Inferring effects of mutations on SARS-CoV-2 transmission from genomic surveillance data

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New and more transmissible variants of SARS-CoV-2 have arisen multiple times over the course of the pandemic. Rapidly identifying mutations that affect transmission could facilitate outbreak control efforts and highlight new variants that warrant further study. Here we develop an analytical epidemiological model that infers the transmission effects of mutations from genomic surveillance data. Applying our model to SARS-CoV-2 data across many regions, we find multiple mutations that strongly affect the transmission rate, both within and outside the Spike protein. Importantly, our model detects lineages with increased transmission even at low frequencies. We rapidly infer significant transmission advantages for the Alpha, Delta, and Omicron variants after their appearances in regional data, when their regional frequencies were only around 1-2%. Our model enables the rapid identification of variants and mutations that affect transmission from genomic surveillance data.

Viruses can acquire mutations that affect how efficiently they infect new hosts, for example by increasing viral load or escaping host immunity[1–4]. The ability to rapidly identify mutations that increase transmission could inform outbreak control efforts and identify potential immune escape variants[5–9]. However, estimating how individual mutations affect viral transmission is a challenging problem.

Current methods to estimate changes in viral transmission generally rely on phylogenetic analyses or fitting changes in variant frequencies to logistic growth models or multinomial extensions[5,10–13]. Phylogenetic analyses for viruses can be challenging due to a high degree of sequence similarity, which implies that the data can be explained equally well by a number of different trees[14]. Phylogenetic analyses also typically rely on extensive Markov chain Monte Carlo sampling that becomes intractable for very large data sets. Growth models can estimate the difference in transmissibility between one variant and others circulating in the same region. However, their estimates may be difficult to compare for variants that arose in other regions or with different genetic backgrounds and they typically do not identify specific mutations responsible for changes in transmission. These approaches also often do not account for superspreading —where a small number of infected individuals cause the majority of secondary infections —which has been observed for viruses like SARS-CoV and SARS-CoV-2[15–18].

To overcome these challenges, we developed a method to infer the effects of single nucleotide variants (SNVs) on viral transmission that systematically integrates genomic data from different outbreak regions and accounts for stochastic effects such as superspreading. Our analytical approach is based on a simple epidemiological model, allowing it to be efficiently applied to large data sets and opening the door to future theoretical extensions. For clarity, we refer to non-reference nucleotides (including deletions or insertions) as SNVs and viral lineages possessing common sets of SNVs as variants. Simulations show that our approach can reliably estimate transmission effects of SNVs even from limited data.

We applied our method to more than 5.6 million SARS-CoV-2 sequences from 126 geographical regions to reveal the effects of mutations on viral transmission throughout the pandemic. While the vast majority of SARS-CoV-2 mutations have negligible effects, we readily observe increased transmission for sets of SNVs in Spike and other hotspots throughout the genome. Importantly, our approach is sensitive enough to identify variants with increased transmission before they reach high frequencies. We demonstrate our capacity for early detection by studying the rise of the Alpha and Delta variants in Great Britain and Omicron in South Africa. We reliably infer increased transmission for these variants rapidly after their emergence, when their frequency in the region was only around 1-2%. An untargeted search for sets of mutations that strongly increase viral transmission also reveals multiple collections of SNVs belonging to well-known variants. Collectively, these data show that our model can be applied for the surveillance of evolving pathogens to robustly identify variants with transmission advantages and to highlight key mutations that may be driving changes in transmission.

Results

Epidemiological Model

To quantify the effects of mutations on viral transmission, we developed a generalized Galton-Watson-like stochastic branching process model of disease spread (Methods). Branching processes have been frequently used to model the stochastic numbers of infections in a population[19–21]. Our model incorporates superspreading by drawing the number of secondary infections caused by an infected individual from a negative binomial distribution with mean R, referred to as

the effective reproduction number, and dispersion parameter k (refs. [15–18,22,23]). Multiple variants with different transmission rates are included by assigning a variant a an effective reproduction number $R_a = R(1 + w_a)$. Under an additive model, the net increase or decrease in transmission for a variant is the sum of the individual transmission effects s_i for each SNV i that the variant contains. In analogy with population genetics, we refer to the w_a and s_i as selection coefficients.

We can then apply Bayesian inference to estimate the transmission effects of SNVs that best explain the observed evolutionary history of an outbreak. To simplify our analysis, we use a path integral technique from statistical physics, recently applied in the context of population genetics [24], to efficiently quantify the probability of the model parameters given the data (for details, see Supplementary Information). This allows us to derive an analytical estimate for the maximum *a posteriori* selection coefficients $\hat{\boldsymbol{s}}$, normalized per serial interval, for a given set of viral genomic surveillance data,

$$\hat{\boldsymbol{s}} = \left[\gamma' I + C_{\text{int}}\right]^{-1} \Delta \boldsymbol{x}\,. \qquad \textbf{(1)}$$

Here $\Delta \boldsymbol{x}$ is the change in the SNV frequency vector over time, γ' is a rescaled regularization term proportional to the precision of a Gaussian prior distribution for the selection coefficients s_i (Methods), and I is the identity matrix. C_{int} is the covariance matrix of SNV frequencies integrated over time, and accounts for competition between variants as well as the speed of growth for different viral lineages (Supplementary Information). Data from multiple outbreaks can be combined by summing contributions to the integrated covariance and frequency change from each individual trajectory (Methods). Our theoretical model could also be extended to incorporate additional features of disease transmission, such as the travel of infected individuals between different outbreak regions.

Validation in simulations

To test our ability to reliably infer selection, we analyzed simulation data using a wide range of parameters. We found that inference is accurate even without abundant data, especially when we combine information from multiple outbreaks (**Fig. 1**, **Supplementary Fig. 1**). Because we model the evolution of relative frequencies of different variants, accurate inference of selection does not require the knowledge of difficult-to-estimate parameters such as the current number of infected individuals or the effective reproduction number (Methods). Simulations also demonstrated that our model is robust to variations in effective reproduction numbers in different regions (**Supplementary Fig. 2**).

Global patterns of selection in SARS-CoV-2

We studied the evolutionary history of SARS-CoV-2 using genomic data from GISAID [25] as of June 9, 2022. We separated data by region and estimated selection coefficients jointly over all regions (Methods). After filtering regions with low or infrequent coverage, our analysis included more than 5.6 million SARS-CoV-2 sequences from 126 different

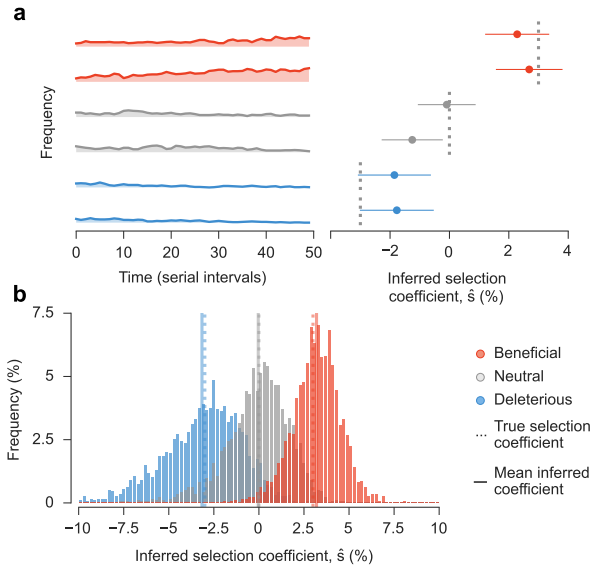

Fig. 1. Our approach accurately estimates transmission effects of mutations in simulations. Simulated epidemiological dynamics beginning with a mixed population containing variants with beneficial, neutral, and deleterious mutations. **a**, Selection coefficients for individual SNVs, shown as mean values ± one theoretical s.d., can be accurately inferred from stochastic dynamics in a typical simulation (Supplementary Information). **b**, Extensive tests on 1,000 replicate simulations with identical parameters show that inferred selection coefficients are centered around their true values. Deleterious coefficients are slightly more challenging to accurately infer due to their low frequencies in data. *Simulation parameters.* The initial population is a mixture of two variants with beneficial SNVs ($s = 0.03$), two with neutral SNVs ($s = 0$), and two with deleterious SNVs ($s = -0.03$). The number of newly infected individuals per serial interval rises rapidly from 6,000 to around 10,000 and stays nearly constant thereafter. Dispersion parameter k is fixed at 0.1.

regions, containing 1,259 nonsynonymous SNVs observed at nontrivial frequencies.

Our analysis revealed that, while the majority of SNVs were nearly neutral, a few dramatically increased viral transmission (**Fig. 2a**, **Table 1**). We observe clusters of SNVs with strong effects on transmission along the SARS-CoV-2 genome (**Fig. 2b**). The highest density of SNVs that increase transmission is in Spike, especially in the S1 subunit (**Supplementary Fig. 3**). Of the top 20 mutations that we infer to be most strongly selected, 13 are in Spike (**Table 1**). However, SNVs with a strong selective advantage are also found in other proteins, especially in N, M, NSP4, and NSP6.

Mutations inferred to substantially increase transmission

The top 50 mutations inferred to increase SARS-CoV-2 transmission the most are given in **Table 1**. Experimental evidence exists to directly or indirectly support most of these inferences. For clarity, we will reference mutations at the amino acid level rather than the underlying SNVs, which are also given in **Table 1**.

Spike mutations/deletions Δ142, P681R/H, R346K, Q498R, L452Q, and K417N comprise seven of the top ten mutations, and all have demonstrated functional effects that could increase transmission [4,26–36]. Similarly, Spike mutations in the receptor binding motif (RBM) such as N440K, L452R, T478K, E484K, F486V, and N501Y appear prominently in our analysis. All of these mutations have been

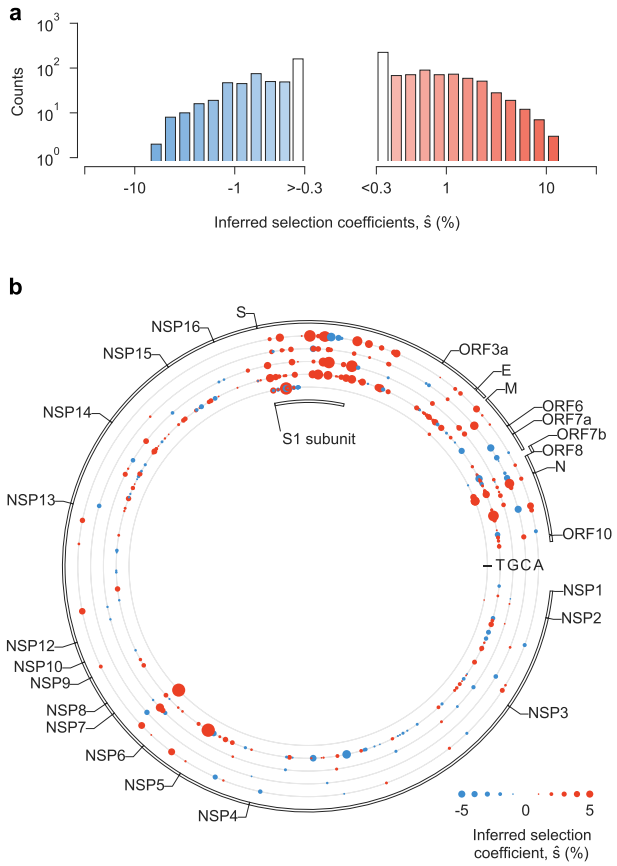

Fig. 2. Inferred transmission effects of SARS-CoV-2 mutations. **a**, The majority of the 1,259 nonsynonymous SNVs included in our study are inferred to have negligible effects on transmission (that is, $\hat{s}$ close to zero). However, a few SNVs have strong effects, as evidenced by a large value of $\hat{s}$. **b**, Patterns of selection across the SARS-CoV-2 genome. Beneficial SNVs often cluster together in the genome. Clustering is especially apparent for the S1 subunit of Spike, where many SNVs that are inferred to have the largest effects on transmission are located.

shown to increase resistance to RBM-specific neutralizing antibodies and the majority also enhance ACE2 receptor binding[4,29,31,34,37–39]. Of these, N501Y ($\hat{s} = 7.2\%$, ranked 17th) is shared by almost all major SARS-CoV-2 variants. Beyond the functional effects above, this mutation is known to increase transmission of infection[40] and to help maintain Spike in an active conformation for receptor recognition[29]. Six Spike N-terminal domain (NTD) mutations/deletions (T19I/R, Δ25, A67V, G142D, and Δ143) are also strongly selected. These lie in the antigenic supersite where mutations have been shown to decrease the neutralization potency of NTD-specific monoclonal antibodies[29,41]. The well-known Spike mutation D614G ($\hat{s} = 3.2\%$, ranked 68th) falls just outside the top 50 mutations in **Table 1**. D614G has been shown to increase binding affinity to the ACE2 receptor, thus increasing viral load and likely contributing to increased transmission[7,42,43].

Research on viral transmission has naturally focused on Spike because of its role in viral entry and as a target of neutralizing antibodies. However, our analysis also reveals strongly selected mutations outside of Spike. These include the Nucleocapsid mutations R203M and D3L. R203M ($\hat{s} = 10.4\%$, ranked 8th) is in the linker region of Nucleocapsid and has been shown to enhance viral RNA replication, delivery, and packaging, which may increase transmission[44]. D3L ($\hat{s} = 8.4\%$, ranked 12th) has been reported to increase production of a non-canonical subgenomic RNA that encodes for ORF9b (ref.[45]), an interferon suppressing gene that can aid innate immune evasion and thereby increase transmission[46].

Our analysis identifies strongly selected mutations in Omicron whose functional effects have not yet been fully explored (**Table 1**). Within Spike, these include mutations near the S1/S2 furin cleavage site, including S704L ($\hat{s} = 6.9\%$, ranked 19th), observed only in Omicron subvariant BA.2.12.1, and N679K ($\hat{s} = 4.9\%$, ranked 37th), observed in all Omicron subvariants. The NTD mutation V213G and deletion Δ25 ($\hat{s} = 4.4\%$ and 4%, ranked 43rd and 48th), both observed in all Omicron subvariants except BA.1, are also strongly selected. Outside Spike, top mutations/deletions in all Omicron subvariants include the NSP4 T492I mutation ($\hat{s} = 15.5\%$, ranked 1st), also found in Lambda and Mu, NSP6 deletion Δ106 ($\hat{s} = 15\%$, ranked 2nd), observed also in all variants of concern (VOCs) except Delta, and Nucleocapsid deletions Δ31-32 ($\hat{s} = 7.3\%$ and 7.2%, ranked 16th and 18th). These mutations may present good targets for future functional studies.

Estimates of selection for major SARS-CoV-2 variants

We estimated the net increase in viral transmission relative to the Wuhan-Hu-1 reference sequence for well-known SARS-CoV-2 variants by adding contributions from the individual variant-defining SNVs (**Fig. 3** and **Supplementary Fig. 4**, see Methods). Because our model uses global data and infers the transmission effects of individual SNVs, variants can be compared to one another directly even if they arose on different genetic backgrounds, or if they appeared in different regions or at different times. This also allows us to infer substantially increased transmission for variants such as Gamma, Beta, Lambda, and Epsilon, which never achieved the level of global dominance exhibited by Alpha, Delta, or Omicron (**Supplementary Fig. 4**).

Our findings are consistent with past estimates that have shown a substantial transmission advantage first for Alpha and then for Delta relative to other pre-Omicron lineages[47–49]. However, past estimates have varied substantially depending on the data source and method of inference. In different analyses Delta has been inferred to have an advantage of between 34% and 97% relative to other pre-Omicron lineages[47,48,50]. Similarly, Alpha has been estimated to increase transmission by 29% to 90% relative to pre-existing lineages in different regions[5,11,12,47,51]. One advantage of our approach is that it can infer selection coefficients that best explain the growth or decline of variants across many regions, allowing selection for different variants to be compared on even footing.

At present, Omicron (BA.1) and related subvariants are clearly inferred to be far more transmissible than past variants (**Fig. 3** and **Table 2**). The transmission advantage of BA.1 ($\hat{w} = 197\%$), which we estimate to be the least transmissible of Omicron subvariants, is still more than twice as large

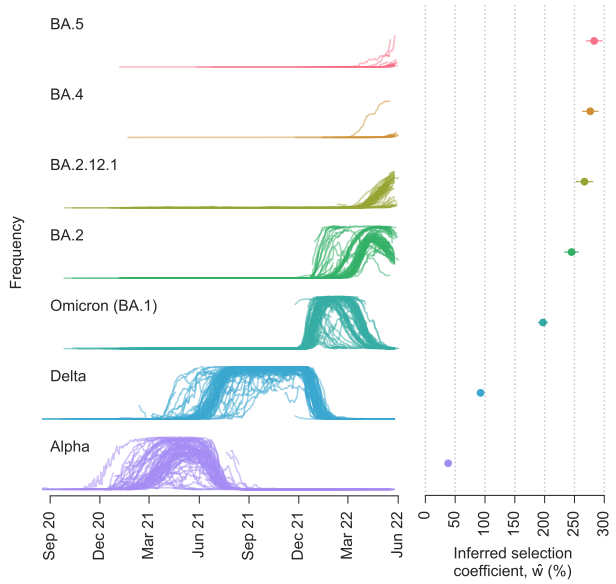

Fig. 3. Multiple SARS-CoV-2 variants strongly increase transmission rate. Frequencies of major variants and their total inferred selection coefficients, shown as mean values ± one s.d. from bootstrap subsampling of regional data (Methods), defined relative to the Wuhan-Hu-1 reference sequence. Selection coefficients for variants with multiple SNVs are obtained by summing the effects of all variant-defining SNVs.

as the inferred selection coefficient for Delta ($\hat{w} = 93\%$). BA.2, the most-prevalent Omicron subvariant worldwide as of June 20, 2022, is estimated to have a transmission advantage of $\hat{w} = 245\%$. We estimate the transmission advantage of emerging Omicron subvariants (BA.2.12.1, BA.4, and BA.5) to be higher than BA.2. Here the Spike RBD mutation Q493R, shared by BA.1, BA.2, and BA.2.12.1, may play an important role. While most Spike mutations in BA.1 are inferred to be beneficial, our analysis suggests that Q493R is deleterious ($\hat{s} = -5.3\%$). This mutation has reverted in Omicron subvariants BA.4 and BA.5 and significantly contributes to their higher inferred transmissibility.

