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REVIEW

Liu: Adaptive evolution of SARS-CoV-2

Is SARS-CoV-2 facing constraints in its adaptive evolution?

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ABSTRACT

The ultimate measure of viral fitness is the ability to maintain high prevalence within its host species. Effective transmission, efficient replication, and rapid immune evasion all contribute to this outcome. Over the past five years, SARS-CoV-2 has successfully adapted to humans, establishing long-term reservoirs and enabling sustained coexistence with the human population. We have observed innovative, synergistic mutations in the spike (S) protein that enhance receptor binding. Adaptation to the upper respiratory tract has shortened the incubation period, thereby facilitating viral spread. These improvements have also enabled immune escape mutations, even when such changes compromise replicative fitness. Adaptive mutations have driven intermittent selective sweeps by dominant variants. However, there are limits to functional enhancement. The receptor binding affinity of the S protein appears to have peaked between 2022 and 2023. The accumulation of fixed mutations plateaued following the emergence of BA.2.86/JN.1 around late 2023 and early 2024. Purifying selection has been the dominant evolutionary force acting on nonsynonymous mutations in the Omicron lineage, and the overall fitness impact of missense mutations in key viral proteins has declined. Additionally, due to weak selection pressure on synonymous mutations, the codon adaptation index in humans has been decreasing among Omicron subvariants. As a result, Omicron lineages have replicated less efficiently in cell cultures compared to the original virus, and recent variants show further attenuation in animal models. In the human population, this attenuation is reflected in declining COVID-19-related mortality, despite persistently high infection rates.

Keywords: SARS-CoV-2; COVID-19; evolution; mutation; Muller’s ratchet.

INTRODUCTION

In the study of real time evolutionary processes, no organism has been observed and documented as intensively and concertedly as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19. Millions of genomic sequences have been analyzed during the pandemic. With its short generation time, high mutation rate, large surplus population, and strong natural selection, variants of SARS-CoV-2 have showcased “speciation”, selective sweeps, and “extinction” in matters of weeks and months. The dazzling drama of alphabetical “dynasties” (Figure 1A and 1B) not only gave us insights about the evolutionary trajectory of a zoonotic RNA virus and its interaction with the human host but also provided us with an opportunity to learn general trends in molecular evolution which would take deep geological time to be appreciable in higher organisms.

SARS-CoV-2 is the third zoonotic coronavirus of the century. While severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) went extinct and Middle East respiratory syndrome coronavirus (MERS-CoV) is now only reported sporadically in Saudi Arabia, SARS-CoV-2 has reigned in every corner of the world and infections remain prevalent year-round. SARS-CoV-2 demonstrated that the ultimate measurement of viral fitness is the ability to maintain high prevalence rates in its host species. Effective human-to-human transmission, efficient replication in human cells, and rapid immune evasion are all means toward this end. On the molecular level, innovative mutations have enabled the S protein of SARS-CoV-2 to enhance binding affinity toward its primary receptor on human cells, angiotensin-converting enzyme 2 (ACE2). Perhaps related to this gain of molecular function, the virus increased its ability to infect cells of the upper airway in preference to the lungs because ACE2 is more concentrated in the nasal epithelium [1]. Tropism toward the nasal tissues facilitated viral shedding and probably contributed to the shortening of the incubation period and increased transmissibility. In addition to its role in receptor binding, the S protein is also the primary target of neutralizing antibodies. For these reasons, the S protein, especially its receptor-binding domain (RBD), has experienced higher rates of adaptive mutations than any other protein of the virus.

However, functional improvements have theoretical and practical limits. Early in the pandemic, Zahradník and colleagues demonstrated by *in vitro* evolution that there is an optimal configuration of the S protein (RBD-62) with an ACE2-binding affinity that is 1000-fold stronger than wild type

[2]. Many variants of SARS-CoV-2 have employed the same mutations predicted by the *in vitro* experiment. Meanwhile, the virus must constantly change the S protein to evade neutralizing antibodies regardless of the effects of the mutations on receptor binding. In addition, evolution of the viral genome is not only driven by functional advantages, but also by the preferred directions of the host-initiated mutagenic mechanisms such as the RNA editing proteins APOBEC (Apolipoprotein B mRNA Editing Catalytic Polypeptide-like), ADAR (Adenosine Deaminase Acting on RNA), and ZAP (zinc finger antiviral proteins) [3-5]. Moreover, a significant proportion of mutations are not selectable and therefore subject to random drifting [6]. For these reasons, ideal conformations such as RBD-62 are probably not achievable in nature.

