SLCoV. (b) SLCoV/ZXC21. (c) SLCoV/WIV1. (d) SLCoV/Shaanxi2011. The sliding window is 250 bp.

with the correlation of dinucleotide motif contents and pathogenicity previously.

The spectra of HCoV-NL63 and HCoV-HKU1 demonstrate extremely high periodicity-2, as well as periodicity-3 in the corresponding regions (Fig. 5(b,d)). HCoV-NL63 and HCoV-HKU1 are the most common human CoVs that cause only a mild cold symptom or no symptom (Pyrce et al., 2007). Again, these high dinucleotide repeats may contribute to the light pathogenicity in these two viruses.

### 3.4. Dinucleotide frequencies of SARS-CoV-2 during evolution

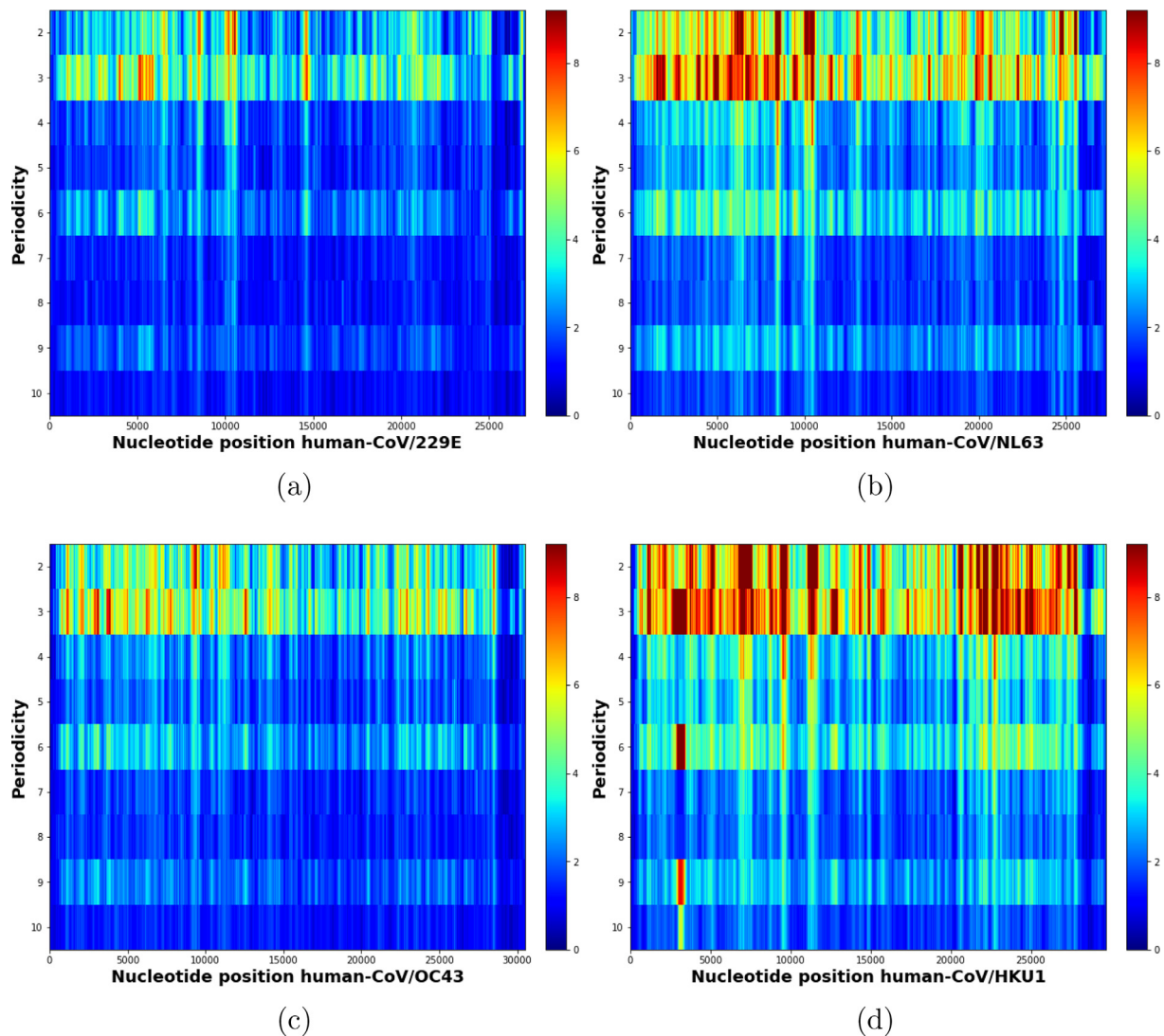
We wish to know whether SARS-CoV-2 originally evolved from SARS-CoV, or SARS-CoV-2 would evolve to SARS-CoV. The answer to this question may help us to predict the evolution of SARS-CoV-2 virus for better disease prediction and control. Because the genomes of SARS-related coronaviruses over a long time period are rarely available, to infer the evolution of coronaviruses, we track the trend of HCoV-229E coronaviruses over the last six decades from the first human infected HCoV-229E identified in 1962 (Thiel et al., 2001). HCoV-229E virus causes common cold but occasionally it can be associated with more severe respiratory infections in children, elderly, and persons with underlying illness.