Though data is currently limited, we estimate some differences in transmissibility between emerging Omicron subvariants BA.4, BA.5, and BA.5.1, which have identical Spike mutations (**Supplementary Fig. 5**). Consistent with preliminary reports[52,53], we estimate BA.5 to be more transmissible than BA.4. Our approach suggests that the Membrane mutation D3N ($\hat{s} = 4.8\%$, ranked 39th, **Table 1**) which is exclusive to BA.5, contributes to the increased transmissibility of BA.5. We also estimate BA.5.1 to be slightly more transmissible than BA.5 due to the ORF10 mutation L37F ($\hat{s} = 2.5\%$, ranked 100th), present only in BA.5.1. While more data will be necessary to fully assess the transmission advantage of these subvariants, our model enables identification of non-Spike mutations that may play a role in conferring selective advantage to emerging variants.

Rapid detection of variants with enhanced transmission

Rapidly identifying variants with increased transmission is important to inform public health efforts to limit viral spread. However, the inherent stochasticity of infection and of genomic surveillance data collection makes accurate inferences difficult. For example, neutral or modestly deleterious variants may initially appear to be beneficial due to a transient rise in frequency despite having no selective advantage.

To quantify how fluctuations affect estimates of selection for neutral variants, we first identified all variants (including both SNVs and collections of SNVs that are strongly linked to one another, see Methods) that are inferred to have selection coefficients with magnitude less than 10% using all of the data gathered until February 1, 2021. Here, we reasoned that changes in transmissibility of 10% or more could fairly be classified as "concerning." We then calculated the selection coefficients that would have been inferred for the SNVs or variants at all earlier time points and in all regions after they were first observed in the data. This "null" distribution (**Fig. 4d**) quantifies fluctuations in inferred selection coefficients for quasi-neutral variants due to stochasticity in viral spread and sampling. Variants with selection coefficients larger than any in the null distribution could then be expected with high confidence to have a significant transmission advantage.

We then tested the effectiveness of our approach for automatically detecting concerning variants using data gathered from all regions until June 9, 2022 (**Table 3**). Ideally, we should specifically identify concerning groups of mutations without flagging ones that have little effect on viral transmission. While it is difficult to conclusively assign particular detection events as true or false positives, we conservatively assumed that all detections of groups of SNVs belonging to major variants denoted by Greek characters are true positives, and detections of any other SNVs (including mutations specific to AY lineages, B.1, and unnamed B.1 sublineages) are false positives. Following this assumption, the positive predictive value of our method is 97%. This value could be yet higher, however, as there is evidence that mutations associated with B.1 and B.1.1.318, including the well-known Spike mutation D614G, increase viral transmission[7,42,54,55].

To further explore our capacity to rapidly detect variants with a transmission advantage, we studied the rise of the Alpha, Delta, and Omicron (BA.1) variants in specific regions. Here, we focused specifically on novel mutations in these variants, i.e., mutations that had not been observed in previous SARS-CoV-2 sequences. Using the above criterion, we find that novel mutations in Alpha are likely to substantially increase transmission using sequence data from London collected on or before November 12, 2020 (**Fig. 4a**). This is roughly three weeks before Public Health England labeled Alpha as a variant of interest (VOI)[56], and more than a month before it was classified as a VOC[57]. At this time the frequency of Alpha in London was around 2%.

Similar analyses show that our model rapidly infers increased transmission for novel mutations in Delta and Omicron. Using data from Great Britain, we infer Delta to significantly increase transmission by April 15, 2021 (**Fig. 4b**). Delta was classified as a VOI on April 4, 2021 and as a VOC more than one month later on May 6, 2021 (ref.[58]). Omicron was inferred to have a significant transmission advantage using sequence data from South Africa collected by October

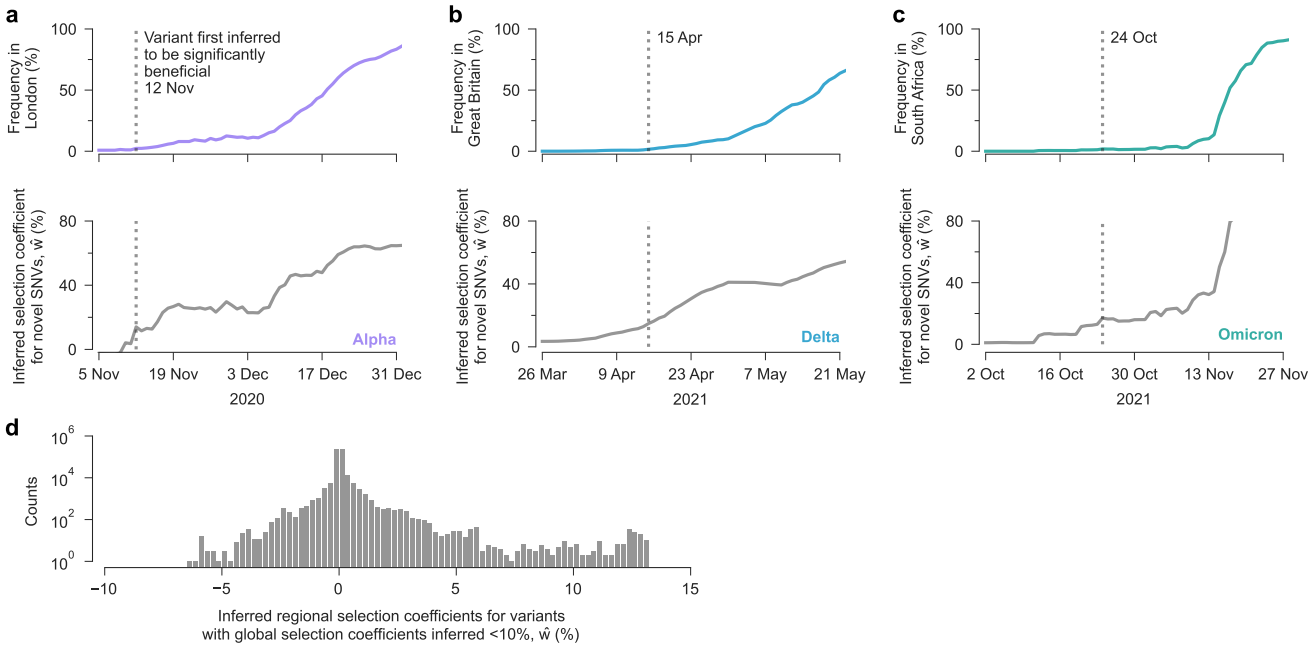

Fig. 4. Our model rapidly infers increased transmission for Alpha, Delta, and Omicron (BA.1). **a**, Frequency of Alpha in London and its inferred selection coefficient over time. The inferred coefficient exceeds the largest one in the null distribution on November 12, 2020. **b**, Frequency of Delta in Great Britain and the inferred selection coefficient for novel Delta SNVs over time. **c**, Frequency of Omicron in South Africa and the inferred selection coefficient for novel Omicron SNVs over time. **d**, The null distribution of regional inferred selection coefficients for variants ultimately inferred to have selection coefficients $|w| < 10\%$ using global data, over all intermediate times and across all regions.

24, 2021 (**Fig. 4c**). Omicron was designated a variant under monitoring (VUM) on November 24, 2021 and a VOC two days later[59]. At the time that we detected increased transmission for Delta and Omicron, their frequencies were still very low ($< 2\%$) in Great Britain and South Africa, respectively. Collectively, these results demonstrate our ability to rapidly identify variants with higher transmission, even when they represent a small fraction of all infections in a region and when the influence of previously-observed mutations is ignored.

Discussion

Quantifying the effects of mutations on viral transmission is an important but challenging problem. To overcome limitations of current methods, we developed a flexible, branching process-based epidemiological model that provides analytical estimates for the transmission effects of SNVs from genomic surveillance data. Applying our model to SARS-CoV-2 data, we identified SNVs that substantially increase viral transmission, including both experimentally-validated Spike mutations and other, less-studied mutations that may be promising targets for future investigation. Importantly, we found that our model is sensitive enough to detect substantial transmission advantages for variants such as Alpha, Delta, and Omicron even when they comprised only a small fraction of the total number of infections in a region, thus providing an "early warning" for more transmissible variants.

The epidemiological model that we have introduced has limitations. We assumed that transmission takes place shortly after infection, which is appropriate for a virus such as SARS-CoV-2. Our approach would need to be modified to consider the spread of viruses where many transmission events are from long-term infections, such as HIV. We also assume that SNVs contribute additively to fitness and that selection coefficients are constant in time. Our model does not delineate intrinsic (e.g., functional) effects of SNVs on transmission from selection advantages due to immune escape; though, for many of the SNVs inferred most strongly to affect transmission, there is independent experimental evidence to suggest that each (or both) of these factors are important (**Table 1**). Simulations show that if selection is time-varying, the constant selection coefficients that we infer reflect averages of time-varying selection over the time that the variant was observed (**Supplementary Fig. 6**). Epistasis could also lead to over- or under-estimation of selection coefficients for specific SNVs, but total contributions to transmission from multiple SNVs are typically estimated accurately (**Supplementary Fig. 7**). We have also assumed that serial intervals are constant in time, but variants may differ in the typical time between infections[60] which could influence relative growth rates. Further studies can extend our technical approach to relax these assumptions.

Our ability to rapidly detect concerning new variants is naturally limited by the public availability of sequence data. Time lags between when sequencing is performed and when sequences are uploaded, in particular, can lead to delays in detection. When we filter sequences based on submission date and repeat the analysis presented in **Fig. 4**, detection of Alpha and Delta is pushed back by approximately 2.5 weeks (**Supplementary Fig. 8**). For Omicron, the delay is larger: inference using sequence data uploaded to GISAID on De-

cember 7th, 2021 clearly shows an enormous transmission advantage for this variant. However, one strength of our approach is that we are able to estimate the effects of individual mutations on viral transmission. Thus, even in cases where sequence data for a novel variant is limited, emerging variants could be classified as concerning purely based on the presence of previously-observed mutations. For example, Alpha, Delta, and Omicron (BA.1) would have had estimated selection coefficients of $\hat{w} = 18\%$, 17%, and 66%, respectively, immediately prior to their first observations in sequence data. Nonetheless, reducing the time between when sequencing is performed and when sequence data is publicly shared could facilitate the detection of new variants with increased transmission and help prepare for growing outbreaks.

While our study has focused on SARS-CoV-2, the epidemiological model that we have developed is very general. The same methodology could be applied to study the transmission of other pathogens such as influenza. Combined with thorough genomic surveillance data, our model provides a powerful method for rapidly identifying more transmissible viral lineages and quantifying the contributions of individual mutations to changes in transmission.

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AUTHOR CONTRIBUTIONS

All authors contributed to methods development, data analysis, interpretation of results, and writing the paper. B.L. and J.P.B. led theoretical analyses. M.S.S. and B.L. led simulations. J.P.B. conceptualized the project. A.A.Q. led validation of SARS-CoV-2 inference results. J.P.B. and M.R.M. supervised the overall project.

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Methods

Epidemiological model

We use a discrete time branching process to model the spread of infection. Individuals can be infected by any one of M viral variants, which are represented by genetic sequences $\boldsymbol{g} = \{g_1, g_2, \ldots, g_L\}$ of length L. For simplicity, we will first assume that alleles at each site i in the genetic sequence for variant a are either equal to the "wild-type" or reference ($g_i^a = 0$) or mutants ($g_i^a = 1$). Later we will relax this assumption to consider genetic sequences with 5 possible states at each site (4 nucleotides or a gap). We call $n_a(t_m)$ the number of individuals infected by variant a at time t_m. To account for super-spreading, the number of newly infected individuals at time t_{m+1} follows a negative binomial distribution[61–66], $P\left(n_a(t_{m+1})|n_a(t_m), k, R_a\right) = P_{NB}\left(r,p\right)$, where $r = n_a k,\ p = k/(k+R_a)$, and $R_a = R(1+w_a)$. Here r and p are the negative binomial distribution parameters, k is the dispersion, R is the effective reproductive number of the reference variant, and w_a encodes the variant dependence of the infectivity. The parameters n, k, and R can be time-varying. For instance, a time-varying R represents change in the number of susceptible and recovered individuals as well as the effects of public health interventions or changes in behavior that modify viral transmission.

Defining the frequency of variant a as $y_a = n_a/\sum_b n_b$, the probability that the frequency vector is $\boldsymbol{y}(t_{m+1}) = \{y_1(t_{m+1}), y_2(t_{m+1}), \ldots\}$ given the initial frequency vector $\boldsymbol{y}(t_0)$, is

$$P((\boldsymbol{y}(t_m))_{m=1}^T|\boldsymbol{y}(t_0)) = \prod_{m=0}^{T-1} P(\boldsymbol{y}(t_{m+1})|\boldsymbol{y}(t_m))\,. \quad \textbf{(2)}$$

Derivation of the estimator

Because (2) is difficult to work with directly, we follow the approach of ref.[67]. We introduce a "diffusion approximation" where we assume that the total number of infected individuals is large and the effects of mutations on transmission are small. Similar approximations have been widely used in population genetics[68–70]. Under these assumptions, the probability distribution for the variant frequencies satisfies a Fokker-Planck equation with terms derived from the first and second moments of the frequency changes $y_a(t_{m+1}) - y_a(t_m)$ under the negative binomial distributions above.

However, the genotype space is high-dimensional (dimension 2^L, with either a mutant or wild-type allele at each site) and undersampled, making inference of selection for genotypes extremely challenging. To simplify the inference problem, we assume that selection is additive, so the total selection coefficient w_a for a variant a is the sum of selection coefficients s_i for mutant alleles at each site i:

$$w_a = \sum_{i=1}^{L} g_i^a s_i\,.$$

We can then derive a Fokker-Planck expression for the dynamics of mutant allele frequencies

$$x_i = \sum_{a=1}^{M} g_i^a y_a\,.$$

At the allele level, the Fokker-Planck equation has a drift vector given by

$$d_i(\boldsymbol{x}) = x_i(1-x_i)s_i + \sum_{j=1, j\neq i}^{L} (x_{ij} - x_i x_j)s_j\,, \quad \textbf{(3)}$$

and a diffusion matrix

$$C_{ij} = \left(\frac{1}{k} + \frac{1}{R}\right) \times \begin{cases} x_{ij} - x_i x_j & i \neq j \\ x_i(1-x_i) & i = j \end{cases},$$

where x_{ij} is the frequency of infected individuals that have mutant alleles at both site i and site j at time t. In deriving (3) we have assumed that the selection coefficients satisfy $s_i \ll 1$ such that $w_a \ll 1$. Despite this technical assumption, our simulations demonstrate that selection can be accurately inferred even when selection is strong (**Supplementary Fig. 9**).

The drift vector describes the expected change in allele frequencies over time. Eq. (3) consists of two terms. The first describes the expected change in the frequency of allele i due to selection at that site. The second term accounts for linkage, that is, it quantifies how the genetic background alters the expected frequency change of an allele.