The purpose of this review is to address such questions as: Where is SARS-CoV-2 in its evolutionary trajectory after five years of circulation in humans? Do we expect more transmissible or more virulent variants in the future or is the virus losing replicative fitness and virulence irreversibly? Here we discuss several observations that indicate that genomic evolution of SARS-CoV-2 has reached a plateau and is probably on the decline.

ACCUMULATION OF FIXED MUTATIONS IS SLOWING DOWN.

While the majority (over 60%) of the ~30,000 nucleotides of the SARS-CoV-2 genome have mutated at least once [4, 7], only a small proportion of the mutations have been selected and fixed [8]. Most synonymous mutations are near neutral, while most nonsynonymous mutations are deleterious [6]. Most of the fixed mutations are in the S protein. By the beginning of 2025, SARS-CoV-2 had accumulated over 60 mutations in the 1273-residue-long S protein, 30 of which in the 223-residue-long RBD [9, 10]. On the other hand, there are altogether about 100 fixed nonsynonymous mutations in the SARS-CoV-2 genome coding for 29 proteins totaling over 9000 amino acid residues [10]. In other words, the overall rate of fixed amino acid mutations is ~1% while there are ~5% fixed mutations in the S protein and over 13% in the RBD.

Cooperative emergence of multiple affinity-enhancing mutations followed by immune escape mutations

Buildup of missense mutations in the *S* gene has been gradual with two brief periods of acceleration, one at the emergence of Omicron BA.1 in late 2021, and the other at the emergence of Omicron BA.2.86 in 2023 (Figure 1C) [10]. The sudden increase in missense mutations in BA.1 also

manifested as an increase in the ratio between nonsynonymous mutations and synonymous mutations (dn/ds or ω, Figure 1D).

Although many missense mutations in the S protein experienced positive selection [8], only about a dozen of them enhanced receptor binding affinity [11-15]. Synergism and epistasis among affinity-enhancing mutations played a major role in the mutation count jumps, meaning certain mutations facilitated the fixation of other mutations, generating new modes of interactions between the RBD and ACE2 [2, 26, 17]. The mutation burst in Omicron in 2021 provides a dramatic example of such epistatic interactions. Most notably, the N501Y mutation turned the affinity-reducing Q498R mutation to an affinity-enhancing mutation. Synergism between N501Y and Q498R reconfigured the RBD-ACE2 interaction in the Omicron variant [18]. Synergistic affinity-enhancing mutations are often followed by immune escape mutations which provide more prominent selective advantages than affinity enhancement [12, 19-21]. The affinity-enhancing mutations in BA.1 (S477N, Q493R, Q498R, N501Y) first appeared in the original Omicron variant (B.1.1.529) and the affinity-reducing mutations (including three deletions) were added subsequently for immune escape, enabling BA.1 to spread quickly, displacing the Delta variant in a few months (Figure 1A) [10, 12].

The second, less dramatic, epistatic mutation burst occurred in 2023. The spike mutation R403K did not increase ACE2-binding affinity in the earlier B.1.1 lineage but did in the BA.2 lineage background [22]. It first appeared in BA.2.86. Although BA2.86, with its high receptor-binding affinity, was recognized for its increase in frequency, it never rose to dominant status (with a peak frequency of ~9% in the US [23]). However, one more mutation, L455S, turned BA2.86 into JN.1, which quickly wiped out all other subvariants to dominate the variant landscape (Figure 1 A and B). L455S significantly reduced the ACE2-binding affinity of the RBD but provided JN.1 with strong immune-evading ability along with enhanced fusogenicity and improved cell entry [20, 21, 24].