Using the measurements of dinucleotides in the genomic regions, the trend of HCoV-229 coronaviruses may infer the evolutionary stages of bat coronaviruses. In a similar method, we may then determine the origin SARS-CoV-2 if the trend of SARS-CoV-2 is compared with SARS-like coronaviruses.

The human coronavirus HCoV-229E strains used in the dinucleotide trend analysis are from different historical periods. The reference genome HCoV-229 in the evolutionary analysis was obtained from the infectious HCoV-229E, the 1973-deposited laboratory-adapted prototype strain of HCoV-229E (VR-740). The HCoV-229E prototype strain was originally isolated in 1962 from a patient in Chicago. The first clinical HCoV-229E isolate from a US patient in 2012 was included in this study (Farsani et al., 2012) (GenBank: JX503060). HCoV-229E (SC3112) isolate in 2015 is included (GenBank: KY983587). The new Human coronavirus strain HCoV-229E was isolated from plasma collected from a Haitian child in 2016 (Bonny et al., 2017) (GenBank: MF542265).

The result in the periodicity trends of the HCoV-229E coronaviruses demonstrates that both periodicity-2 (Fig. 6(a)) and periodicity-3 (Fig. 6(b)) in the coronaviruses are increasing with evolutionary time. SARS-CoV-2 has relatively high periodicity-2 and periodicity-3. This result suggests that the trends of





**Fig. 5.** Genomic spectra of common human coronaviruses. (a) HCoV-229E. (b) HCoV-NL63. (c) HCoV-OC43. (d) HCoV-HKU1. The sliding window is 250 bp.

periodicity-2 and periodicity-3 are increasing with time. The evolutionary origin of coronaviruses can be inferred by the trends of periodicity-2 and periodicity-3. Therefore, we may compare the periodicity-2 and periodicity-3 in SARS-CoV-2 and SARS-like coronaviruses to understand the evolutionary origin of SARS-CoV-2.