The Fokker-Planck equation can then be used to derive a path integral, which expresses the probability of an entire evolutionary history or "path" (i.e., frequencies of genetic variants over time, $\boldsymbol{x}(t_m)_{m=1}^T$). In Supplementary Information, we derive the path integral expression following a similar approach to the one described in ref.[67]. The path integral is

$$P\left((\boldsymbol{x}(t_m))_{m=1}^T|\boldsymbol{x}(t_0), \boldsymbol{s}, n\right) \approx \left(\prod_{m=0}^{T-1} \frac{1}{\sqrt{\det C}} \left(\frac{n}{2\pi\Delta t_m}\right)^{L/2} \prod_{i=1}^{L} dx_i(t_{m+1})\right) \exp\left(-\frac{n}{2} S((\boldsymbol{x}(t_m)_{m=0}^T)\right), \quad \textbf{(4)}$$

$$\text{where} \quad S((\boldsymbol{x}(t_m)_{m=0}^T) = \sum_{m=0}^{T-1} \left[\frac{\boldsymbol{x}(t_{m+1}) - \boldsymbol{x}(t_m)}{\Delta t_m} - \boldsymbol{d}(\boldsymbol{x}(t_m))\right] C^{-1}(\boldsymbol{x}(t_m)) \left[\frac{\boldsymbol{x}(t_{m+1}) - \boldsymbol{x}(t_m)}{\Delta t_m} - \boldsymbol{d}(\boldsymbol{x}(t_m))\right].$$

Here $n = \sum_{a=1}^{M} n_a$ is the total number of individuals infected by all variants and $\Delta t_m = t_{m+1} - t_m$. The path integral in (4) has a form that is similar to the one obtained in ref.[67]. The path integral quantifies the probability density for paths of mutant allele frequencies in the evolutionary history of the pathogen. We can then use Bayesian inference to find the maximum *a posteriori* estimate for the selection coefficients given the frequencies, the infected population size, the parameters R and k. The posterior probability of the selection coefficients is

$$P\left(\boldsymbol{s}|(\boldsymbol{x}(t_m),n)_{m=0}^{T}\right) \propto P\left((\boldsymbol{x}(t_m))_{m=1}^{T}|\boldsymbol{x}(t_0),\boldsymbol{s},n\right) \times P_{\text{Prior}}(\boldsymbol{s}), \tag{5}$$

where $P\left((\boldsymbol{x}(t_m))_{m=1}^{T}|\boldsymbol{x}(t_0),\boldsymbol{s},n\right)$ is the probability of a path given by (4) and the $P_{\text{Prior}}(\boldsymbol{s})$ is a Gaussian prior probability for the selection coefficients with zero mean and covariance matrix $\sigma^2 I$. Here, I is the identity matrix and σ^2 is the variance of the prior. We call the precision $\gamma = 1/\sigma^2$. In Supplementary Information we show that the selection coefficients that maximize (5) are

$$\hat{\boldsymbol{s}} = \left[\gamma I + \sum_{m=0}^{T-1} \frac{nk^2R^2}{(k+R)^2}\Delta t_m C(t_m)\right]^{-1} \left[\sum_{m=0}^{T-1} \frac{nkR}{k+R}\left(\boldsymbol{x}(t_{m+1}) - \boldsymbol{x}(t_m)\right)\right], \tag{6}$$

where the parameters k, R, and n are implicitly functions of t.

There are two interesting limiting forms of the estimator. First, we define the new matrix $\bar{C}$ whose entries are

$$\bar{C}_{ij} = \begin{cases} x_{ij}(t_m) - x_i(t_m)x_j(t_m) & i \neq j \\ x_i(t_m)(1 - x_i(t_m)) & i = j \end{cases}. \tag{7}$$

In the limit that $k \to \infty$, the negative binomial distribution for new infections becomes a Poisson distribution with rate $\lambda = R$. In this special case, the model is equivalent to the Wright-Fisher model from population genetics. The estimator reduces to

$$\hat{\boldsymbol{s}} = \left[\gamma I + \sum_{m=0}^{T-1} nR\,\bar{C}\right]^{-1} \left[\sum_{m=0}^{T-1} nR\left(\boldsymbol{x}(t_{m+1}) - \boldsymbol{x}(t_m)\right)\right].$$

The opposite limit $k \to 0$ corresponds to a distribution for new infections with extremely heavy tails, i.e., one where super-spreading is dominant. In this case the drift in (3), which quantifies expected frequency changes due to selection, is unchanged. However, the diffusion matrix, which encodes linkage as well as the changes in frequency that are due to the stochastic nature of infection transmission, diverges. In this case, diffusion dominates the process entirely.

Simplifying the estimator and robustness to incomplete knowledge of time-varying parameters

While our model has the ability to account for the time dependence of parameters appearing in (6), such as the infected population size n, the dispersion k, and the mean reproductive number R, these can be challenging to reliably estimate from data. However, we generally do not require full knowledge of these time-dependent parameters to accurately estimate selection.

In fact, due to finite sampling noise, estimates of selection produced by assuming constant (and incorrect) parameters are more accurate than estimates that use the true time-varying parameters (**Supplementary Fig. 10**). The naive estimator in (6) implies that time points or regions with larger R, n, or k should be weighted more heavily in the estimate. However, frequency information is always inaccurate due to noise from finite sampling, so weighing some time points or regions significantly more than others based upon the parameters alone means that undue weight is given to the uncertain information available from these times and regions.

For this reason, we assume parameters that are spatially and temporally constant in all of the following analysis as discussed below. This allows the estimator to be simplified substantially. If we assume constant parameters and scale the regularization γ by $nkR/(k+R)$ in the numerator in (6), the parameter dependence in the numerator and the denominator is identical and cancels out (due to the additional factor of $(k+R)/kR$ in the definition of the covariance matrix). With the same definition of the matrix $\bar{C}$ as above, and additionally defining $\bar{C}_{\text{int}} = \sum_{m=0}^{T-1} \Delta t_m \bar{C}$ and $\gamma' = \gamma nkR/(k+R)$, the simplified estimator is given by

$$\hat{\boldsymbol{s}} = \left[\gamma' I + \bar{C}_{\text{int}}\right]^{-1} \left[\boldsymbol{x}(t_T) - \boldsymbol{x}(t_0)\right]. \tag{8}$$

This form of the estimator is similar to the estimator for selection coefficients in the Wright-Fisher model[67], except that it omits contributions from the mutation term, because the mutation rate for SARS-CoV-2 is small. Practically, (8) has significant advantages over (6). The most important is that the difficult-to-estimate parameters k and n are no longer required. In addition, R does not need to be estimated. For methods of inferring these parameters as well as discussions about the difficulty of inferring them, see refs.[71–80].

Extension to multiple regions and multiple SNVs at each site

The model can easily account for outbreaks in multiple regions or outbreaks at different times. If the probability of the

evolutionary path in each region is independent, which is the case if there is no travel between regions, then the probability of all of the evolutionary paths in all of the regions is simply the product of the probabilities of the paths in each region, given by (4). Bayesian inference can be applied in the same way as before, resulting in the estimator

$$\hat{\boldsymbol{s}} = \left[\gamma' I + \sum_{r=1}^{Q} \bar{C}_{r,\text{int}}\right]^{-1} \left[\sum_{r=1}^{Q} \boldsymbol{x}_r(t_{r,T_r}) - \boldsymbol{x}_r(t_{r,0})\right], \quad \textbf{(9)}$$

where Q is the number of regions, t_r is the time in region r, t_{r,T_r} is the final time in region r, $t_{r,0}$ is the initial time in region r, $\boldsymbol{x}_r$ is the frequency in region r, and $\bar{C}_{r,\text{int}}$ is the scaled integrated covariance matrix in region r given by integrating (7) over time. The estimator can further be extended to allow for multiple different nucleotides at each site by simply letting each different nucleotide have its own entry in the frequency vector x_i. If there are J mutations at each site this results in a frequency vector of length LJ, and a covariance matrix of size $LJ \times LJ$. By convention, reference sequence alleles have selection coefficients of zero, so the mutant allele selection coefficients at each site are normalized by subtracting the inferred coefficient for the reference allele.

Branching process simulations

We implemented the superspreading branching process for the number of infected individuals in Python. We used a negative binomial distribution for the number of secondary infections caused by a group of individuals infected with the same pathogen variant. To test how finite sampling affects model estimates, we sampled n_s genomes per time point to use for analysis. We computed the single and double mutant frequencies, x_i and x_{ij}, respectively, from the sampled sequences and estimated the selection coefficients from these using (1), possibly extended to account for multiple outbreaks or multiple alleles at each locus as described above. For the analysis of how finite sampling affects estimates, shown in **Supplementary Fig. 10**, we use the full version of the estimator given by (6). For all other simulations, we assume that the parameters n, k, and R are not known for inference and so we use the simplified estimator in (9) for inferring selection.

Regions and time-series for SARS-CoV-2 analysis

We used sequence alignments and metadata downloaded from GISAID (ref.[81]) on June 9th, 2022, which includes more than 5.6 million sequences. One potential caution in interpreting this data is that not all sequences in the database will have been generated from unbiased surveillance efforts.

Ideally, we would like to divide this data into the smallest separate areas that have outbreaks that are largely independent of those in the surrounding regions, so as to avoid biases due to travel between regions or unequal sampling in different locations. However, this needs to be balanced with the limitations of the data, since regions with poor sampling could contribute more noise than signal. We therefore divided data into the smallest regions available in the metadata that are still large enough such that infections resulting from travel outside of the region are likely to be far less frequent than transmission within the region. This results in the inclusion of mostly separate countries in Europe and Asia and states in North America. Two exceptions to this are that we separate northern and southern California due to the geographical separation of population centers, and we separate Northern Ireland from the rest of the United Kingdom due to its geographical isolation.

To minimize the effects of sampling noise, we chose regions and time-series within these regions based on the following criteria:

1. In any period of 5 days within the time-series there are at least 20 total samples.
2. The number of days in the time-series is greater than 20.
3. The number of new infections per day is at least 100.

The last criterion ensures that there are enough infected individuals that transmission is not driven overwhelmingly by stochasticity. We assessed the number of newly infected individuals by using the estimates provided by the Institute of Health Metrics and Evaluations[82]. Since the dates provided in their estimates correspond to dates when individuals were infected, and dates in the GISAID sequence data correspond to dates when individuals were sequenced, we shifted the dates in the IHME data 5 days forward to roughly compensate for delays between infection and sequencing. We then eliminated days on which the estimated number of new infections was smaller than 100.

Our results are robust to reasonable variation in these parameters. Comparing the number of locations used and the sample sizes shown in **Supplementary Fig. 11** in the data to those used in the simulations shown in **Supplementary Fig. 1**, we expect our inference to accurately distinguish beneficial, deleterious, and neutral SNVs from one another.

Data processing

We perform a number of preprocessing steps to ensure data quality. We first eliminated incomplete sequences with gaps or ambiguous nucleotides at more than 1% of the genome. We then removed sites from our analysis where gaps are observed at $> 95\%$ frequency, since these sites may represent very rare insertions or sequencing errors. We also removed sites in noncoding regions of the SARS-CoV-2 genome and ones where all observed SNVs are synonymous. We imputed gaps that are not associated with known variants and ambiguous nucleotides with the nucleotide at the same site that occurs most frequently in other sequences from the same region.

For the remaining sites, in each region we excluded rare SNVs whose frequency is not larger than 1% for at least 5 consecutive days. These sites, if included, are almost always inferred to have extremely small selection coefficients. Furthermore, since their frequencies are so small, their covariance with other sites is also small and is therefore unlikely to have a large effect on inference. We verified that different

reasonable values for these cutoffs result in essentially identical selection coefficients (**Supplementary Fig. 12**).

Calculating frequency changes and covariances

To increase robustness to finite sampling in time, we integrated terms in (6) and other time-dependent equations over time by assuming that frequencies are piecewise linear, rather than summing contributions from each time point[67]. This results in diagonal terms of the integrated covariance being calculated as

$$\sum_{m=0}^{T-1} \Delta t_m \left[\frac{(3-2x_i(t_{m+1}))(x_i(t_m)+x_i(t_{m+1}))}{6} - \frac{x_i^2(t_m)}{3} \right],$$

and off-diagonal elements being calculated as

$$\sum_{m=0}^{T-1} \Delta t_m \left[\frac{x_{ij}(t_m)+x_{ij}(t_{m+1})}{2} - \frac{x_i(t_m)x_j(t_m)+x_i(t_{m+1})x_j(t_{m+1})}{3} - \frac{x_i(t_m)x_j(t_{m+1})+x_i(t_{m+1})x_j(t_m)}{6} \right].$$

For obtaining reliable estimates of the changes in SNV frequencies (the term $x(t_T) - x(t_0)$ in (8)), it is important to have enough sequences to avoid large errors due to finite sampling. On the other hand, if a large number of days are used at the end or the start of the time-series to calculate the frequencies, then the frequency changes are likely underestimates. To balance these competing issues, we calculated $\boldsymbol{x}(t_T)$ as the frequencies in the window of the final 15 days and $\boldsymbol{x}(t_0)$ as the frequencies in the window of the first 15 days for each time-series and region with poor sampling. This smoothing is necessary especially in regions where sampling is sparse, where the number of genomes sampled on a particular day may be as small as 1 or 2. If there are at least 200 sampled sequences in a period of less than 15 days at the start or the end of the time-series, then the window size was taken as the smallest number of days in which there was a total of at least 200 sequences. We confirmed that our results are robust to reasonable changes of this window size of 15 days (**Supplementary Fig. 12**).

We also normalized time in units of serial intervals or "generations" by dividing the integrated covariance matrix by 5, following results that the serial interval for SARS-CoV-2 is roughly 5 days[83–85]. This allows us to convert from units of time in days to generations, as in (8).

Calculating selection coefficients

After the above preprocessing there remain 1,259 SNVs observed at a frequency above 1% for at least 5 consecutive days in at least one region and observed at least 5 times. We assume constant values for R, n, and k in all regions, and use (9) to estimate selection. When R, n, and k are constant, these terms can be effectively absorbed into the regularization γ'.

We normalize selection coefficients such that the nucleotide for the Wuhan-Hu-1 reference sequence at each site has a selection coefficient of 0. To do this, we subtract the selection coefficient for the reference nucleotide from the inferred coefficient for each other allele at that site after all selection coefficients have been computed.

In order to quantify selection for groups of SNVs that frequently appear together, one must be able to determine what SNVs are closely linked. To determine sets of strongly linked SNVs, we considered the following statistics. If the number of genomes with a SNV at site i is called h_i and the number of genomes with SNVs at both site i and site j is h_{ij}, then we say that two sites i and j are strongly linked if h_{ij}/h_i and h_{ij}/h_j are both greater than 80%.

To form sets of strongly linked SNVs, we combined all pairs of strongly linked SNVs that share SNVs in common. For example, if SNV i is strongly linked with SNV j, and SNV j is strongly linked with SNV k, then $\{i,j,k\}$ forms one set of strongly linked SNVs. With the frequency cutoff that we have used for the definition of strongly linked SNVs (80%), the great majority of SNVs in each set of strongly linked SNVs are strongly linked to all other SNVs in the same set.

As for the major variants, we computed selection coefficients for sets of strongly linked SNVs by summing the contributions from individual SNVs. Selection coefficients for strongly linked SNVs were used to compute the "null" distribution that we use as a metric for early detection of variants with increased transmission.

We used these estimates for the selection coefficients for nonsynonymous SNVs to estimate the corresponding selection coefficients for amino acid substitutions (**Table 1**). If there were multiple SNVs in a codon that result in the same amino acid variant, but are not strongly linked to one another, then the selection coefficient for the amino acid was calculated as the largest (in absolute value) of the SNVs. If there were multiple SNVs in the same codon that yield the same amino acid and these SNVs are strongly linked to one another, then the selection coefficient for the mutant amino acid was calculated as the sum of the selection coefficients for the SNVs.

We calculated selection coefficients for major variants by summing the individual nucleotide SNVs that define the variant, which follows from our assumption of additive fitness. The SNVs for major named variants such as Alpha and Delta were identified according to the mutations provided by

https://covariants.org.

We also computed selection coefficients for collections of strongly linked SNVs that may not be officially-designated variants.

Computational complexity

Here we briefly discuss the computational complexity of our method. The steps in our data processing are

1. Clean the data (eliminate sequences with large numbers of Ns or gaps, etc.).
2. Separate the data by time and region.
3. Identify SNVs observed above the minimum frequency threshold.
4. Compute SNV covariance matrices/changes in SNV frequencies in each region and integrate them over time.
5. Infer the selection coefficients, which involves inverting the total integrated SNV covariance matrix.

Let L be the length of the SARS-CoV-2 sequence (roughly 3×10^4 bps) and let M be the total number of sequences (roughly 10^7 including data taken up until June 9th, 2022). Then, steps 1 and 2 involve computations that scale as $\mathcal{O}(M)$. Step 3 is $\mathcal{O}(ML)$. This step also introduces a new parameter relevant for the scaling of the problem, which is the fraction of SNVs that are observed at high enough frequencies to be included in our analysis. Let us call this fraction p, which is roughly 0.35 with our current settings. Naively, step 4 then involves a computation that scales like $\mathcal{O}(M(pL)^2)$. However, the calculation of the covariance can easily be parallelized across regions. In each individual region, the fraction of SNVs that are observed at high enough frequencies to be included is a different parameter q and the number of sequences in the region is a parameter M_r. The largest q that we find in the regions analyzed is around 0.05. For N_r separate regions (127 in our analysis), step 4 then involves N_r parallel computations that scale like $\mathcal{O}(M_r(qL)^2)$. Due to the matrix inversion, step 5 requires $\mathcal{O}((pL)^3)$ computations to complete.

Choice of regularization

In principle, the regularization strength γ' is related to the width of the prior distribution for SNV selection coefficients. The regularization strength also plays a role in reducing noise in selection coefficient estimates due to finite sampling of viral sequences. This is especially important for SNVs that are observed only briefly in data, as they will have small integrated variances in the "denominator" of (6). Larger values of the regularization more strongly suppress noise, but they also shrink inferred selection coefficients towards zero.

We use a regularization strength of $\gamma' = 40$. For much smaller values of γ', selection coefficient estimates are unstable due to sampling noise. However, inferred selection coefficients stabilize and become insensitive to the precise value of γ' for $\gamma' \gtrsim 10$ (**Supplementary Fig. 12**). Larger values of γ' will result in selection coefficients with smaller absolute values, but for large enough γ' the rank ordering of inferred selection coefficients is highly reliable. In summary, the coefficients that appear to be the most beneficial or deleterious remain this way regardless of reasonable choices for γ', though their precise values scales with the regularization strength.