Epistatic mutations continued in the JN.1 sublineages but did not result in comparable mutation leaps or selective sweeps. Q493E enhanced ACE2 binding only in the presence of L455S and F456L, found in some JN.1 sublineages. Q493E emerged independently in KP.3 and LP.8 [17]. Subsequently, a deletion, S31del, developed in KP.3.1 to produce KP.3.1.1. Although the deletion itself reduced the ability of the virus to infect cells by membrane fusion, it granted KP.3.1.1 a

significant competitive edge over its parent by immune evasion [25, 26]. S31del also independently emerged in other JN.1 sublineages such as KP.2.3 and LP.8, showing that S31 was a prominent immune target in the human population during 2024.

The historical pattern of SARS-CoV-2 evolution seems to indicate that there was more room for revolutionary innovations through synergistic mutations during the pioneering stages of a zoonotic epidemic. Later attempts of epistatic changes ended up only reformative.

Convergence, flip, and reversion

Even though the S protein experienced strong positive selection [8], natural mutations fixed during the pandemic indicated that there are limited modes of receptor binding that the virus can evolve into. For this reason, mutations in the *S* gene tend to be convergent, recurrent, and cyclical. Table 1 lists some of the recurrent S mutations. Multiple variants resorted to the same tricks again and again.

The FLip mutants showed that the virus can swap positions of two adjacent amino acids in the S protein to evade neutralizing antibodies and to improve receptor binding. Multiple XBB and JN.1 sublineages swapped the positions of L455 and F456 via the FLip mutations (L455F + F456L) [16, 27]. Both mutations, especially L455F, dampened receptor binding when acting alone [16], and F456L also reduced viral infectivity [15], which is probably why they did not develop early in the pandemic. However, more than three years later, F456L showed up, presumably because it enabled the virus to evade antibodies developed against earlier lineages. Subsequent L455F mutation effectively restored receptor binding affinity and further helped the virus to penetrate herd immunity [16].

RBD mutations also reverted depending on the genetic background. The Q493R mutation in BA.1 and BA.2 was found to enhance receptor binding [28]. However, the mutation reverted in BA.2.75 and its XBB sublineages as well as in BA.2.86 ant its JN.1 sublineages. The reversal reduced receptor-binding affinity [29] but was allowed in the context of the overall strong binding of the BA.2.75 and BA.2.86 lineages for the purpose of immune evasion. Beside Q493, six other amino acids in the RBD are known to experience “Yo-Yo” mutations. Notably, G446 and N501 had mutated between two forms three times, and L452 four times by August of 2023. Meanwhile, deletion of HV69-70 in the N-terminal domain of the S protein had appeared and disappeared three times within the same time frame [30].

Natural restrictions on the improvement of receptor-binding affinity

Although *in vitro* evolution produced an RBD with a 1000-fold increase in receptor-binding affinity, the highest affinity we have observed in natural variants was no more than 20-fold stronger than wild type. The strongest receptor-binding virus in various reports was BA.2.75, XBB.1.5, or BA.2.86, depending on measurement methods and samples analyzed [14, 19-21, 24, 31]. Figure 1F shows dissociation constants (*Kd*) of the major variants according to [19] and [20], with lower *Kd* indicating higher binding affinity. The discrepancy between *in vitro* evolution and the actual *in vivo* outcomes attests to the differences between isolated molecular interactions on the surface of yeast cells and natural selection of replicating viruses with competing functional priorities in the host cell and the need to compromise under immune pressure. For example, the D614G mutation, which became dominant globally within the first few months of the pandemic [32], resulted in an RBD that bound less tightly to ACE2 than wild type, but the mutation increased the density of intact S trimers on the viral surface by preventing premature dissociation of S1 from S2 following furin cleavage in the producing cell [33, 34]. As discussed earlier, after high-affinity RBDs were produced, they were quickly deoptimized by immune escape mutations that gave the lineages bigger advantages in human populations. The current trend shows an overall tendency of decrease in receptor-binding affinity (Figure 1F) [20, 21, 25, 35].