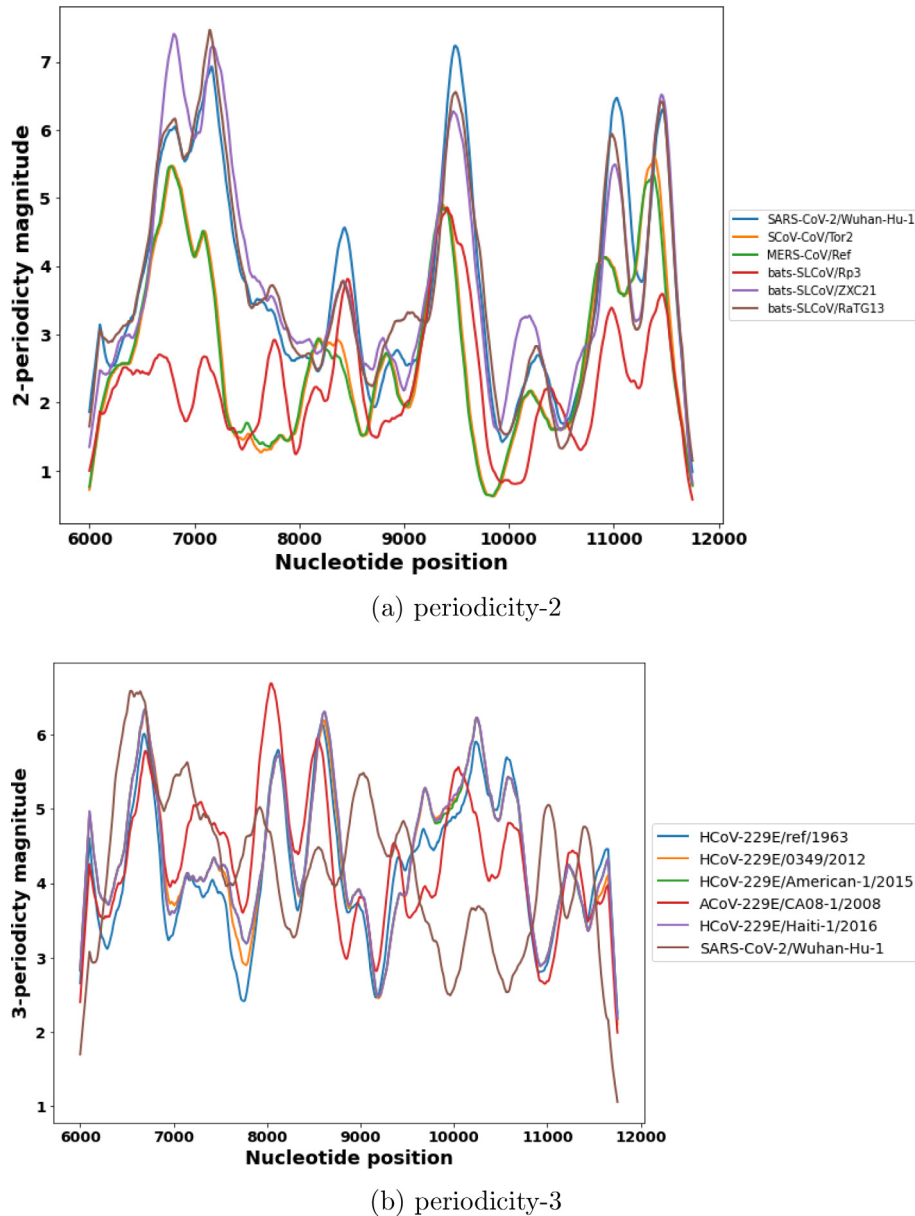
To investigate the correlation of dinucleotide repeats with virus virulence in SARS-related coronaviruses, we compare the spectra of the genomic region at coordinates 6k–12k bp of five SARS-related coronaviruses. The genomic regions contain abundant dinucleotide repeats. The region 6k–12k of the genome contains three dinucleotide repeats approximately located at 6k–8k, 8k–10k, and 10k–12k sub-regions. The five SARS-related coronaviruses have different levels of virulence. The most virulent virus is MERS-CoV, followed by SARS-CoV. The periodicity-2 magnitudes, which reflect the distribution of dinucleotides, are compared and shown in (Fig. 7(a,b)). We may see that MERS-CoV genomic region has the lowest dinucleotide level in all three dinucleotide sub-regions. SARS-CoV also has a low dinucleotide level but is higher than MERS-CoV. Fig. 8 also shows the depleted CG and GC contents in SARS-CoV-2 genome, which is consistent with recent study (Xia, 2020). We, therefore, may infer that the low dinucleotide level correlates with high virulence. The lower the dinucleotide level is, the higher virus virulence is. This postulation is supported by the

observation of the dinucleotide in three SARS-related coronaviruses. Compared with the SARS-CoV, the SARS-like bats-SLOV/Rp3 has similar dinucleotide distributions in sub-regions 1 (6k–8k) and 3 (10k–12k), but higher dinucleotide distribution in sub-region 2 (8k–10k). SLCoV/Rp3 has lower virus virulence than SARS. The SARS-like SLCoV/ZXC21, which shares the highest sequence identity with SARS-CoV-2, shows slightly lower dinucleotide distributions in the two sub-regions 2 and 3, and slightly higher dinucleotide distribution than sub-region 1.

We notice that trends of the periodicity-2 and periodicity-3 from MERS-CoV, SARS-CoV, SARS-like CoVs, and SARS-CoV-2 are increasing (Fig. 8 (a,b)). Based on the previous analysis of the periodicity trends of coronaviruses at different times, we may infer that SARS-CoV-2 originates from the SARS-like CoVs.

### 3.5. The dynamics of latent periodicity-2 in SARS-CoV-2 genome during evolution

It is well studied that dinucleotide composition imbalance in RNA viruses may impact the virus replications, specifically, attenuating or strengthening virus virulence during evolution (Fros et al., 2017; Gu et al., 2019). Clinical evidences have suggested that SARS-CoV-2 has lower virulence than SARS-CoV. To investigate if



**Fig. 6.** Comparison of periodicities in genomic regions (6k–12k) of human HCoV-229E coronaviruses. (a) periodicity-2. (b) periodicity-3.

the increased dinucleotide repeats correlate with virus virulence, we compare the dinucleotide frequencies in the three dinucleotide motif rich islands of SARS-CoV-2 and SARS-CoV/Tor2.