Rapid detection of variants with increased transmission

To estimate how quickly we can detect a transmission advantage for a new SNV or variant, selection coefficients are calculated only in the specific region where the variant arose. Since inference is only done in a single region, SNVs that appear only briefly at low frequencies —and which therefore are unlikely to change transmission rate —only appear once, whereas in the global analysis such SNVs may appear at low frequencies in multiple regions. For this reason we use a lower regularization of 10 for regional analysis. The null distribution is calculated by first finding all variants (including one or more SNVs) that are inferred to have a selection coefficient of absolute value less than 10% using the joint inference over all regions with data collected until February 1st, 2021. We then calculated the selection coefficients that would have been inferred for these variants at all earlier time points in each region after they were first observed in that region. We can then say with high confidence that a variant significantly increases transmission once the inferred coefficient for that variant in a specific region surpasses any of the inferred coefficients in the null distribution.

Data and code

Sets of processed data, computer code, and scripts that we have used in our analysis are available in the GitHub repository located at https://github.com/bartonlab/paper-SARS-CoV-2-inference. This repository also contains Jupyter notebooks that can be run to reproduce the results presented here, using sequence data and metadata from GISAID. A full list of originating and submitting laboratories for the sequences used in our analysis can be found at https://www.gisaid.org using the EPI-SET-ID: EPI_SET_20220719kb.

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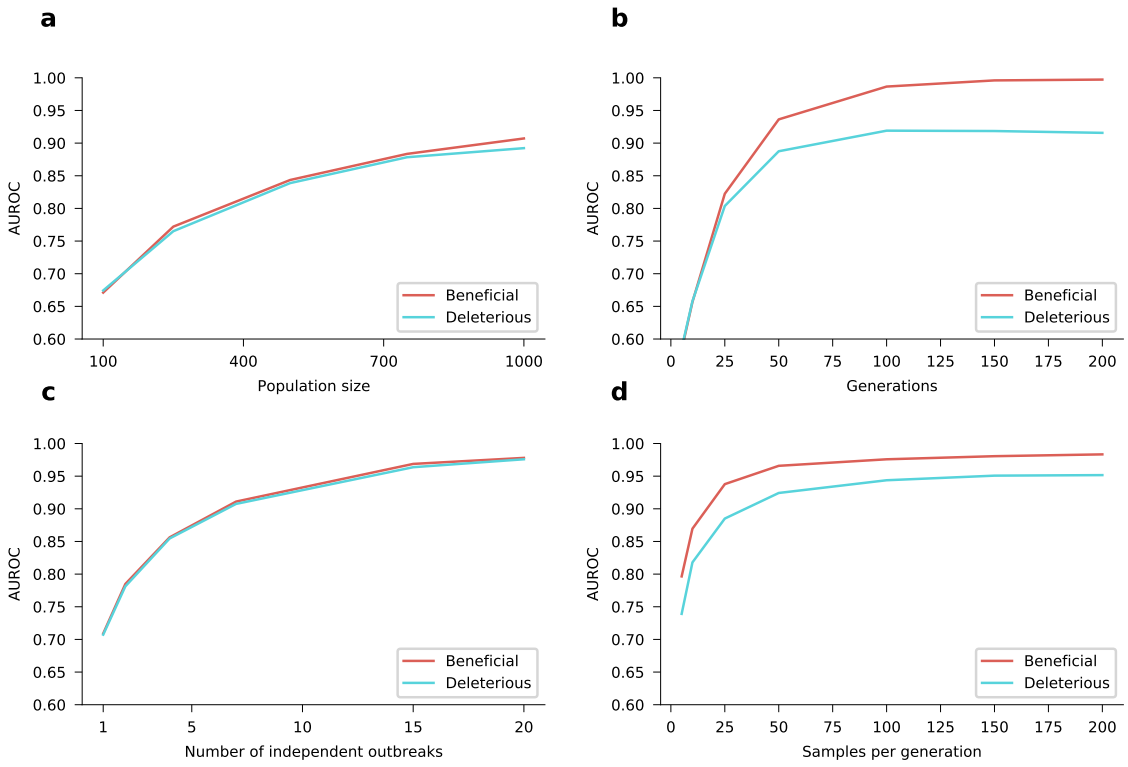
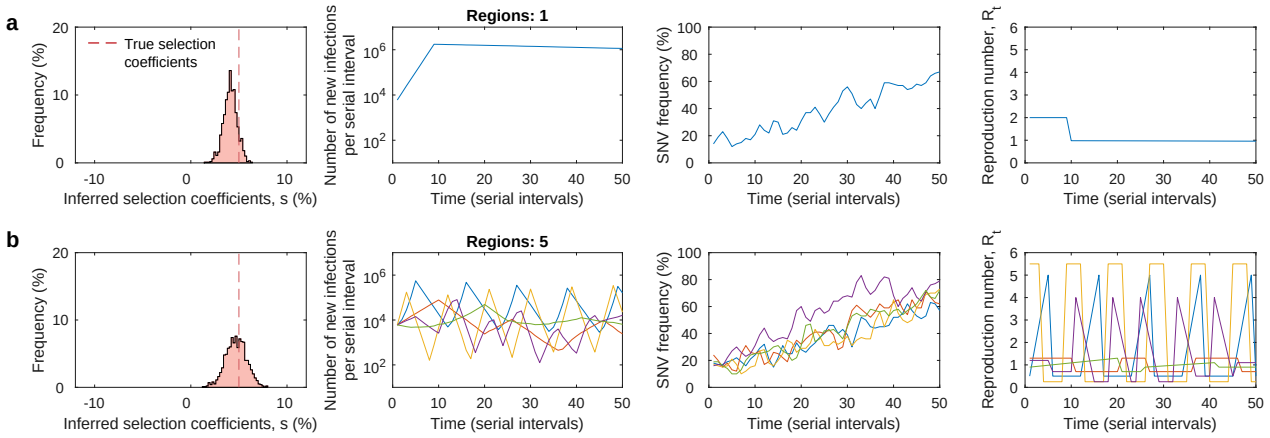
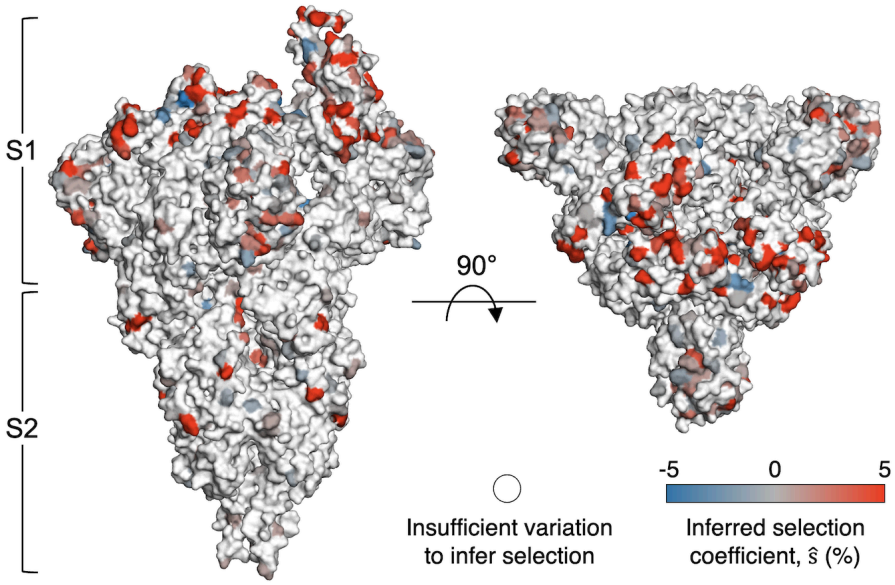


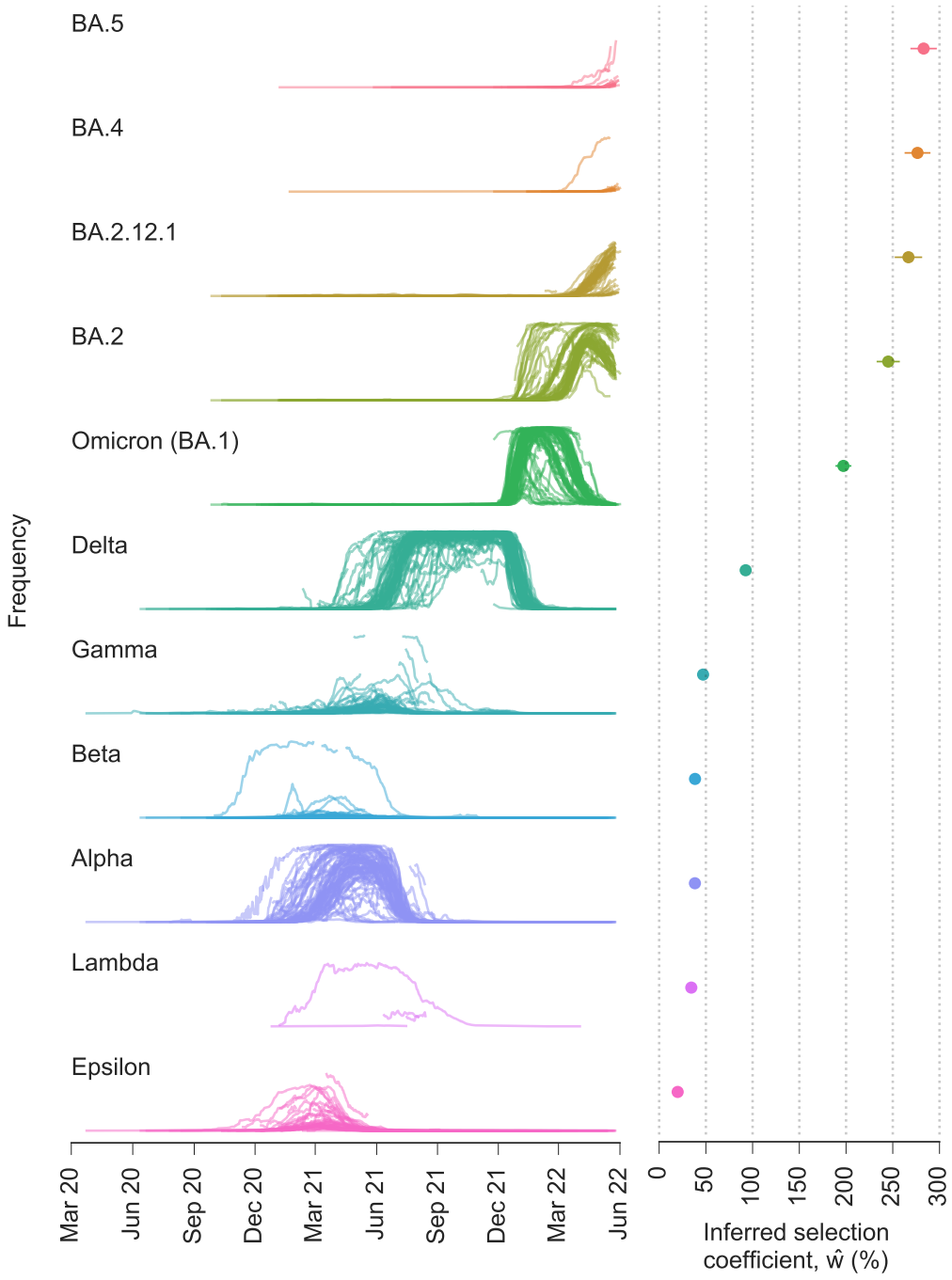
Supplementary Fig. 1. Accuracy of inference for different parameters. How the AUROC scores for both beneficial SNVs (in red) and deleterious SNVs (in blue) depends upon the different model parameters. **a**, Inference accuracy for different values of newly-infected population size. The parameters used are 10 simulations each with 50 sampled genomes per generation for 25 generations. **b**, Inference accuracy for different numbers of generations (serial intervals). Data is from a single simulation with 25 samples per generation and a newly-infected population size of 10,000. **c**, Inference accuracy for different numbers of independent outbreaks (simulations). The parameters used are 50 samples per generation for 10 generations and a newly-infected population size of 10,000. **d**, Inference accuracy for different values of samples per generations. Data is from a single simulation with 50 generations with a newly-infected population size of 10,000. The initial population is a mixture of two variants with beneficial SNVs ($s = 0.03$), two with neutral SNVs ($s = 0$), and two with deleterious SNVs ($s = -0.03$). Dispersion parameter k is fixed at 0.1. This is the same initial population composition as described in **Fig. 1**. All AUROC scores are calculated by averaging over 1,000 replicate simulations.



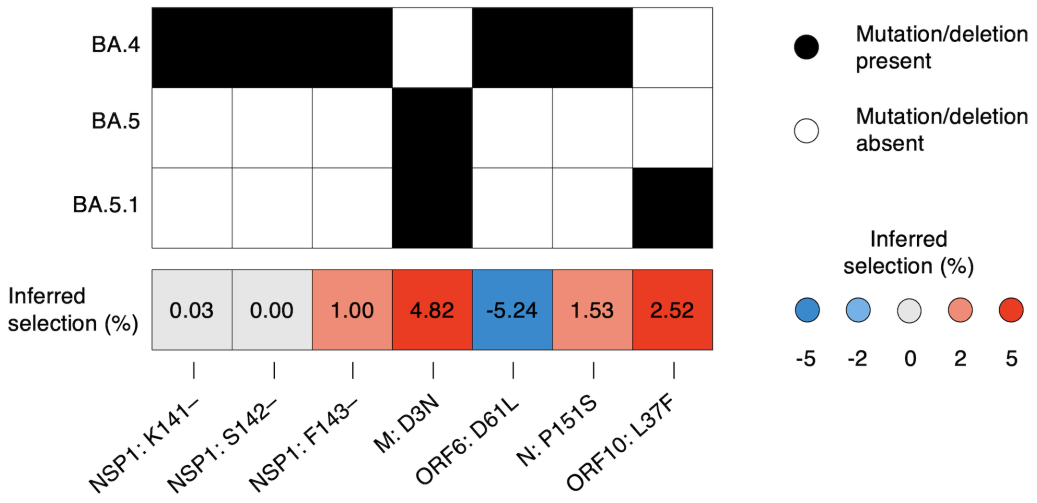
Supplementary Fig. 2. Inference is robust to variation of reproduction number, R, across regions. Our approach provides a systematic way to combine data from outbreaks in multiple regions. Simulations show that the estimator in (9) has good performance whether the selection coefficients are inferred based on data from, **a**, a single region or, **b**, five regions. *Simulation parameters.* The initial population in each region is a mixture of a neutral variant with no mutations and a variant with a beneficial SNV ($s = 0.05$). The same beneficial SNV appears in all 5 regions. Each region has a different profile of the time-varying reproduction number, R (rightmost panel). In the first simulation, the number of newly infected individuals per serial interval rises rapidly from 6,000 to around 10,000 and stays nearly constant thereafter. While in the second simulation it has a different profile for each region, all the while staying between 100 and 100,000. Dispersion parameter k is fixed at 0.1 for both simulation scenarios.



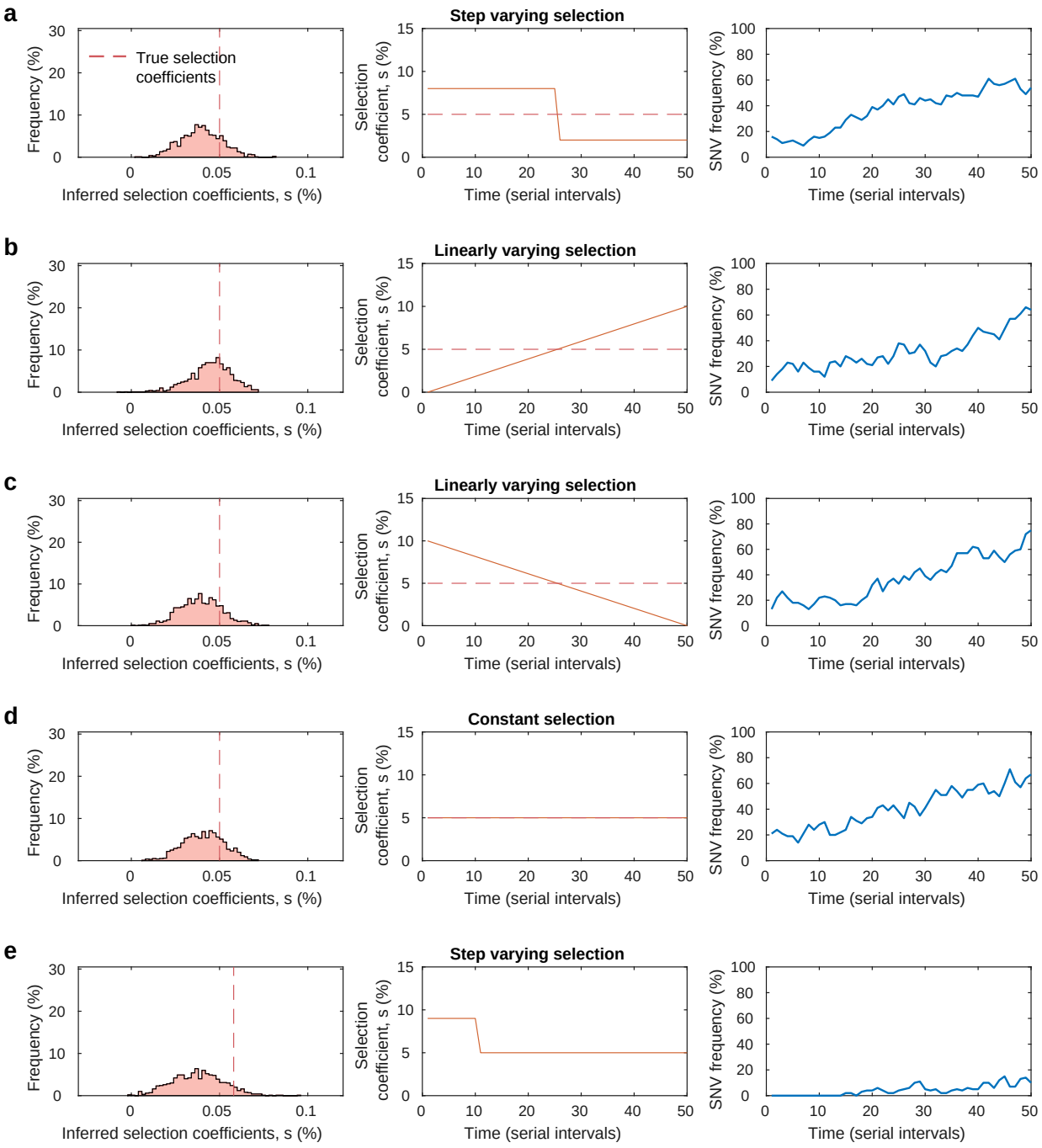
Supplementary Fig. 3. Inferred selection coefficients for Spike mutations mapped on the crystal structure. The majority of the inferred strongly selected mutations are in the S1 subunit of Spike. For sites with multiple mutations, the mutation with the largest magnitude of inferred selection coefficient was used for mapping. Structure of the Spike protein was obtained from http://rcsb.org/ (PDB ID: 7WG7) (ref.[86]).