Of the nine RBD mutations in the optimal *in vitro* evolution product, RBD-62 [**Error! Bookmark not defined.**], five of them are found in the current LP.8.1* lineage. Interestingly, although V445K in RBD-62 has never been reported in nature, mutation of V445 to another basic amino acid, histidine, has been fixed in the BA.2.86/JN.1 lineage. Recently, H445 was replaced by the third basic amino acid, arginine, in LP.8 and enhanced its receptor-binding affinity [35]. Another mutation in RBD-62, I468F, was found in early variants but was not fixed because it rendered the virus more susceptible to antibody neutralization [36]. The other two mutations in RBD-62 (I358F and T470M) have never been reported in natural variants, suggesting that these residues must be conserved for effective viral replication or for infection of the human host. Two mutations found in another *in vitro* evolution product with improved ACE2-binding (RBD-71), R408D and K417V, have not been reported in nature, but R408S and K417N/T have been fixed in multiple lineages. The fact that the mutations are tolerated suggests that chances of R408D and K417V emerging in the future exist, but they are unlikely because aspartic acid (D) is categorically different from serine

(S), so is valine (V) from asparagine (N) and threonine (T). Nevertheless, it is worthwhile to analyze the impact of D408 and V417 in the context of the current JN.1 sublineages.

DELETERIOUS MUTATIONS LEAD TO DEGENERATION AND ATTENUATION.

Even with enormous population sizes, exponential growth, and strong natural selection, viruses still experience significant genetic drift [37]. Viruses are known to be subject to Muller's ratchet, the process of irreversible accumulation of deleterious mutations due to random genetic drift, especially in asexual organisms, leading to reduced fitness [37, 38]. Beside the high error rates of viral RNA-dependent RNA polymerases (RdRp) [39], mammalian cells use mutagenesis as a defense mechanism to dampen viral fitness [3-5, 38]. All the Variants of Concern (VOC) derived directly from the ancestral virus instead of evolving from a previous VOC, suggesting lowered adaptability of the variants compared to the ancestral virus. The ancestral virus and most of the early variants are long gone from the human population, leaving the extant subvariants at the mercy of Muller's ratchet, even with occasional recombination between circulating lineages.

Declining mutational fitness effects

Bloom and Neher [6] estimated the fitness effects of SARS-CoV-2 mutations by comparing independent occurrences of each mutation to an expected number based on mutation rates at the third nucleotide of four-fold degenerate codons. They found that the overall effects of nonsynonymous mutations in each gene were negative. Moreover, plotting their calculated average fitness effects of nonsynonymous mutations in the *RdRp* gene and in the *S* gene over time, we show that the overall fitness effects of missense mutations declined during the reign of Omicron from the beginning of 2022 to the end of 2024 (Figure 1E), which is consistent with the observation of Maiti and colleagues of a dn/ds decline within the Omicron VOC [8]. In other words, further mutations are increasingly detrimental to viral fitness rather than being adaptive.

The large genome of SARS-CoV-2 contains regions where natural selection is relaxed. Bloom and Neher's study also found that fitness effects of nonsynonymous mutations in the accessory proteins were comparable to those of synonymous mutations, i.e., near neutral, consistent with the observed accumulation of missense and nonsense mutations in these genes [8, 40].