The result shows that both SARS-CoV-2 and SARS-CoV have an abundance of dinucleotides TT and TA in the whole genome, and three prominent dinucleotide motif regions (Fig. 8(a)–(d)), and extreme CG deficiency in sub-region 3 (Fig. 8(d)). The dinucleotides TT and TA are increased in SARS-CoV-2 compared with SARS-CoV (Fig. 8(a)–(d)).

The role of increased dinucleotides TT and TA in the SARS-CoV-2 genome is to possibly attenuate virus replication. One mechanism for attenuating virus by dinucleotide bias is that the dinucleotide regions fold a special structure as a target for cell RNA cleavage, which is a fundamental host response for controlling viral infections (Zhou et al., 1993). The 2,5-oligoadenylate synthetase/RNase L system is an innate immunity pathway that responds to a pathogen-associated molecular pattern (PAMP) to induce degradation of viral and cellular RNAs, thereby blocking viral infection. In higher vertebrates, this process is often regulated by interferons

(IFNs). Ribonuclease L (RNase L, L is for latent) is an interferon (IFN)-induced antiviral ribonuclease which, upon activation, destroys all RNA within the cellular and viral (Silverman, 2007). RNase L cleaves hepatitis C virus (HCV) RNA at single-stranded TT and TA dinucleotides throughout the open reading frame (ORF). An interesting discovery is that in bacterium *Mycoplasma pneumoniae*, which is a respiratory infection agent, the genomes also have relative abundance extremes dinucleotides TT and TA (Karlin, 1998). Therefore, we may postulate that the dinucleotides TT and TA regions in SARS-CoV-2 are possibly cleaved by RNase L during infection.

### 3.6. Increases in periodicities in SARS-CoV-2 genomes during the COVID-19 pandemic

During the ongoing COVID-19 pandemic in the year 2020, large numbers of SARS-CoV-2 isolate genomes with transmission dates from the globe have been accumulated in the GISAID database. To gather additional evidence supporting our hypothesis that the