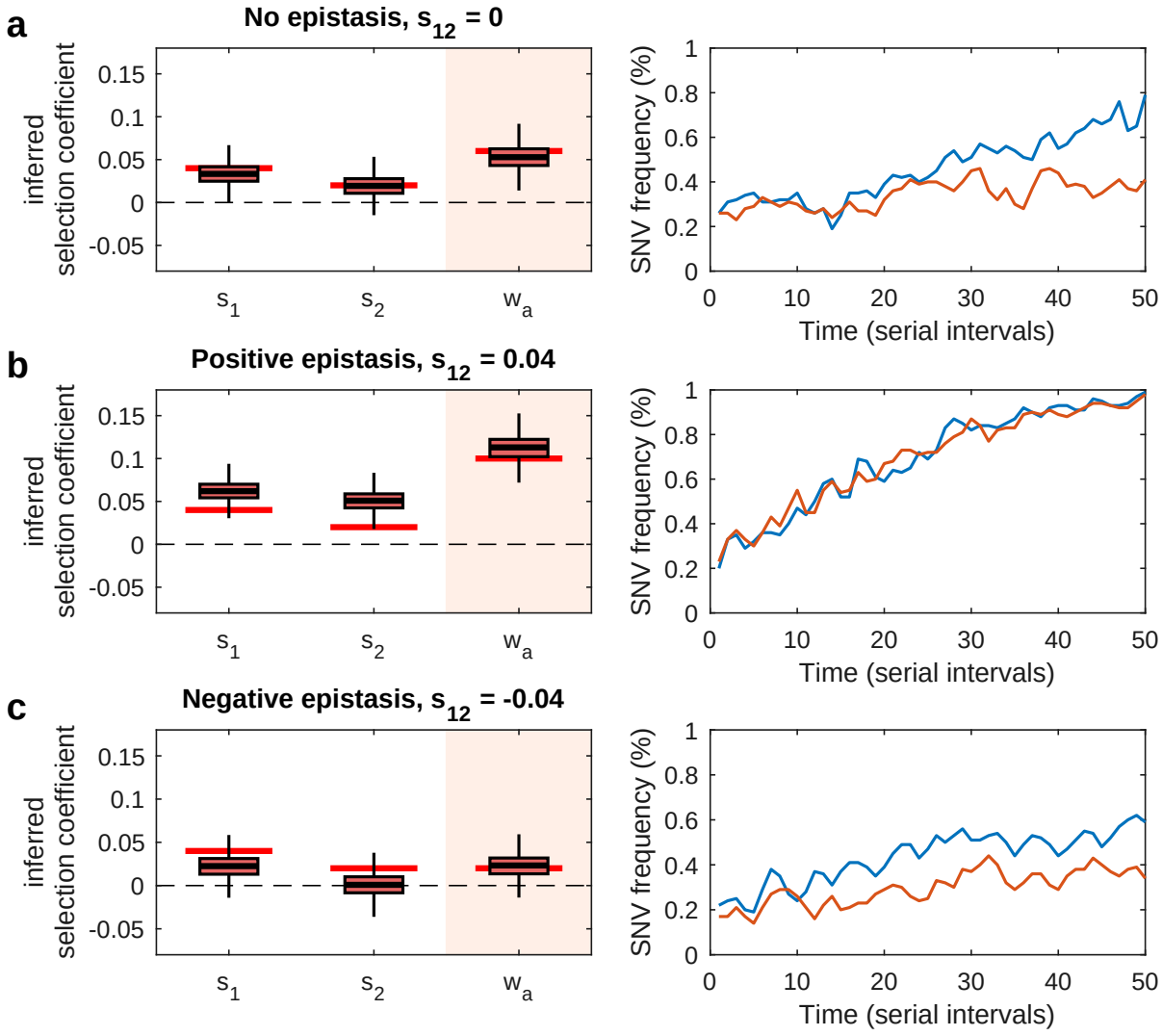
Supplementary Fig. 4. Multiple SARS-CoV-2 variants strongly increase transmission rate. Frequencies of major variants and their total inferred selection coefficients, shown as mean values ± one s.d. from bootstrap subsampling of regional data (Methods), defined relative to the Wuhan-Hu-1 reference sequence. Selection coefficients for variants with multiple SNVs are obtained by summing the effects of all variant-defining SNVs. Because our method uses global data and accounts for competition between variants, we infer large transmission advantages even for variants such as Gamma, Beta, Lambda, and Epsilon, which never achieved the same level of global dominance as variants such as Alpha and Delta.



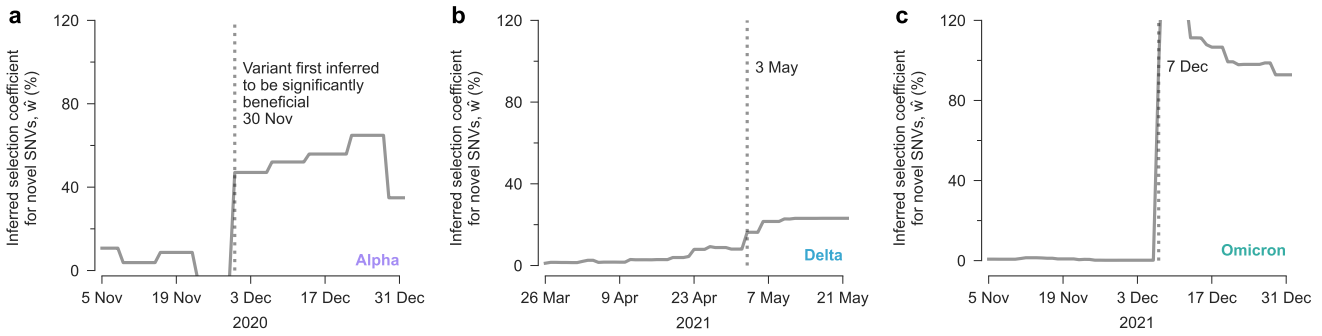
Supplementary Fig. 5. Non-Spike mutations/deletions in the emerging Omicron sub-variants BA.4, BA.5, and BA.5.1 and their associated inferred selection. The list of all mutations (both Spike and non-Spike) associated with these variants is provided in **Table 2.**



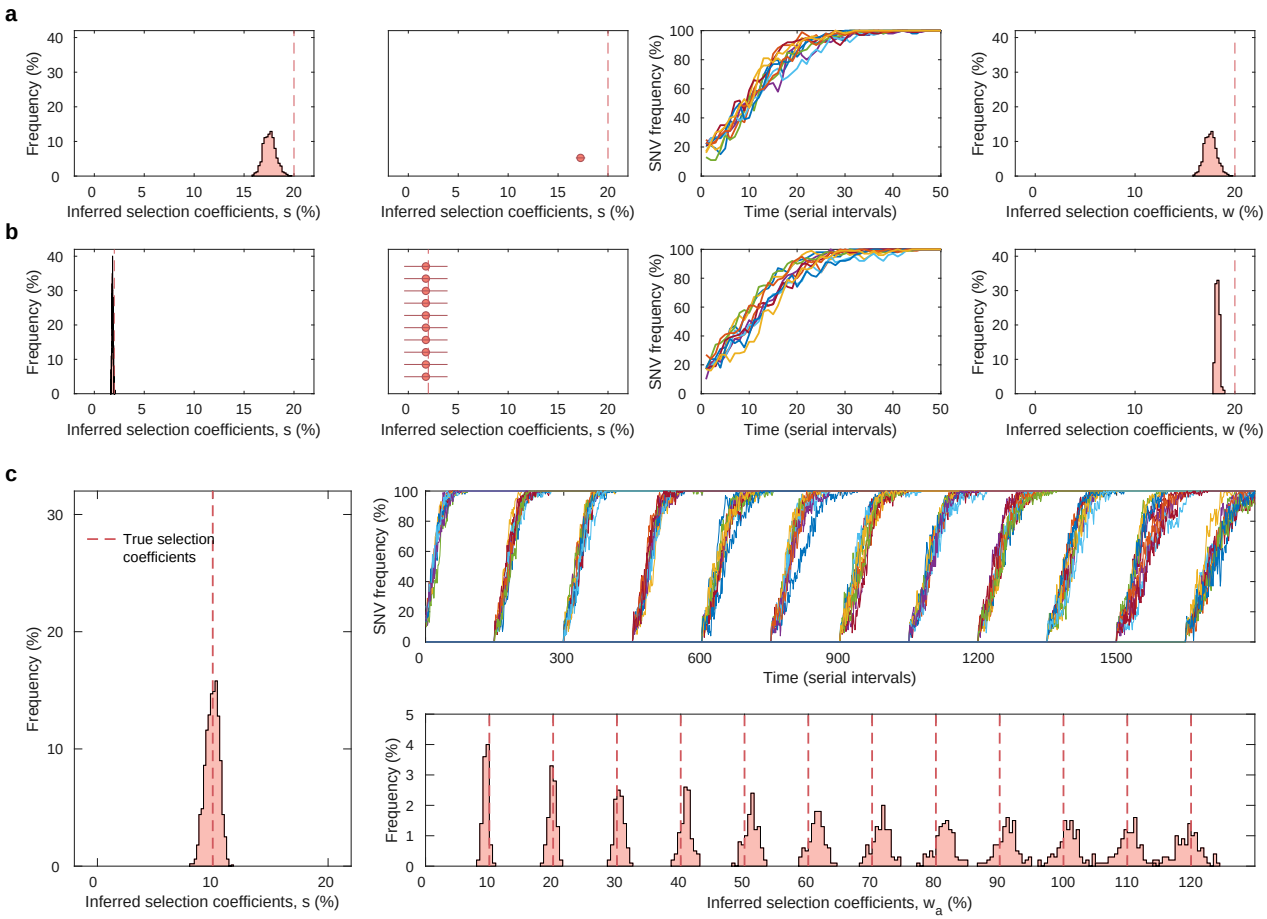
Supplementary Fig. 6. Average value inferred for time-varying selection coefficients. We simulated five scenarios of time-varying selection coefficients: **a**, step varying, **b**, linearly increasing, **c**, linearly decreasing, **d**, constant over time and, **e**, step varying where the SNV appears in the population after the true selection coefficient has changed. In each case, the inferred selection coefficient is close to the average of the time-varying selection coefficient over the time when the SNV was present in the population. *Simulation parameters.* The initial population in the first four simulation scenarios is a mixture of a neutral variant with no mutations and a variant with a beneficial SNV with a time-varying selection coefficient (center panels). In the fifth simulation scenario, the initial population consists entirely of the neutral variant with the beneficial mutant appearing after 15 serial intervals. The number of newly infected individuals per serial interval rises rapidly from 6,000 to around 10,000 and stays nearly constant thereafter. Dispersion parameter k is fixed at 0.1 for all simulation scenarios.



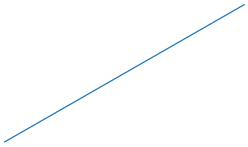
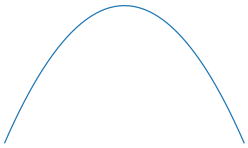
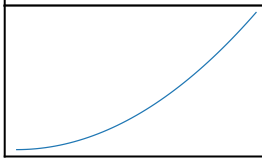
Supplementary Fig. 7. Accurate inference of variant fitness in presence of epistasis. a, Both SNV selection coefficients and variant selection coefficients are inferred accurately in absence of epistasis. Inferred selection coefficients over 1,000 runs are shown in box plots, with true values for the parameters shown with solid bars in red. The lower and upper edge of the box plot correspond to the 25th to 75th percentiles, the bar inside the box plot corresponds to the median while the top and bottom whiskers show the maximum and minimum value within 1.5 times the interquartile range. In scenarios with positive epistasis (**b**) or negative epistasis (**c**), our method attributes the effect of epistasis to selection coefficients. Thus, while the inferred SNV selection coefficients may be under- or over-estimated, the inferred variant selection coefficients are recovered. *Simulation parameters.* We simulate a two-locus system where the initial population consists of a mixture of all four variants, i.e., a neutral variant with no mutations, a variant with two beneficial SNVs ($s_1 = 0.04$, $s_2 = 0.02$), and both single SNV variants. The initial frequencies in the population of the neutral, the two single mutant variants, and the double mutant variants are set to 67%, 10%, 10% and 13%. We simulate three scenarios with the epistasis term taking on values s_{12} = {0, 0.04, -0.04}. Here the selection coefficient for the double mutant is $s_1 + s_2 + s_{12}$. The number of newly infected individuals per serial interval rises rapidly from 6,000 to around 10,000 and stays nearly constant thereafter. Dispersion parameter k is fixed at 0.1 for all simulation scenarios.



Supplementary Fig. 8. Detection of significantly increased transmission for Alpha, Delta, and Omicron with GISAID sequence data filtered by submission date. **a**, Inferred selection coefficient for novel Alpha SNVs over time using sequence data from London, filtered by submission date to GISAID. **b**, Inferred selection coefficient for novel Delta SNVs using sequence data in Great Britain, filtered by GISAID submission date. **c**, Inferred selection coefficient for novel Omicron SNVs over time using sequence data from South Africa, filtered by GISAID submission date.