Declining codon adaptation index in the human host

Beside functional improvements and immune escape, another direction of viral evolution after entering a new host species is optimization of codon usage in the cells of the host, which involves synonymous changes. Selections of nonsynonymous mutations typically take priority over codon usage optimization because the fitness effects of the latter are more subtle. Therefore, it is conceivable to expect sacrifices in codon usage in genes where selective pressure for adaptive evolution is high. Codon adaptation index (CAI) of SARS-CoV-2 in the human population experienced a decline in the early variants, restored in Omicron [41-45], and decreased again [44]. After analyzing the CAI and the codon pair adaptation index (CPAI), Padhiar and colleagues reported no drastic net changes in SARS-CoV-2 genes in codon usage from December of 2019 to July of 2024. However, when I plotted the temporal curves with their supplementary data, we found that the *S* gene experienced two phases of decline in both CAI and CPAI with a sharp increase in the middle, and there was an overall negative correlation between CPAI and time ($p = 0.0028$, Figure 1G) [46]. This suggests that evolution of the *S* gene not only has to strike compromises between receptor-binding and immune evasion but also must bear the cost of deoptimized codon usage. Timing of the jump in CAI and CPAI in the *S* gene coincided with the emergence of Omicron. The large number of adaptive mutations and simultaneous optimization of codon usage in the original Omicron variant suggest a unique evolutionary mechanism which is still a puzzle today [38]. Using the same data set, we also observed significant codon degeneration in the ORF1ab (which includes the *RdRp* gene) and the *N* gene, where multiple adaptive mutations have been documented [40, 47-49]. The CPAI of the whole genome also showed a decreasing trend, although not statistically significant ($p = 0.15$).

Loss of proofreading function

Effects of mutations outside of the S protein on viral evolution are generally less well understood. Some of them have been fixed in the current lineages. Mutations in transcriptional regulatory sequences (TRSs) may alter the number and quantity of subgenomic transcripts, most of which leading to truncations and deletions [40]. Of the extra-spike variations, mutations in non-structural protein 14 (NSP14) is most pertinent to viral evolution and relatively well studied.

NSP14 is a multifunctional protein. It's N-terminal exoribonuclease domain is responsible for proofreading during viral RNA replication. Mutations in the enzyme disrupt proofreading and

accelerate mutation accumulation throughout the viral genome [50-52]. The effect of NSP14 mutations on viral mutation load is stronger than that of NSP7, NSP8, and NSP12, even though the latter proteins form the core RNA polymerase complex [51]. One NSP14 mutation, I42V, was fixed in BA.1 and all subsequent Omicron subvariants. A humanized mouse model revealed that I42V contributed to the attenuation of BA.1, along with other Omicron mutations in NSP5, NSP6, M, and E, sparing infected mice from lethal brain invasion [53]. The only mutations in BA.1 that might have increased virulence are those in the N protein, but they failed to make a difference in the context of the Omicron NSP14, M, and E. The attenuating effect of the NSP14 mutation attests to the destructive nature of uncontrolled mutagenesis.

Unlike in the S protein which, as a major immune target, experiences positive selection based on dominant antibodies in the human population, mutations in non-structural proteins are mostly neutral [8, 40, 52] and prone to random drifting. Consequently, there is more divergence in proteins such as NSP14 than reflected by the number of fixed mutations. In the study of Hassan and colleagues [52], 962 nonsynonymous point mutations were found among the 1581 nucleotides of the *nsp14* gene, with only 110 of the 527 amino acids conserved. Yet, there has only been one fixed mutation in NSP14 after five years. Therefore, degeneration of non-structural genes may be more pronounced than we see in the lineage-defining mutation spectrums.

Phenotypical attenuation

Accumulation of deleterious synonymous and nonsynonymous mutations resulted in attenuation of SARS-CoV-2 as the pandemic unfolded. There was a decline in replication efficiency in cell cultures from the early B.1.1 variant to Omicron [26, 54]. The Omicron subvariants, including some dominant XBB family members, BA.2.86, and JN.1, were less pathogenic in hamsters and in mice than the ancestral B.1 variant [24]. Later Omicron subvariants were less virulent than earlier Omicron subvariants, although BA.2.86 and JN.1 demonstrated more efficient replication in human nasal epithelial cells [22, 24].

Attenuation of SARS-CoV-2 in humans manifested as reduced mortality rates over time. There seemed to be a rapid decline in mortality rate during the first few months of the pandemic [55]. COVID-19 related mortality in the United States has been declining continuously even though viral prevalence remains high as evidenced by high test positivity rates (Figure 1H) [56]. The mortality decrease does not correlate with vaccination efforts, as intensive vaccination in 2021

failed to prevent the death peaks later in the year, and the federal government completely stopped free vaccination in September of 2023 [57]. The mortality rate correlates partially with natural immunity. After the Omicron wave in the winter of 2021-2022, most Americans had turned seropositive [58], and mortality has dropped dramatically ever since. However, the subsequent gradual decline from 2022 till now probably has more to do with viral attenuation than with herd immunity because of declining vaccine coverage and rapid immune escape of the virus.