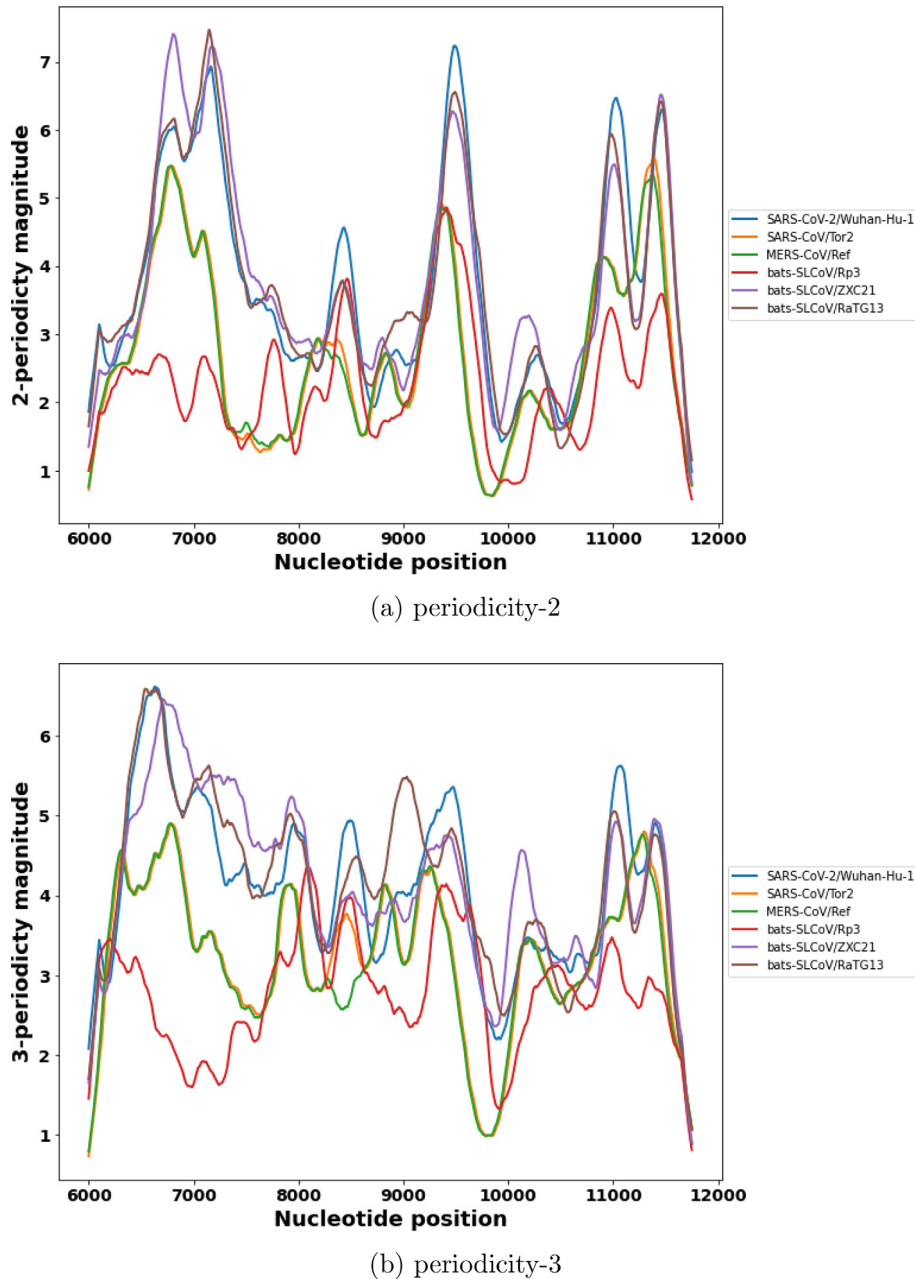


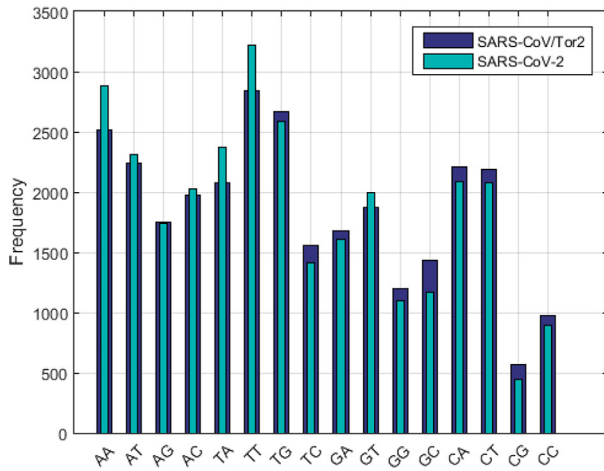
Fig. 7. Comparison of periodicities in the genomic regions (6 kb–12 kb) of SARS-CoV and SARS-like coronaviruses. (a) periodicity-2. (b) periodicity-3.

latent periodicity-2 in SARS-CoV-2 is increased during human infection, we measure the latent periodicities 2 and 3 in SARS-CoV-2 genomes during the COVID-19 pandemic (Fig. 9). The high-quality SARS-CoV-2 genomes are randomly selected from the global in each month of 2020. The results show that in the infection periods from Jan. to Sept./2020, the SARS-CoV-2 population has been increasing latent periodicity-2 (Fig. 9(a)) and periodicity-3 (Fig. 9(b)) in the genome variants. Notably, the periodicity-2 and periodicity-3 in the period Oct.–Dec./2020 are slightly lower than Jul.–Sept./2020 (Fig. 9). The decreases of periodicities 2 and 3 of the SARS-CoV-2 genomes in Oct.–Dec./2020 can probably explain the virulence increase in the second wave of the global COVID-19 pandemic in Oct.–Dec./2020. Therefore, these results further validate our hypothesis that the latent periodicity-2 and periodicity-3 in an RNA virus are increasing during human infection. The changes of periodicity magnitude can be

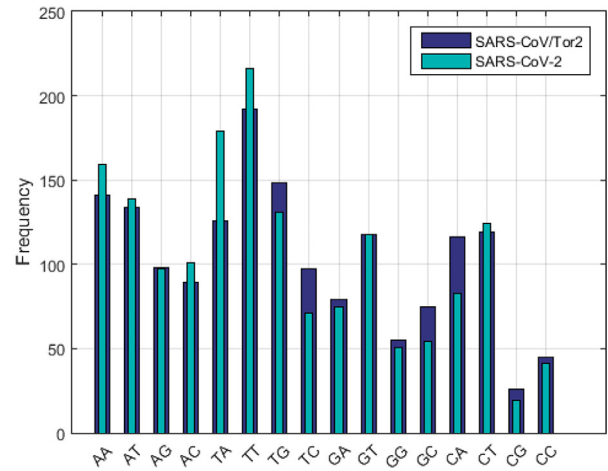
global indicators of the genome in tracking virus evolution. Nevertheless, further analysis of the dynamics of periodicities 2 and 3 in different geographic regions and clinical outcomes will be needed to understand the correlation of the periodicities and SARS-CoV-2 virulence during the infection and evolution.