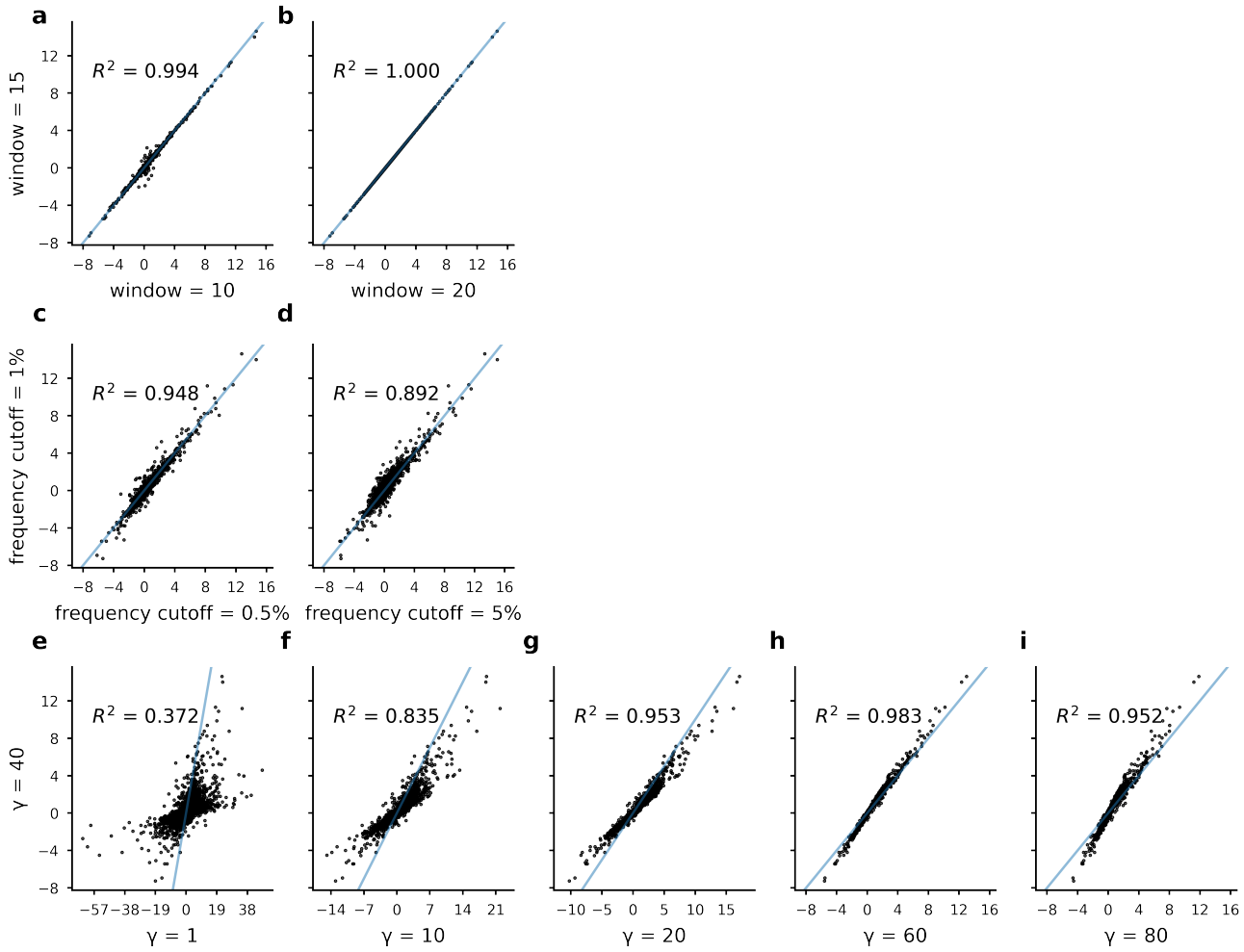


Supplementary Fig. 9. Ability to estimate large variant selection coefficients, w_a. While the estimate (9) is derived assuming selection coefficients are small, simulations show that combining data from multiple regions allows for accurate estimation of both large SNV selection coefficients, s, and variant selection coefficients, w_a. **a**, A scenario with a variant containing a single strongly beneficial SNV ($s = 0.2$) and, **b**, a scenario with a variant containing 10 mildly beneficial SNVs ($s = 0.02$). The true variant selection coefficient w_a has the same magnitude in both simulation scenarios ($w_a = 0.2$). **c**, Simulating a scenario where 12 beneficial SNVs ($s = 0.1$) appear and fixate successively (top right panel), such that w_a ranges from 0.1 to 1.2, both the SNV (left panel) and variant selection coefficient (bottom right panel) were estimated accurately. Results are obtained by combining data from 10 regions. Histograms are obtained from $1,000$ replicate simulations. *Simulation parameters.* In the simulation scenarios considered in **a** and **b**, the initial population in each region consists of a mixture of a neutral variant with no mutations along with a variant with a single strongly beneficial SNV (s = 0.2), or a variant with 10 beneficial SNVs (s = 0.02) respectively. In the simulation in **c**, each region's initial population consists of a mixture of a neutral variant with no mutations along with a variant with beneficial mutations. In this latter variant, 12 beneficial mutations (s = 0.1) appear and fixate in succession such that the variant selection coefficient varies from w_a = 0.1 to w_a = 1.2. The same variant appears in 10 independent regions in all simulation scenarios. The number of newly infected individuals per serial interval is nearly constant around 10,000. Dispersion parameter k is fixed at 0.1.

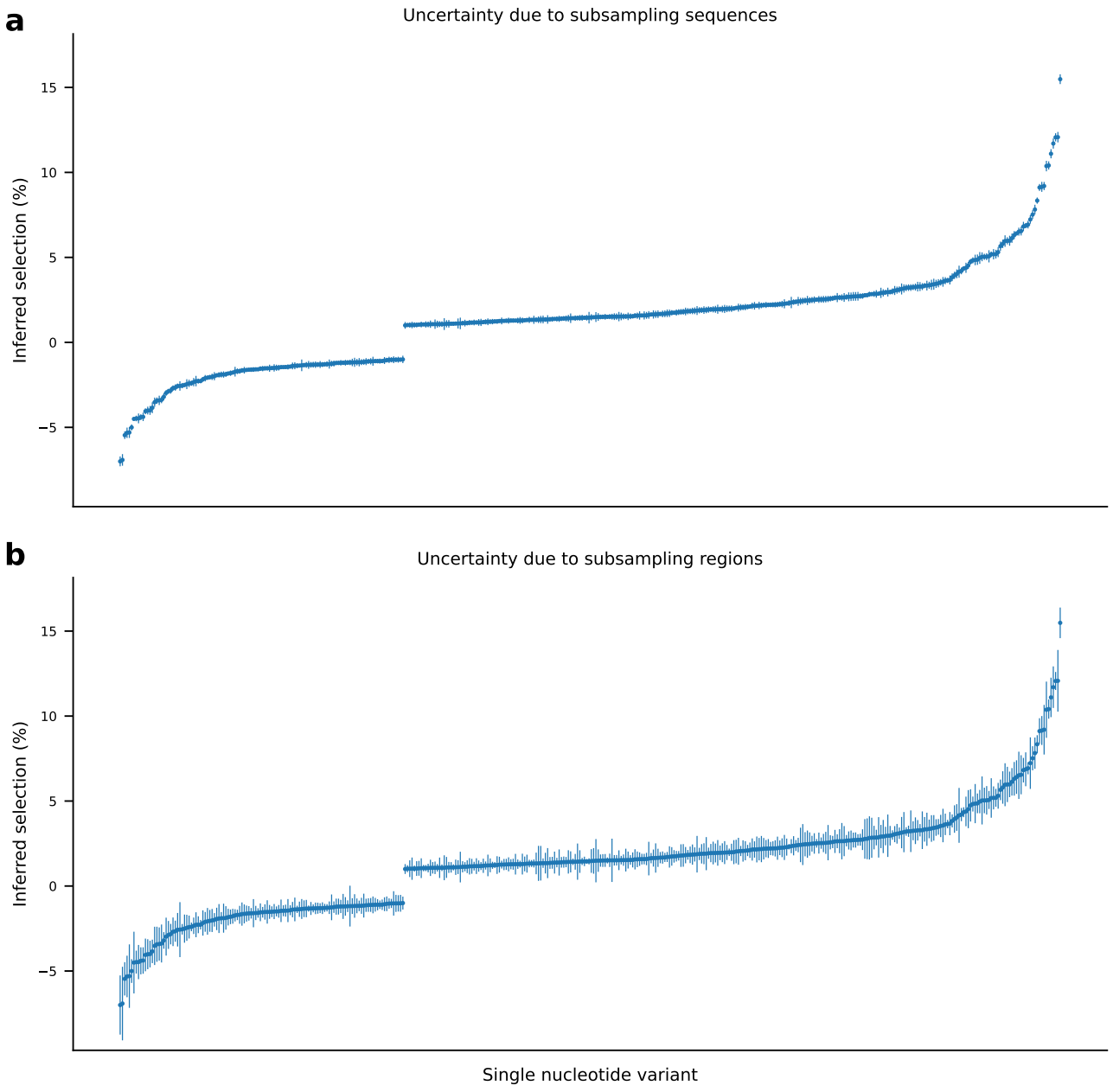
Number of new infections per serial interval	Sampling	Inference Parameter (n)	AUROC Beneficial	AUROC Deleterious
	Finite	Time-Varying	0.832	0.779
		Constant	**0.937**	**0.881**
	Perfect	**Time-Varying**	**0.999**	**0.992**
		Constant	0.973	0.940
	Finite	Time-Varying	0.873	0.821
		Constant	**0.944**	**0.882**
	Perfect	**Time-Varying**	**1.0**	**0.999**
		Constant	0.986	0.950
	Finite	Time-Varying	0.798	0.736
		Constant	**0.873**	**0.824**
	Perfect	**Time-Varying**	**0.981**	**0.935**
		Constant	0.905	0.863

Supplementary Fig. 10. Effects of finite sampling on inference using constant and time-varying parameters. The ability of the model to distinguish beneficial and deleterious SNVs, as measured by the AUROC score, depending on whether the sampling is perfect or finite and whether constant parameters or the true time-varying parameters are used for the number of new infections per serial interval n in the inference. If parameters are considered to be constant, then these parameters are not required for inference using (8). Both simulations use constant values of $k = 0.01$ and $R = 1$. The results are similar but less dramatic if the correct time-varying values are used for k or R as well. Results are shown for different trajectories of numbers of infections and are consistent regardless of the trajectory. In the upper panel, the number of new infections per serial interval, n, starts at $5,000$ and rises linearly to $100,000$. In the middle panel, n starts at $10,000$, rises quadratically to a maximum of $200,000$, and then falls back to the original number. In the final panel, n rises from an initial size of $1,000$ to a final size of $65,000$. All simulations are run for 50 serial intervals. Rows that yield better inference are marked by bold text. If sampling is finite, then it is better to use constant parameters; if sampling is perfect, then it is better to use the real time-varying parameters. The initial population of individuals are infected with a mixture of two variants with beneficial SNVs ($s = 0.03$), two with neutral SNVs ($s = 0$), and two with deleterious SNVs ($s = -0.03$), as in **Fig. 1**. Simulations are run for 50 simulations with 25 samples in each serial interval, and AUROC scores are averaged over 1,000 replicate simulations.

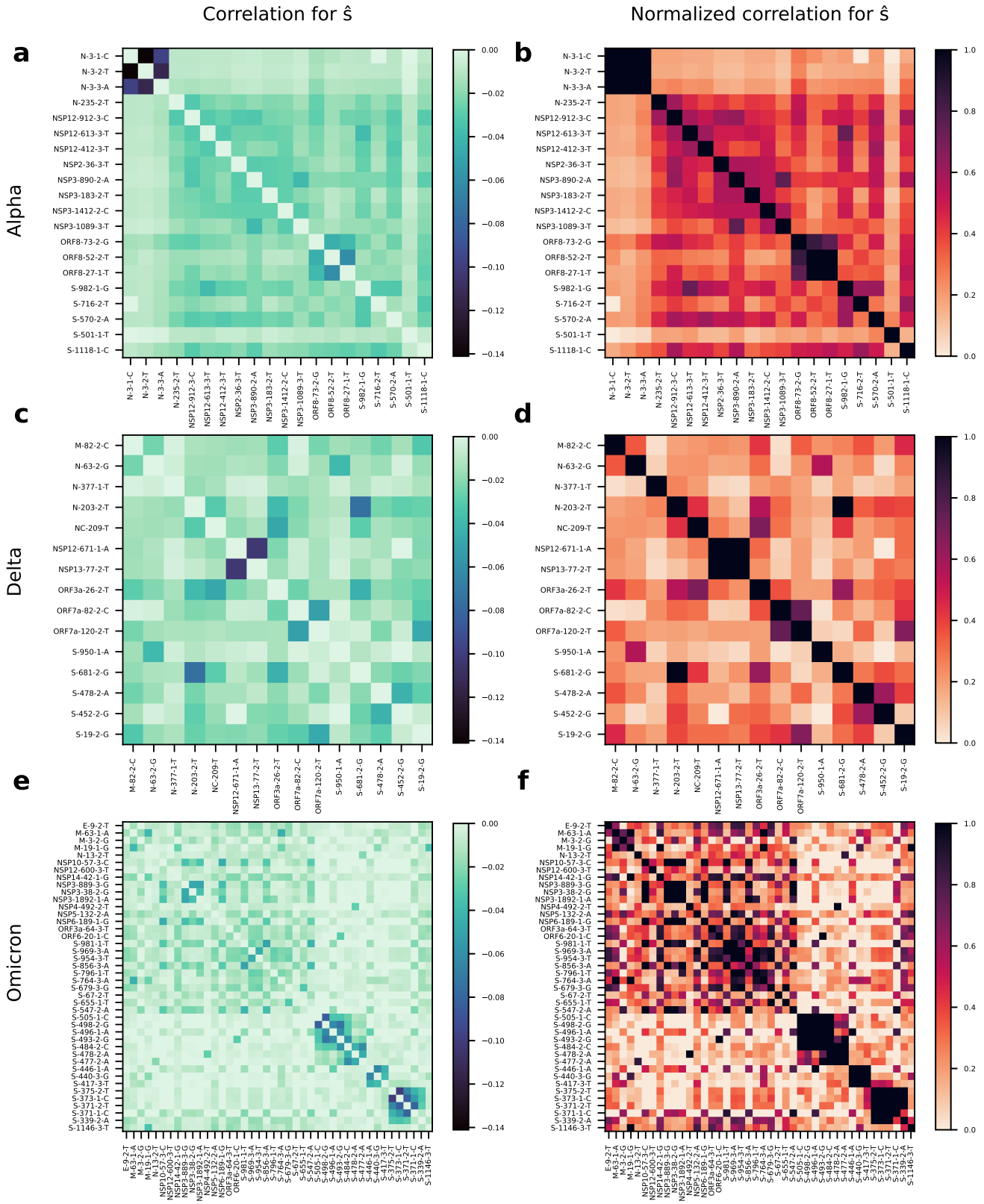
Supplementary Fig. 11. Sampling Distributions. The number of genomes per day in the regions that are used for inference.



Supplementary Fig. 12. Inferred selection coefficients are robust to different values of the regularization γ', different frequency cutoffs, and different numbers of days used to calculate the frequency changes. a-b, Comparison of inferred coefficients when the number of days at the beginning and end of the time-series are used in order to calculate the frequency changes. Inferred coefficients are largely robust to these changes **c-d**, Comparison of inferred coefficients for different frequency cutoffs. Including more or less sites does not alter the order of inferred coefficients. **e-i**, Comparison of inferred coefficients for different values of the regularization. Altering the regularization value has little effect upon the distribution of inferred selection coefficients, and selection coefficients for different values of the regularization are highly correlated.



Supplementary Fig. 13. Selection coefficient estimates and uncertainty. Plots of all inferred selection coefficients with absolute values greater than 1%. **a**, Selection coefficients with uncertainty estimates from bootstrapping the sequences in each region. 20 sequences were sampled per time point per region, with replacement. Error bars represent standard deviations of the inferred coefficients computed over 100 bootstrap samples. **b**, Selection coefficients with uncertainty estimates from subsampling the regions used. For each run, we inferred selection coefficients using a random subsample of 80% of the total number of regions. Error bars represent standard deviations of the inferred coefficients computed over 100 samples.



Supplementary Fig. 14. Correlations between $\hat{s}$ for strongly linked subsets of mutations defining major variants. As discussed in **Supplementary Information**, the covariance of the inferred parameters is given by the matrix in (S5). The correlation matrix of the inferred parameters is easily calculated from this covariance. SNV labels are in the format of xxx-yyy-z-n, where xxx is the protein, yyy is the codon in the protein, z is the index of the nucleotide in the codon, and n is the nucleotide. **a, c, e**, The correlation matrix for SNVs that are strongly linked to one another in Alpha, Delta, and Omicron, respectively. The diagonal elements, all equal to 1 in a correlation matrix, are set to zero for visualization purposes. **b, d, f**, Correlation matrices from **a**, **c**, and **e**, normalized by the maximum possible correlation for a group of linked SNVs, as discussed in **Supplementary Information**, with the same number of SNVs. The (i, j)th element of these matrices represents the percent of linkage between the selection coefficients for SNVs i and j.