A global meta-analysis revealed that the global case fatality rates of the ancestral virus, the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron VOCs were 3.64%, 2.62%, 4.19%, 3.60%, 2.01%, and 0.70%, respectively [59]. Note that the more lethal Beta and Gamma variants emerged before Alpha, and Delta was not as virulent as initially reported in Asia [60]. The case fatality rates of the ancestral virus, the Alpha, Delta, and Omicron VOCs in North America were 4.77%, 2.67%, 2.50%, and 0.73%, respectively, which is roughly consistent with Figure 1H considering the lag time between infection and death and the different prevalence rates of the variants [59]. Viral attenuation and immunity buildup probably both contributed to the decrease in case fatality rate from the original virus to Omicron.

Effect of vaccination on viral evolution

Vaccination is unlikely to affect mutagenic mechanisms but enhanced immune responses in vaccinees help to suppress the wild type, giving mutants selective advantages even though they may have reduced replicative fitness. A retrospective study showed that vaccination was associated with an increase in viral diversity in India in 2021 and 2022 [61]. Moreover, viral lineage diversity, measured as Shannon entropy, was higher in patients of vaccine breakthrough infections than in unvaccinated patients. Higher incidence of intra-host single nucleotides variants (iSNVs) in breakthrough infections indicated that vaccination accelerated viral evolution in the human host. Immune escape mutations were fixed at higher frequencies in vaccinated patients. Higher dn/ds ratios of viral isolates from vaccinees indicated stronger positive selection. Interestingly, when iSNVs of various viral lineages were compared, the early variants, B.1 and B.1.1, yielded much higher diversity than the VOCs did in both vaccinated and unvaccinated hosts, indicating faster evolution in the early phases of the pandemic as the virus adapted to humanity.

We should not be concerned that vaccination facilitates selection of immune escape mutations. Mutations occur anyway. Just as we do not refrain from using molnupiravir as an antiviral

treatment even though it acts through mutagenesis, we should not avoid vaccination, which causes eventual reduction of the replicative fitness of the virus by forcing it to mutate.

CONCLUSION

Right now, in the sixth year of the COVID-19 pandemic, there are increasing signs indicating that adaptive evolution of SARS-CoV-2 has plateaued, as reflected in slower accumulation of fixed mutations, recurrent and cyclic mutations at the same sites, declining mutational fitness effects, declining receptor-binding affinity of the S protein, diminishing codon optimization in the human host, and decreasing mortality rates. Moreover, evolution of SARS-CoV-2 has been gradual since the JN.1 sweep early in 2024, and there has only been one addition to WHO's Variants under Monitoring (VUM, i.e., LP.8.1*) since September of 2024. The last addition to the Variants of Interest (VOI) was JN.1 in December of 2023 and the last addition to the Variants of Concern (VOC) was Omicron (B.1.1.529) in November of 2021. Currently, LP.8.1.1 is posed to slowly displace the other JN.1 sublineages to dominate in the future months. Like its predecessors, the new variant is expected to experience genetic drift under immune pressure as well as Muller's ratchet.

SARS-CoV-2 may become like the four current human coronaviruses that cause the common cold [62]. If the Russian flu of 1889-1894 was indeed caused by a coronavirus which became today's HCoV-OC43, the five-year timeframe of the historical pandemic provides an interesting reference point for COVID-19. In spite of airplanes helping SARS-CoV-2 spread globally, and vaccines to "flatten the curve", the natural courses of the two pandemics of respiratory illnesses ended up comparable.