#### 4. Discussion and perspectives

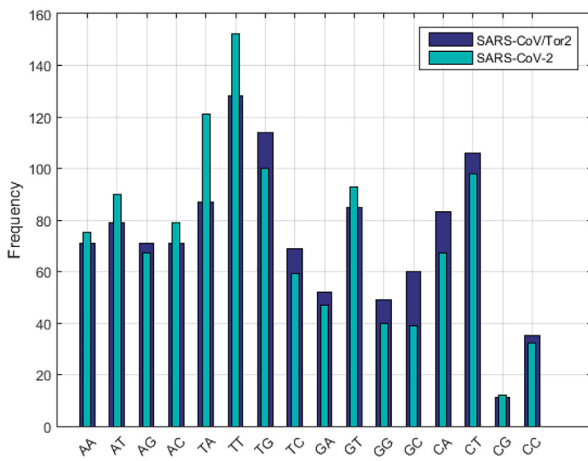
In this study, we investigate the latent periodicity-2 and periodicity-3 which are caused by approximate tandem repeats in the SARS-CoV-2 genome. The approximate dinucleotide motifs in the genomic spectra are revealed by the periodicity analysis. We discover that the strength of these repeats correlates with the evolutionary fitness of the virus to a human host for maximizing its survival in epidemics, instead of destroying the host. Therefore, RNA viruses simulate host mRNA composition such as the



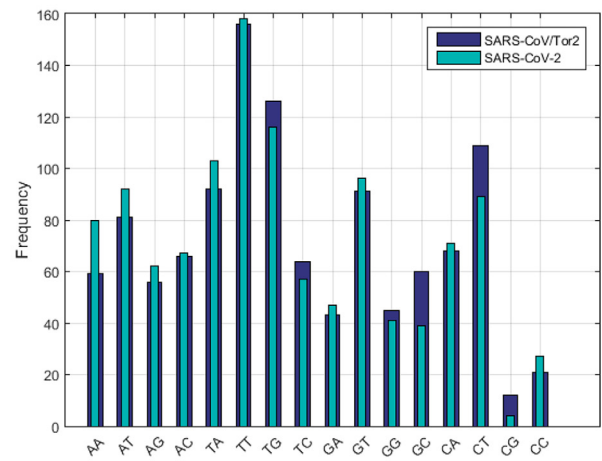
(a) whole genome



(b) sub-region 1



(c) sub-region 2



(d) sub-region 3

**Fig. 8.** The dinucleotide frequency distributions in the three genomic regions of SARS-CoV-2 and SARS-CoV/Tor2. (a) whole genome, (b) sub-region 1. (c) sub-region 2. (d) sub-region 3. The sub-regions are listed in Table 1.

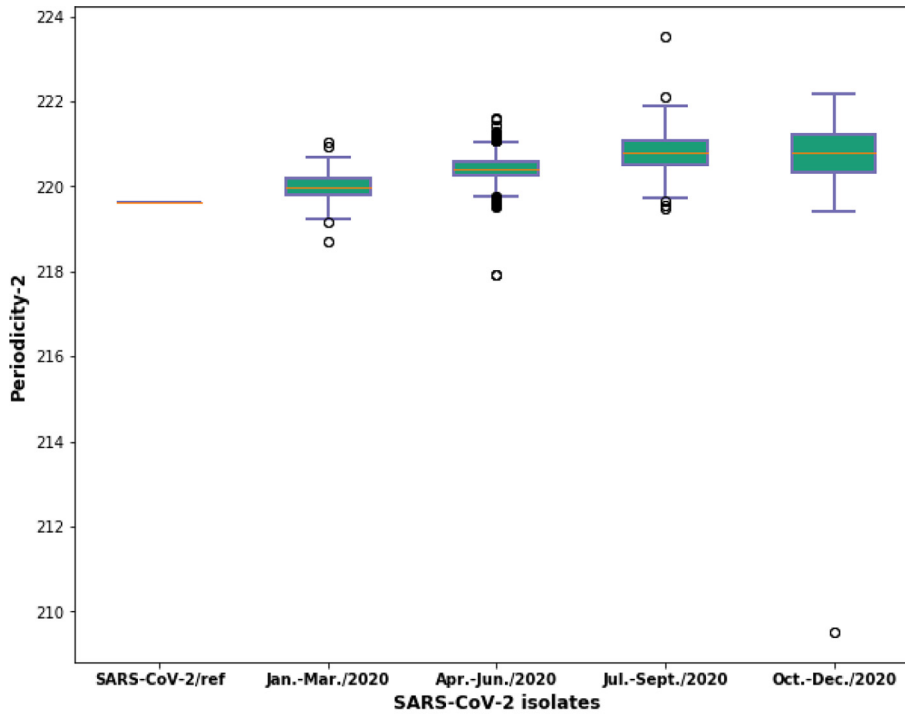
dinucleotide compositions (Fros et al., 2017). Most vertebrate RNA and small DNA viruses suppress genomic CG and TA dinucleotide frequencies, apparently mimicking host mRNA composition. The abundance of dinucleotides TT and TA are most likely common pathogenicity islands in microbial genomes. This study on SARS-CoV-2 provides additional evidence that increased content of dinucleotides TT and TA in SARS-CoV-2 is the result of interaction with the host during virus evolution. We consider these three dinucleotide abundance regions as pathogenicity islands of the SARS-CoV-2 genome. In addition, these special regions may contribute RNA replications, and can be recognized by cell RNAase L for RNA degradation in the immune response. However, this study is only a theoretical analysis of the genomes. The actual functional consequences and the impacts on transmissibility and pathogenesis of these dinucleotide imbalances should be determined by biochemical experiments and animal models.