Rank	Protein	Mutation(s) (nt)	Mutation (aa)	Selection (%)	Location	Associated variant(s)	Phenotypic effect
1	NSP4	C10029T	T492I	15.5 ± 0.9		Lambda, Mu, BA.1, BA.2, BA.4, BA.5, BA.2.12.1	
2	NSP6	T11288-90	S106-	15 ± 1.0		Alpha, Beta, Gamma, Eta, Iota, Lambda, BA.1, BA.2, BA.4, BA.5, BA.2.12.1	*Increased transmission by interferon antagonism [87]
3	S	Δ21986-88	G142-	13.5 ± 1.2	NTD	BA.1	*Increased resistance to NTD-specific nAbs [88]
4	S	C23604G	P681R	12.1 ± 1.8	FCS	Delta, Kappa	Enhanced cleavage, fusogenicity, and pathogenicity [89]
5	S	G22599A	R346K	12.1 ± 0.5	RBD	Mu	Reduced neutralization [90]
6	S	A23055G	Q498R	11.7 ± 1.2	RBM	BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Increased ACE2 binding and resistance to nAbs [86,91]
7	S	T22917A	L452Q	10.4 ± 0.6	RBM	Lambda, BA.2.12.1	Increased RBD expression (stability) [92], increased resistance to nAbs [93,94], and increased cell entry [95]
8	N	G28881T	R203M	10.4 ± 1.7		Delta, Kappa	Enhanced replication, RNA delivery and packaging [96]
9	S	G22813T	K417N	9.2 ± 0.9	RBD	Beta, BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Increased ACE2 binding [92] and resistance to nAbs [86,97]
10	S	C23604A	P681H	9.1 ± 0.8	FCS	Alpha, Mu, BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Enhanced cleavage [98] and increased resistance to interferon-induced immunity [99], leading to increased replication and/or transmission
11	S	Δ21989-91	V143-	8.7 ± 1.2	NTD	BA.1	Increased resistance to NTD-specific nAbs [88]
12	N	G28280C, A28281T, T28282A[#]	D3L	8.4 ± 0.7		Alpha	Increased transmissibility by introducing a transcription regulatory sequence upstream of ORF9b [100]
13	S	T23018G	F486V	8.3 ± 0.5	RBM	BA.4, BA.5	Increased ACE2 binding and resistance to nAbs [101,102]
14	S	T22882A	N440K	7.8 ± 0.8	RBM	BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Increased resistance to nAbs [86,97]
15	S	T22917G	L452R	7.5 ± 0.7	RBM	Delta, Kappa, Epsilon, BA.4, BA.5	Increased RBD expression (stability) [92], increased resistance to nAbs [93], and increased cell entry [94]
16	N	Δ28364-66	E31-	7.3 ± 0.6		BA.1, BA.2, BA.4, BA.5, BA.2.12.1	
17	S	A23063T	N501Y	7.2 ± 1.5	RBM	Alpha, Beta, Gamma, Mu, BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Increased infection, transmission, ACE2 binding, and resistance to nAbs [86]
18	N	Δ28367-69	R32-	7.2 ± 0.3		BA.1, BA.2, BA.4, BA.5, BA.2.12.1	
19	S	C23673T	S704L	6.9 ± 0.4	FCS	BA.2.12.1	
20	M	T26767C	I82T	6.8 ± 0.7		Delta, Eta	
21	S	C21618T	T19I	6.5 ± 1.2	NTD	BA.2, BA.4, BA.5, BA.2.12.1	*Increased resistance to NTD-specific nAbs [103,104]
22	NSP6	T11296G	F108L	6.5 ± 1.4			*Increased transmission by interferon antagonism [87]
23	S	G21987A	G142D	6.4 ± 1.0	NTD	BA.2, BA.4, BA.5, BA.2.12.1	Increased resistance to NTD-specific nAbs [86,88]
24	S	A24424T	Q954H	6.3 ± 1.0	HR1	BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Increased infectivity in vitro [105]
25	S	C22674T	S371F	6.1 ± 0.8	RBD	BA.2, BA.4, BA.5, BA.2.12.1	Increased resistance to nAbs [94,97]
26	S	C22995A	T478K	6 ± 0.8	RBM	Delta, BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Increased ACE2 binding [86] and resistance to nAbs [93]
27	S	C23854A	N764K	6 ± 1.3		BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Improved structural stability [106,107]
28	S	T24469A	N969K	5.8 ± 1.0	HR1	BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Improved structural stability [86]
29	S	C23525T	H655Y	5.7 ± 0.6	FCS	Gamma, BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Increased viral replication, spike protein cleavage, and transmission in vivo [108]
30	S	C22686T	S375F	5.3 ± 0.8	RBD	BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Increased resistance to nAbs [86]
31	N	C28311T	P13L	5.2 ± 0.5		Lambda, BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Escape from a HLA-B*27:05 CD8+ T cell epitope [109]
32	S	C21618G	T19R	5.2 ± 0.7	NTD	Delta	*Increased resistance to NTD-specific nAbs [103,104]
33	S	G24368T	D936Y	5.2 ± 1.2			
34	S	G22578A	G339D	5.1 ± 0.5	RBD	BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Increased resistance to nAbs [86]
35	S	G23012A	E484K	5 ± 0.8	RBM	Beta, Gamma, Eta, Iota, Mu	Increased ACE2 binding [110] and resistance to nAbs [93]
36	S	G23948T	D796Y	5 ± 0.7		BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Improved structural stability [86]
37	S	T23599A	N679K	4.9 ± 0.8	FCS	BA.1, BA.2, BA.4, BA.5, BA.2.12.1	*Increased proteolytic activation [86]
38	NSP5	C10449A	P132H	4.8 ± 1.2		BA.1, BA.2, BA.4, BA.5, BA.2.12.1	
39	M	G26529A	D3N	4.8 ± 0.4		BA.5	
40	NSP6	T11288A	S106T	4.7 ± 0.9			*Increased transmission by interferon antagonism [87]
41	N	G28881A	R203K	4.7 ± 1.5		Alpha, Gamma, Lambda, BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Enhanced replication, RNA delivery and packaging [96]
42	ORF8	Δ28251-53	F120-	4.4 ± 0.5		Delta	
43	S	T22200G	V213G	4.4 ± 0.9	NTD	BA.2, BA.4, BA.5, BA.2.12.1	*Increased resistance to NTD-specific nAbs [86]
44	NSP12	G14030A	R197Q	4.3 ± 0.3			
45	ORF3a	C26060T	T223I	4.2 ± 0.4		BA.2, BA.4, BA.5, BA.2.12.1	
46	S	C21762T	A67V	4.2 ± 1.6	NTD	Eta, BA.1	Increased resistance to NTD-specific nAbs [86]
47	E	C26270T	T9I	4 ± 0.8		BA.1, BA.2, BA.4, BA.5, BA.2.12.1	
48	S	Δ21635-37	P25-	4 ± 1.1	NTD	BA.2, BA.4, BA.5, BA.2.12.1	
49	S	C22227T	A222V	3.8 ± 0.4	NTD	20E (EU1)	*Slightly increased cell entry [111]
50	S	T22679C	S373P	3.7 ± 0.7	RBD	BA.1, BA.2, BA.4, BA.5, BA.2.12.1	Increased resistance to nAbs [112]

Table 1. Table of most highly selected amino acid substitutions across the SARS-CoV-2 genome. Error bars were found by taking random sub-samples of 80% of the original regions and re-estimating the selection coefficients. Error bars are the standard deviation of the inferred coefficient for each site over 100 replicates. * represents the cases where phenotypic effect of an amino acid variant has not been reported explicitly in the literature. Instead, it is either based on the function of the encompassing gene, for a mutation to a different amino acid or deletion at the same position. [#] all three mutations appear together; RBM = receptor binding motif; RBD = receptor binding domain; NTD= N-terminal domain; FCS = S1/S2 furin cleavage site; HR1 = heptad repeat 1; nAbs = neutralizing antibodies.

Variant	Pango Lineage	Selection (%)	Mutations
B.1	B.1	7.7 ± 1.5	ORF1b:P314L, S:D614G
20E(EU1)	B.1.177	14.5 ± 2.4	S:A222V,ORF10:V30L,N:A220V
Epsilon	B.1.427/429	19.8 ± 2.4	S:S13I, S:W152C, S:L452R, S:D614G, NSP9:I65V, NSP13:D260Y
Lambda	C.37	34.4 ± 2.9	S:G75V, S:T76I, S:R246-, S:S247-, S:Y248-, S:L249-, S:T250-, S:P251-, S:G252-, S:D253N, S:L452Q, S:F490S, S:D614G, S:T859N, NSP3:T428I, NSP3:P1469S, NSP3:F1569V, NSP4:L438P, NSP4:T492I, NSP5:G15S, NSP6:S106-, NSP6:G107-, NSP6:F108-, NSP12:P323L, N:P13L, N:R203K, N:G204R, N:G214C
Alpha	B.1.1.7	38.3 ± 3.0	S:H69-, S:V70-, S:Y144-, S:N501Y, S:A570D, S:D614G, S:P681H, S:T716I, S:S982A, S:D1118H, NSP3:T183I, NSP3:A890D, NSP3:I1412T, NSP6:S106-, NSP6:G107-, NSP6:F108-, N:D3L, N:R203K, N:G204R, N:S235F, NSP12:P323L, ORF8:Q27*, ORF8:R52I, ORF8:Y73C
Beta	B.1.351	38.4 ± 3.0	S:D80A, S:D215G, S:L241-, S:L242-, S:A243-, S:K417N, S:E484K, S:N501Y, S:D614G, S:A701V, ORF3a:Q57H, NSP2:T85I, NSP3:K837N, NSP5:K90R, NSP6:S106-, NSP6:G107-, NSP6:F108-, N:T205I, NSP12:P323L, E:P71L
Eta	B.1.525	40.7 ± 3.6	S:Q52R, S:A67V, S:H69-, S:V70-, S:Y144-, S:E484K, S:D614G, S:Q677H, S:F888L, NSP12:P323F, N:S2-, N:D3Y, N:A12G, N:T205I, M:I82T, NSP3:T1189I, NSP6:S106-, NSP6:G107-, NSP6:F108-, E:L21F, ORF6:F2-
Kappa	B.1.617.1	43.1 ± 5.7	S:E154K, S:L452R, S:E484Q, S:D614G, S:P681R, S:Q1071H, NSP12:P323L, NSP13:G206C, NSP13:M429I, NSP15:K259R, NSP15:S261A, N:R203M, N:D377Y, M:I82S, ORF3a:S26L, NSP3:T749I, NSP6:T77A, ORF7a:V82A
Iota	B.1.526	45.3 ± 4.9	S:Q52R, S:A67V, S:H69-, S:V70-, S:Y144-, S:E484K, S:D614G, S:Q677H, S:F888L, NSP12:P323F, N:S2-, N:D3Y, N:A12G, N:T205I, M:I82T, NSP3:T1189I, NSP6:S106-, NSP6:G107-, NSP6:F108-, E:L21F, ORF6:F2-
Gamma	P.1	47.0 ± 3.4	S:L18F, S:T20N, S:P26S, S:D138Y, S:R190S, S:K417T, S:E484K, S:N501Y, S:D614G, S:H655Y, S:T1027I, S:V1176F, ORF3a:S253P, NSP3:S370L, NSP3:K977Q, NSP6:S106-, NSP6:G107-, NSP6:F108-, N:P80R, N:R203K, N:G204R, NSP12:P323L, NSP13:E341D, ORF8:E92K
Mu	B.1.621	53.2 ± 3.6	S:T95I, S:Y144S, S:Y145N, S:R346K, S:E484K, S:N501Y, S:D614G, S:P681H, S:D950N, NSP3:T237A, NSP3:T720I, NSP4:T492I, NSP6:Q160R, NSP12:P323L, NSP13:P419S, N:T205I, ORF3a:Q57H, ORF3a:V256I, ORF3a:N257Q, ORF3a:P258*, ORF8:T11K, ORF8:P38S, ORF8:S67F
Delta	B.1.617.2	92.6 ± 5.6	S:T19R, S:E156-, S:F157-, S:R158G, S:L452R, S:T478K, S:D614G, S:P681R, S:D950N, NSP12:P323L, NSP12:G671S, NSP13:P77L, M:I82T, N:D63G, N:R203M, N:D377Y, ORF3a:S26L, ORF7a:V82A, ORF7a:T120I, ORF8:D119-, ORF8:F120-, ORF9b:T60A
Omicron	BA.1	197.2 ± 8.5	S:A67V, S:H69-, S:V70-, S:T95I, S:G142-, S:V143-, S:Y144-, S:Y145D, S:N211-, S:L212I, S:G339D, S:S371L, S:S373P, S:S375F, S:K417N, S:N440K, S:G446S, S:S477N, S:T478K, S:E484A, S:Q493R, S:G496S, S:Q498R, S:N501Y, S:Y505H, S:T547K, S:D614G, S:H655Y, S:N679K, S:P681H, S:N764K, S:D796Y, S:N856K, S:Q954H, S:N969K, S:L981F, N:P13L, N:E31-, N:R32-, N:S33-, N:R203K, N:G204R, NSP3:K38R, NSP3:S1265-, NSP3:L1266I, NSP3:A1892T, NSP4:T492I, NSP5:P132H, NSP6:L105-, NSP6:S106-, NSP6:G107-, NSP6:I189V, NSP12:P323L, NSP14:I42V, ORF9b:P10S, ORF9b:E27-, ORF9b:N28-, ORF9b:A29-, E:T9I, M:D3G, M:Q19E, M:A63T
Omicron	BA.2	245.1 ± 12.4	S:T19I, S:L24-, S:P25-, S:P26-, S:A27S, S:G142D, S:V213G, S:G339D, S:S371F, S:S373P, S:S375F, S:T376A, S:D405N, S:R408S, S:K417N, S:N440K, S:S477N, S:T478K, S:E484A, S:Q493R, S:Q498R, S:N501Y, S:Y505H, S:D614G, S:H655Y, S:N679K, S:P681H, S:N764K, S:D796Y, S:Q954H, S:N969K, N:P13L, N:E31-, N:R32-, N:S33-, N:R203K, N:G204R, N:S413R, NSP1:S135R, NSP3:T24I, NSP3:G489S, NSP4:L264F, NSP4:T327I, NSP4:T492I, NSP5:P132H, NSP6:S106-, NSP6:G107-, NSP6:F108-, NSP12:P323L, NSP13:R392C, NSP14:I42V, NSP15:T112I, ORF3a:T223I, ORF6:D61L, ORF9b:P10S, ORF9b:E27-, ORF9b:N28-, ORF9b:A29-, E:T9I, M:Q19E, M:A63T
Omicron	BA.2.12.1	273.9 ± 15.1	S:T19I, S:L24-, S:P25-, S:P26-, S:A27S, S:G142D, S:V213G, S:G339D, S:S371F, S:S373P, S:S375F, S:T376A, S:D405N, S:R408S, S:K417N, S:N440K, S:L452Q, S:S477N, S:T478K, S:E484A, S:Q493R, S:Q498R, S:N501Y, S:Y505H, S:D614G, S:H655Y, S:N679K, S:P681H, S:S704L, S:N764K, S:D796Y, S:Q954H, S:N969K, N:P13L, N:E31-, N:R32-, N:S33-, N:R203K, N:G204R, N:S413R, NSP1:S135R, NSP3:T24I, NSP3:G489S, NSP4:L264F, NSP4:T327I, NSP4:L438F, NSP4:T492I, NSP5:P132H, NSP6:S106-, NSP6:G107-, NSP6:F108-, NSP12:P323L, NSP13:R392C, NSP14:I42V, NSP15:T112I, ORF3a:T223I, ORF6:D61L, ORF9b:P10S, ORF9b:E27-, ORF9b:N28-, ORF9b:A29-, E:T9I, M:Q19E, M:A63T
Omicron	BA.4	279.2 ± 14.3	S:T19I, S:L24-, S:P25-, S:P26-, S:A27S, S:H69-, S:V70-, S:G142D, S:V213G, S:G339D, S:S371F, S:S373P, S:S375F, S:T376A, S:D405N, S:R408S, S:K417N, S:N440K, S:L452R, S:S477N, S:T478K, S:E484A, S:F486V, S:Q498R, S:N501Y, S:Y505H, S:D614G, S:H655Y, S:N679K, S:P681H, S:N764K, S:D796Y, S:Q954H, S:N969K, N:P13L, N:E31-, N:R32-, N:S33-, N:P151S, N:R203K, N:G204R, N:S413R, NSP1:S135R, NSP1:K141-, NSP1:S142-, NSP1:F143-, NSP3:T24I, NSP3:G489S, NSP4:L264F, NSP4:T327I, NSP4:T492I, NSP5:P132H, NSP6:S106-, NSP6:G107-, NSP6:F108-, NSP12:P323L, NSP13:R392C, NSP14:I42V, NSP15:T112I, ORF3a:T223I, ORF6:D61L, ORF7b:L11F, ORF9b:P10S, ORF9b:E27-, ORF9b:N28-, ORF9b:A29-, E:T9I, M:Q19E, M:A63T
Omicron	BA.5	283.0 ± 14.1	S:T19I, S:L24-, S:P25-, S:P26-, S:A27S, S:H69-, S:V70-, S:G142D, S:V213G, S:G339D, S:S371F, S:S373P, S:S375F, S:T376A, S:D405N, S:R408S, S:K417N, S:N440K, S:L452R, S:S477N, S:T478K, S:E484A, S:F486V, S:Q498R, S:N501Y, S:Y505H, S:D614G, S:H655Y, S:N679K, S:P681H, S:N764K, S:D796Y, S:Q954H, S:N969K, N:P13L, N:E31-, N:R32-, N:S33-, N:R203K, N:G204R, N:S413R, NSP1:S135R, NSP3:T24I, NSP3:G489S, NSP4:L264F, NSP4:T327I, NSP4:T492I, NSP5:P132H, NSP6:S106-, NSP6:G107-, NSP6:F108-, NSP12:P323L, NSP13:R392C, NSP14:I42V, NSP15:T112I, ORF3a:T223I, ORF9b:P10S, ORF9b:E27-, ORF9b:N28-, ORF9b:A29-, E:T9I, M:D3N, M:Q19E, M:A63T

Table 2. Table of selection coefficients for groups of amino acid mutations. The selection coefficient for a variant is calculated as the sum of the selection coefficients for the individual mutations that the variant contains.