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TABLES AND FIGURES WITH LEGENDS

Table 1. S mutations fixed in multiple independent lineages

Mutations	**Pre-Alpha**	**Alpha**	**Beta**	**Gamma**	**Delta**	**Lambda**	**Omicron**
HV69-70del	B.1.258, B.1.375	x					BA.1
Y144del		x					XBB.1.5

R346							BQ.1.1, XBB.1.5
K417N/T			x	x			x
G446S							BA.1, BA.2.75
L452R/Q					x	x	BA.4/5
F456L							Multiple XBB and JN.1 sublineages
T478K					x		x
E484A/K			x	x			x
F490S						x	XBB.1.5
N501Y		x	x	x			x
D614G	A and B.1						
P681R/H		x			x		x

Fixation is defined as complete replacement in an entire population. Presence in all isolates of a variant is indicated with "x". If fixed only in some subvariants, names of selected subvariants are given.

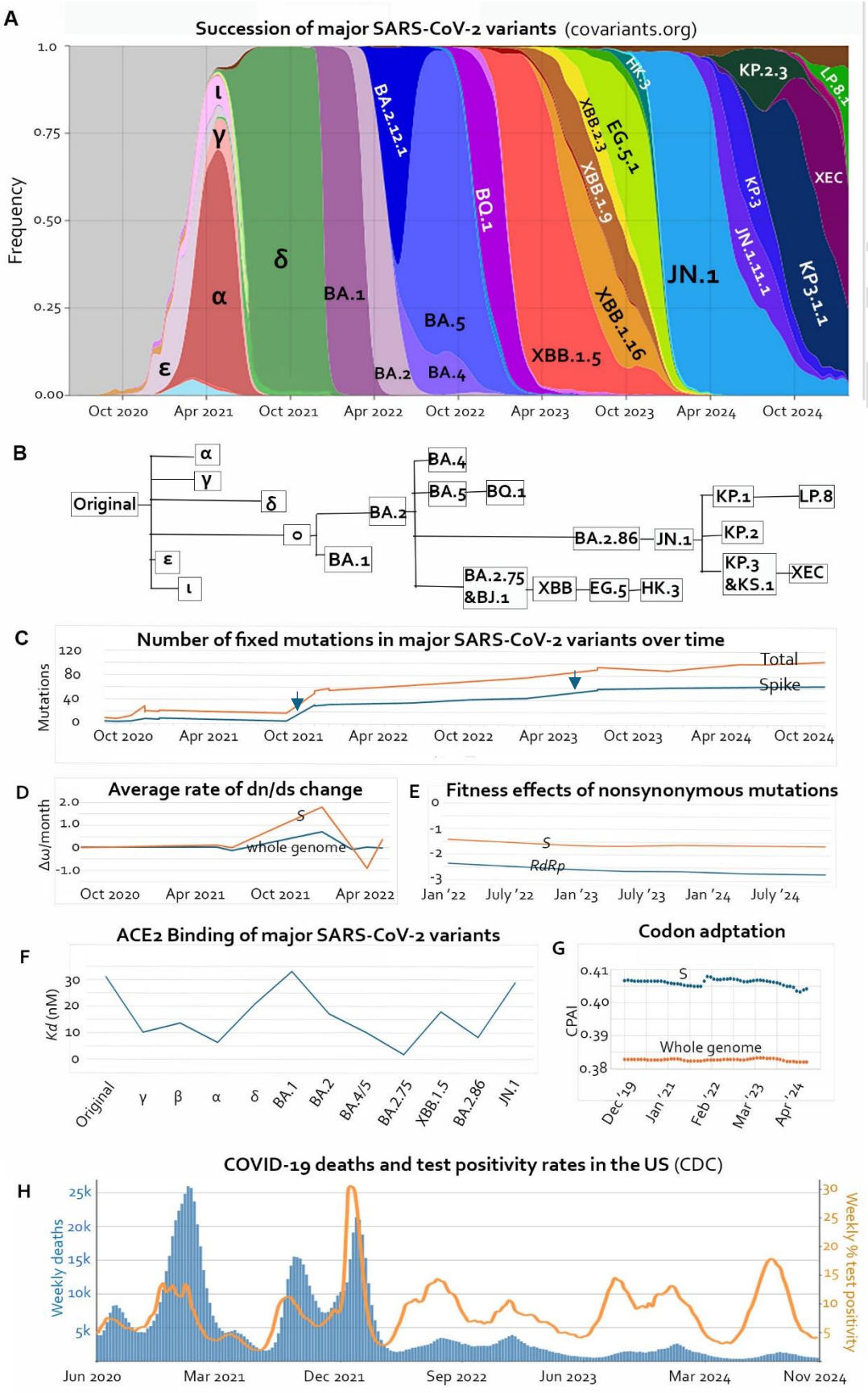
A
Succession of major SARS-CoV-2 variants (covariants.org)
Frequency
1.0
0.75
0.50
0.25
0.00
Oct 2020
Apr 2021
Oct 2021
Apr 2022
Oct 2022
Apr 2023
Oct 2023
Apr 2024
Oct 2024
ι
γ
α
ε
δ
BA.1
BA.2.12.1
BA.2
BA.4
BA.5
BQ.1
XBB.1.5
XBB.2.3
XBB.1.9
XBB.1.16
EG.5.1
HK.3
JN.1
JN.1.11.1
KP.3
KP.2.3
LP.8.1
XEC
KP3.1.1
B
Original
α
γ
δ
ο
BA.1
BA.2
BA.4
BA.5
BQ.1
BA.2.86
JN.1
KP.1
LP.8
KP.2
KP.3 &KS.1
XEC
BA.2.75 &BJ.1
XBB
EG.5
HK.3
C
Number of fixed mutations in major SARS-CoV-2 variants over time
Mutations
120
80
40
0
Total
Spike
Oct 2020
Apr 2021
Oct 2021
Apr 2022
Oct 2022
Apr 2023
Oct 2023
Apr 2024
Oct 2024
D
Average rate of dn/ds change
Δω/month
2.0
1.0
0
-1.0
S
whole genome
Oct 2020
Apr 2021
Oct 2021
Apr 2022
E
Fitness effects of nonsynonymous mutations
0
-1
-2
-3
S
RdRp
Jan '22
July '22
Jan '23
July '23
Jan '24
July '24
F
ACE2 Binding of major SARS-CoV-2 variants
Kd (nM)
30
20
10
0
Original
γ
β
α
δ
BA.1
BA.2
BA.4/5
BA.2.75
XBB.1.5
BA.2.86
JN.1
G
Codon adptation
CPAI
0.41
0.40
0.39
0.38
S
Whole genome
Dec '19
Jan '21
Feb '22
Mar '23
Apr '24
H
COVID-19 deaths and test positivity rates in the US (CDC)
Weekly deaths
25k
20k
15k
10k
5k
Weekly % test positivity
30
25
20
15
10
5
Jun 2020
Mar 2021
Dec 2021
Sep 2022
Jun 2023
Mar 2024
Nov 2024