The choice of latent periodicity-2 as the genome signature is the simplest, but combining the periodicity-2 and periodicity-2 would be more informative (Karlin and Burge, 1995). The coding periodicity-3 is natural and is the most pronounced periodicity in

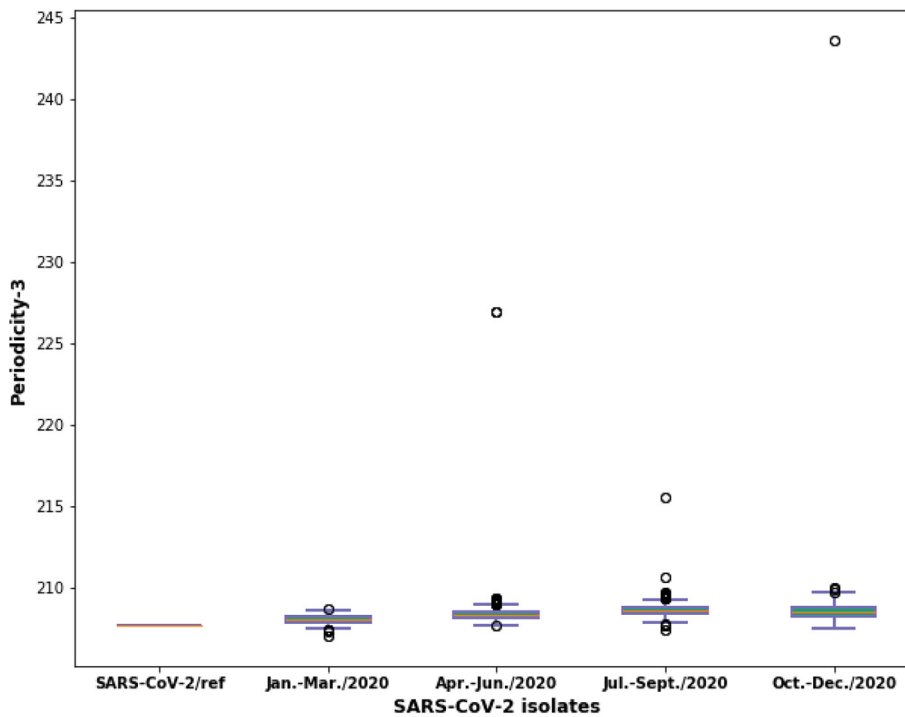
viral genomes. As we have shown in SARS-CoV-2 genomes during COVID-19 pandemic period year 2000, both periodicity-2 and periodicity-3 are increasing with the infection time. We will investigate the contributions of the two periodicities to the virulence and transmission of SARS-CoV-2 in detail as future research.

In humans and mammals, APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like3) systems help protect the organisms from viral infections. For the origin of dinucleotide repeats in the SARS-CoV-2 genome, we speculate that the molecular mechanism of increased dinucleotide repeats during evolution fitness is possibly APOBEC3-mediated editing of viral RNA, in which Cytosine (C) is often mutated to Uracil (U) by deamination (Bishop et al., 2004). Therefore, many dinucleotide TN motif regions could be generated as the periodicity-2 island in the RNA genome by the APOBEC defense system.