Figure 1. Evolutionary timeline of major SARS-CoV-2 variants. (A) Succession of dominant SARS-CoV-2 variants and dominant Omicron subvariants. Source: GISAID, via CoVariants.org (2025). (B) Phylogenetic relationship of lineages labelled in A. (C) Accumulation of fixed mutations in SARS-CoV-2 over time. Red: all proteins; Blue: the S glycoprotein. Arrows point to periods of acceleration. Mutation counts were obtained from outbreak.info [10]. (D) Average rate of dn/ds change from September 2020 to May 2022. Red: the *S* gene; Blue: whole genome. Replotted with data from [8]. (E) Temporal change of fitness effects of nonsynonymous mutations from January 2022 to November 2024. Red: the *S* gene; Blue: the *RdRp* gene. Plotted with data from Bloom and Neher [6]. (F) Dissociation constants (Kd) of the S protein of major SARS-CoV-2 variants. Lower *Kd* values indicate higher receptor-binding affinity. Data obtained from [19] and [20]. (G) Change in codon pair adaptation index (CPAI) of SARS-CoV-2 between December 2019 and July 2024. Blue: coding sequence of the *S* gene; Red: all coding sequences. Plotted with data from [46]. (H) Weekly COVID-19 deaths and nucleic acid amplification test percent positivity in the United States from June 2020 to November 2024. Blue: deaths; Orange: test positivity. Data obtained from the Center for Disease Control and Prevention [56].