Cross-species transmission of coronaviruses from wildlife reservoirs may lead to disease outbreaks in humans, posing a severe threat to human health. To date, most studies on the zoonotic origin of SARS-CoV-2 primarily focus on the Spike protein, which is essential for the entry of virus particles into the cell. Mutations



(a) periodicity-2



(b) periodicity-3

**Fig. 9.** Box-plots of trends periodicities 2 and 3 in SARS-CoV-2 genomes during the infection periods in year 2020. (a) periodicity-2. (b) periodicity-3. The x-axis is for SARS-CoV-2 reference genome, and the SARS-CoV-2 genomes of the virus samples collected during the COVID-19 pandemic in year 2020.

or acquisition of potential cleavage site for furin proteases in the Spike protein may confer zoonotic transmissibility of SARS-CoV-2 (Andersen et al., 2020; Hoffmann et al., 2020). However, the spike protein is required but not sufficient for zoonotic coronavirus

transmission. For instance, the Spike protein in SARS-like SHC014-CoV can only enable the chimeric virus SHC014-MA15 to infect human cells when the Spike protein is integrated into a wild-type SARS-CoV backbone (Menachery et al., 2015). The

SARS-CoV backbone contains ORF1ab replication components. Our study provides evidence for the importance of ORF1ab replication regions in evolutionary fitness. Consequently, these ORF1ab replication components are critical for zoonotic transmissions. Especially, the results in this study suggest that when these dinucleotide repeat regions in ORF1ab of SARS-CoV-2 genome are increased, the virus fits more in human hosts, therefore, virulence of the virus is decreased. Modification of these repeat regions, therefore, could produce the attenuated virus, which can be effective and safe for vaccine development.

This study on the genomic spectrum of SARS-CoV-2 reveals high dinucleotides TT regions in ORF1a might contribute to evolution fitness in host immune evasion. Accordingly, monitoring SARS-CoV-2 and developing antiviral drugs should envisage molecular characteristics and changes of nsps 3–6, which are vital components in interacting with human hosts. These special dinucleotide motif regions should be investigated in detail for its functions and phenotype changes in SARS-CoV-2. Importantly, these high periodicity-2 regions can possibly be the prophylactic and therapeutic targets for controlling COVID-19.

At last, we clarify some aspects of the method used in this study. In genome analysis, Fourier transform is often used to detect the periodicities and repeats in a DNA sequence. The Fourier transform method computes the power spectrum of each frequency on the four binary indicator sequences that correspond to four nucleotide positions in the DNA sequence. In the periodicity detection method in this study, we introduce the mean of four nucleotides, i.e.,  $n/4p$ , in Eq. (2), instead of the mean of individual nucleotide composition. If we take the mean of individual nucleotide composition in Eq. (2), the periodicity-2 and periodicity-3 spectra by the NDU method are the same as the corresponding Fourier power spectra (Yin and Wang, 2016). Therefore, we consider using the mean  $n/4p$  in Eq. (2) is an advantage to capture periodicity-2 in our original method (Yin, 2017). In addition, the periodicity detection method uses a parallel four-nucleotide analysis. Yet the periodicity spectrum is only in the form of the sum over nucleotides of four types (Eq. (2)). The periodicity profiles for the nucleotides of particular types could be more informative. In particular, the rankings for the intensities of periodicity-2 of SARS-CoV-2 and SARS-CoV are T>C>G>A (SARS-CoV-2: periodicity-2(A) = 36.5391, periodicity-2(T) = 75.2827, periodicity-2(C) = 65.8607, periodicity-2(G) = 43.5443; SARS-CoV/Tor2: periodicity-2(A) = 18.3036, periodicity-2(T) = 48.8777, periodicity-2(C) = 37.7693, periodicity-2(G) = 26.2949). The ranking for the intensity of coding periodicity-3 for the complete genome of SARS-CoV-2 is T>C>G>A (periodicity-3(A) = 25.2457, periodicity-3(T) = 70.5814, periodicity-3(C) = 48.1901, periodicity-3(G) = 45.9472), whereas for SARS-CoV/Tor2 the counterpart ranking is T>G>C>A (periodicity-3(A) = 14.1041, periodicity-3(T) = 47.2578, periodicity-3(C) = 26.2882, periodicity-3(G) = 29.4027). We see that difference in intensities for the nucleotides of the same types is also significant between the two viruses. Such four-nucleotide profiles of human CoVs are worth investigating in the future.

#### CRediT authorship contribution statement

**Changchuan Yin:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jtbi.2021.110604>.

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