Group of linked SNVs	Associated lineage(s)/variant(s)	Number of regions
NSP5:P132H, NSP14:I42V, S:G339D, S:S371F, S:S373P, S:S375F, S:K417N, S:N440K, S:S477N, S:E484A, S:Q493R, S:Q498R, S:Y505H, S:H655Y, S:N679K, S:N764K, S:D796Y, S:Q954H, S:N969K, S:D1146D, ORF3a:T64T, E:T9I, M:Q19E, M:A63T, ORF6:R20R, ORF7b:Y18Y, NC:28271, N:P13L	Omicron (BA.1, BA.2, BA.2.12.1, BA.4, BA.5)	100
NSP6:T77A, NC:210, NSP3:A488S, NSP3:P1228L, NSP3:P1469S, NSP4:D144D, NSP4:V167L, NSP6:V120V, NSP12:G671S, NSP13:P77L, NSP14:A394V, S:T19R, S:L452R, S:P681R, S:D950N, ORF3a:S26L, M:I82T, ORF7a:V82A, ORF7a:T120I, ORF7b:L40L, N:D63G, N:R203M, N:G215C, N:D377Y	Delta	92
NSP6:I189V, NSP3:K38R, NSP3:A889A, NSP3:A1892T, NSP10:V57V, NSP12:N600N, S:A67V, S:-214mE, S:-214nE, S:-214oE, S:-214pP, S:-214rP, S:-214sE, S:-214tE, S:-214uE, S:S371P, S:G446S, S:G496S, S:T547K, S:N856K, S:L981F, M:D3G	Omicron (BA.1)	91
NSP6:S104-, NSP3:S1265-, NSP3:L1266-, NSP6:L105-, S:G142-, S:V143-, S:Y144-, S:Y145D, S:N211-, S:L212I	Omicron (BA.1)	88
NSP5:D48D, NSP1:S135R, NSP3:T24I, NSP3:G489S, NSP3:A534A, NSP4:L264F, NSP4:V290V, NSP4:T327I, NSP4:L438F, NSP5:R131R, NSP9:I65I, NSP12:L758L, NSP13:R392C, NSP15:T112I, NSP15:E145E, S:T19I, S:V213G, S:T376A, S:D405N, S:R408S, ORF3a:T223I, M:F112F, ORF6:D61L, N:S413R	Omicron (BA.2, BA.2.12.1, BA.4, BA.5)	83
N:G30-, N:E31-, N:R32-, N:S33-	Omicron (BA.1, BA.2, BA.2.12.1, BA.4, BA.5)	83
S:E156-, S:F157-, S:R158G, ORF8:D119-, ORF8:F120-	Delta	81
S:L24-, S:P25-, S:P26-, S:A27S	Omicron (BA.2, BA.2.12.1, BA.4, BA.5)	57
NSP12:P412P, NSP2:S36S, NSP3:T183I, NSP3:A890D, NSP3:F1089F, NSP3:I1412T, NSP12:H613H, NSP12:T912T, S:A570D, S:T716I, S:S982A, S:D1118H, ORF8:Q27*, ORF8:R52I, ORF8:Y73C, N:D3L, N:S235F	Alpha	44
NSP9:Y31Y, NSP1:D156D, NSP3:D10D, NSP3:S370L, NSP3:K977Q, NSP3:P1200P, NSP3:V1298V, NSP12:D140D, S:T20N, S:P26S, S:D138Y, S:R190S, S:K417T, S:T1027I, S:V1176F, ORF3a:S253P, ORF8:E92K, N:P80R, NSP13:E341D	Gamma	36
NSP6:Y234Y, NSP12:D523D, S:D53D, S:L452Q, S:S704L	Omicron (BA.2.12.1)	30
S:I68-, S:H69-, S:V70-, S:V143-, S:Y144-	Alpha, Eta, Omicron (BA.1, BA.4, BA.5)	24
NSP6:G107-, NSP6:F108-	Alpha, Beta, Gamma, Eta, Iota, Lambda, Omicron (BA.2, BA.2.12.1, BA.4, BA.5)	9
NSP6:S106-, NSP6:G107-	Alpha, Beta, Gamma, Eta, Iota, Lambda, Omicron (BA.1, BA.2, BA.2.12.1, BA.4, BA.5)	8
NSP10:S11S, NSP3:T237A, NSP3:T720I, NSP3:S1106S, NSP13:P419S, NSP14:I332I, S:Y144S, S:Y145N, NC:26492, ORF8:T11K, ORF8:P38S	Mu	7
S:N501Y, S:P681H, N:R203K, N:G204R	Alpha, Omicron (BA.1, BA.2, BA.2.12.1, BA.4, BA.5)	6
NSP12:P323L, NC:241, NSP3:F106F, S:D614G	B.1	4
S:C166S		2
S:D80A, NSP3:K837N, S:D215G, S:T240-, S:L241-, S:L242-, S:A243-, E:P71L	Beta	2
NSP15:V320M, NSP3:I414I, NSP4:T173I, S:A575A, S:T1238T, ORF7b:P44-, NC:27888, NC:27889, NC:27891, NC:27892, NC:27893, ORF8:M1-, ORF8:K2-, ORF8:F3-, N:A208-, N:R209-, NSP3:E378V	B.1.1.318	2
NSP5:L89F, NSP14:N129D, NSP16:R216C, ORF3a:G172V, ORF8:S24L, N:P67S	B.1.2	2
S:G142D	Omicron (BA.2)	2
NSP6:V149A, NSP3:P822L, NSP4:A446V, NSP6:T181I, NSP3:T955T, NSP9:L112L	AY.9	2
NSP7:C32C, NSP2:H532Q, NSP12:G345G, NSP13:V34V, M:A81S, N:S412R	AY.27	2
NSP12:L308L, NSP3:P192L, S:D253A, S:D979E, ORF3a:C130C, M:P123P, ORF7b:L41L		1
NC:28271	Delta	1
NSP10:M137K, NSP3:R1868P, NSP4:S358Y, NSP10:L138R, NSP14:P128P	AY.4.5	1
NSP12:F694Y	AY.4	1
NSP2:R370C, NSP2:E575K, ORF3a:Y109C	B.1.279	1
NSP4:T492I, S:T478K	Delta, Omicron (BA.1, BA.2, BA.2.12.1, BA.4, BA.5)	1
NSP9:M101I, NSP3:I1683T, NSP12:V720I, NSP13:A598S, S:T1116T, M:R150R, ORF7b:*15*, NSP13:H290Y, NSP3:Y1776Y, S:N439K	B.1.258.22	1
NSP8:N43H, NSP3:P1403S, S:M1237T	L.1	1
NSP12:D481A, S:D1259Y	AY.4.5	1
NSP2:M117V, NSP3:V1811V, NSP8:P10P, ORF3a:L95F, NC:28272, N:D401Y	B.1.438.1	1
NSP16:A199A, NSP1:V60V, NSP3:T1189T, M:L93L, N:A220V, ORF10:V30L	EU1	1
S:G75V, NSP3:F1569V, S:T76I, S:P251-, N:G214C, S:R246-, S:S247-, S:Y248-, S:L249-, S:T250-, S:G252-, S:D253N	Lambda	1
ORF3a:D265D, N:D216D (synonymous)		1

Table 3. Table of groups of linked SNVs that are detected as increasing transmission. Their associated variants and the number of times these groups are detected is also listed.

Supplementary Information

1. Summary

Here we discuss two main topics. First, we give a detailed introduction of our epidemiological model as well as a derivation of the estimator (1) and an important simplification of it. Second, we describe simulations of an outbreak and show that selection coefficients can be accurately recovered from simulation data even with relatively poor sampling.

2. Epidemiological model

2.1. Introduction

In epidemiology, the spread of infection can be modeled as a branching process where each infected individual (also referred to as a case) infects n additional individuals [113]. The distribution of n is often taken to be Poisson, but differences in the number of contacts with susceptible individuals, disease course within an individual, and other factors mean that the Poisson rate λ is not generally the same for all cases [114]. Below, we first follow ref. [114] to explore families of distributions for the number of new cases per infected individual. Next, we extend these models to consider multiple variants of the pathogen that differ in their spreading efficiency. We seek to characterize how the distribution of pathogen variant frequencies is expected to change over time, and how such data can be used to estimate the relative spreading efficiency of different variants.

2.2. Distributions for the number of infected individuals

As noted above, the basic distribution of the number of new cases n caused by one case in a susceptible population is Poisson,

$$P_{\mathrm{P}}(n|\lambda) = \frac{\lambda^n e^{-\lambda}}{n!}\,.$$

Typically we might take the Poisson rate λ to be R, the effective reproduction number, which is the expected number of cases directly caused by one case. In that case, the average number of cases following the Poisson distribution is

$$\langle n \rangle_{P_{\mathrm{P}}(n|R)} = \sum_{n=0}^{\infty} n P_{\mathrm{P}}(n|R) = R\,.$$

To account for variability in transmission dynamics, the basic Poisson distribution with a single rate R can be replaced with a continuous mixture of Poisson distributions, where the rate parameter λ follows a gamma distribution,

$$P_{\Gamma}(\lambda|\alpha,\beta) = \frac{\beta^{\alpha}}{\Gamma(\alpha)}\lambda^{\alpha-1}e^{-\beta\lambda}\,,$$

with shape parameter α and rate parameter β. The average value of λ is

$$\langle \lambda \rangle_{P_{\Gamma}(\lambda|\alpha,\beta)} = \frac{\alpha}{\beta}\,,$$

and its variance is

$$\left\langle \left(\lambda - \frac{\alpha}{\beta}\right)^2 \right\rangle_{P_{\Gamma}(\lambda|\alpha,\beta)} = \frac{\alpha}{\beta^2}\,.$$

In this context, it is natural to take $\alpha = k$ and $\beta = k/R$. With these choices, the gamma distribution reads

$$P_{\Gamma}(\lambda|k,R) = \frac{1}{\Gamma(k)}\left(\frac{k}{R}\right)^k \lambda^{k-1}e^{-k\lambda/R}. \tag{S1}$$

The parameter k is a dispersion parameter that determines how long-tailed the distribution is. The mean value of λ is always R, but when k is smaller its variance increases. In the limit that $k \to \infty$, we recover the pure Poisson distribution with rate $\lambda = R$. When $k = 1$, the distribution of the number of cases n is geometric,

$$\int_0^{\infty} d\lambda\, P_{\Gamma}(\lambda|k=1,R)\, P_{\mathrm{P}}(n|\lambda) = P_g(n|p) = (1-p)^n\, p\,,$$

where $p = 1/(1+R)$. For arbitrary values of $k > 0$, the number of cases follows a negative binomial distribution,

$$P_{\mathrm{NB}}(n|k,R) = \frac{\Gamma(k+n)}{n!\,\Gamma(k)}\left(\frac{k}{k+R}\right)^k\left(\frac{R}{k+R}\right)^n\,.$$

The standard parameters of the negative binomial distribution are r and p, which are set to k and $k/(k+R)$ in our parameterization above.

2.3. Dynamics for variant frequencies

Let us assume that there exist multiple variants of a pathogen, which are distinguished by an index a. The number of cases infected with variant a is n_a. We assume that different variants have slightly different transmission probabilities, so that $R_a = R(1+w_a)$, with $|w_a| \ll 1$. The term w_a is analogous to a selection coefficient in population genetics.

2.3.1. Dynamics of multiple cases infected by a single variant

First, let us assume that n individuals, each labeled by an index i, are all infected by the same variant of a pathogen. How many cases will be generated from these individuals? The number of new cases for all individuals is

$$n' = \sum_{i=1}^{n} n'_i ,$$

where the numbers of cases n'_i generated by individual i follows a negative binomial distribution. Because all individuals are infected by the same variant, the negative binomial parameter $p = k/(k+R)$ is the same for each of them. Then, assuming that all of the infection events are independent, it can be shown that the probability distribution for the total number of new cases n' also follows a negative binomial distribution with the same value of p, and with $r = nk$ (that is, the new r parameter value is the sum of the individual r parameter values). Thus, the distribution of n' is

$$P_{\mathrm{NB}+}(n'|k,R,n) = \frac{\Gamma(nk+n')}{n'!\,\Gamma(nk)} \left(\frac{k}{k+R}\right)^{nk} \left(\frac{R}{k+R}\right)^{n'} .$$

2.3.2. Dynamics for multiple cases infected by multiple variants

Let us extend the previous example to consider m variants of a pathogen. At the starting point, the number of individuals infected by a given variant a is n_a, with $a \in \{1,\ldots,m\}$. The fraction of cases infected by variant a is

$$y_a = \frac{n_a}{\sum_{b=1}^{m} n_b} .$$

Now, we would like to know how the fraction of individuals infected by each variant is expected to change with each round of infections. In other words, for variant a, we would like to compute

$$\left\langle y'_a \right\rangle = \left\langle \frac{n'_a}{\sum_{b=1}^{m} n'_b} \right\rangle = \sum_{\boldsymbol{n'}} \left(\prod_{b=1}^{m} P_{\mathrm{NB}+}(n'_b|k, R(1+w_b), n_b) \right) \frac{n'_a}{\sum_{c=1}^{m} n'_c}$$

where the outer sum is over all vectors $\boldsymbol{n'}$ with entries $\{n'_1, n'_2, \ldots\}$, and with $n'_b \geq 0$ for all b. Here, we have assumed that the n'_b's are independent across b.

To proceed, it is convenient to write the negative binomial distributions as mixtures of Poisson distributions (as indicated above), giving

$$\begin{aligned}
\left\langle y'_a \right\rangle &= \sum_{\boldsymbol{n'}} \left(\prod_{b=1}^{m} \int_0^\infty d\lambda_b \, P_\Gamma\left(\lambda_b | n_b k, R(1+w_b)\right) P_{\mathrm{P}}(n'_b|\lambda_b) \right) \frac{n'_a}{\sum_{c=1}^{m} n'_c} \\
&= \left(\prod_{b=1}^{m} \int_0^\infty d\lambda_b \, P_\Gamma\left(\lambda_b | n_b k, R(1+w_b)\right) \right) \sum_{\boldsymbol{n'}} \left(\prod_{b=1}^{m} P_{\mathrm{P}}(n'_b|\lambda_b) \right) \frac{n'_a}{\sum_{c=1}^{m} n'_c} .
\end{aligned}$$

Next, we use the fact that the sum of independent Poisson-distributed random variables is also Poisson with rate parameter equal to the sum of the individual rates, and that the distribution of independent Poisson random variables conditioned on their sum is multinomial, to write

$$\begin{aligned}
\left\langle y'_a \right\rangle &= \left(\prod_{b=1}^{m} \int_0^\infty d\lambda_b \, P_\Gamma\left(\lambda_b | n_b k, R(1+w_b)\right) \right) \sum_{n'=0}^{\infty} P_{\mathrm{P}}\left(n'|\lambda\right) \sum_{\boldsymbol{n'}:\sum_{c=1}^{m} n'_c = n'} P_{\mathrm{M}}\left(\boldsymbol{n'} \middle| n', \frac{\boldsymbol{\lambda}}{\lambda}\right) \frac{n'_a}{n'} \\
&= \left(\prod_{b=1}^{m} \int_0^\infty d\lambda_b \, P_\Gamma\left(\lambda_b | n_b k, R(1+w_b)\right) \right) \sum_{n'=0}^{\infty} P_{\mathrm{P}}\left(n'|\lambda\right) \frac{\lambda_a}{\lambda} \\
&= \left(\prod_{b=1}^{m} \int_0^\infty d\lambda_b \, P_\Gamma\left(\lambda_b | n_b k, R(1+w_b)\right) \right) \frac{\lambda_a}{\lambda} .
\end{aligned}$$

Here $\boldsymbol{\lambda}$ is a vector with entries $\{\lambda_1, \lambda_2, \ldots\}$, and we have also introduced $\sum_a \lambda_a = \lambda$. Note also that the outer sum on the first line is over all vectors $\boldsymbol{n}'$ whose (non-negative) entries sum to n'.

Computing the remaining integrals exactly is challenging, largely because the Gamma distributions have different rate parameters. To address this, next we will expand our expression to first order in the w_a, since these are assumed to be small parameters. Referring back to Eq. (S1), the expansion gives

$$\begin{aligned}\left\langle y_a' \right\rangle &= \left(\prod_{b=1}^{m} \int_0^\infty d\lambda_b \, P_\Gamma\left(\lambda_b | n_b k, R\right) \left[1 - k w_b \left(n_b - \frac{\lambda_b}{R} \right) \right] \right) \frac{\lambda_a}{\lambda} + \mathcal{O}\left(w^2\right) \\ &= \left(\prod_{b=1}^{m} \int_0^\infty d\lambda_b \, P_\Gamma\left(\lambda_b | n_b k, R\right) \right) \left[1 - \sum_{c=1}^{m} k w_c \left(n_c - \frac{\lambda_c}{R} \right) \right] \frac{\lambda_a}{\lambda} + \mathcal{O}\left(w^2\right) .\end{aligned}$$

Next we change variables to $\{\lambda, q_1 = \lambda_1/\lambda, q_2 = \lambda_2/\lambda, \ldots, q_{m-1} = \lambda_{m-1}/\lambda\}$, because the distribution of the sum of gamma-distributed random variables, λ, with the same rate parameter and the ratios of the individual variables to the total (λ_a/λ) follow independent gamma and Dirichlet distributions [115]. The mth ratio $q_m = 1 - \sum_{a=1}^{m-1} q_a$ by conservation. By convention we will also set $w_m = 0$, which can be thought of as normalizing the value of R relative to a reference genotype. The transformation then gives

$$\begin{aligned}\left\langle y_a' \right\rangle &= \int_0^\infty d\lambda \, P_\Gamma\left(\lambda | nk, R\right) \left(\prod_{b=1}^{m-1} \int dq_b \right) P_\mathrm{D}\left(\boldsymbol{q} | \boldsymbol{n} k\right) \left[1 - \sum_{c=1}^{m} k w_c \left(n_c - \frac{\lambda q_c}{R} \right) \right] q_a \\ &= \left(\prod_{b=1}^{m-1} \int dq_b \right) P_\mathrm{D}\left(\boldsymbol{q} | \boldsymbol{n} k\right) \left[1 - \sum_{c=1}^{m} k w_c \left(n_c - n q_c \right) \right] q_a \\ &= \left(1 - k \sum_{c=1}^{m} n_c w_c \right) y_a + \left(\prod_{b=1}^{m-1} \int dq_b \right) P_\mathrm{D}\left(\boldsymbol{q} | \boldsymbol{n} k\right) nk \left(\sum_{c \neq a} w_c q_c q_a + w_a q_a^2 \right) \\ &= \left(1 - nk \sum_{b=1}^{m} w_b y_b \right) y_a + \frac{nk}{nk+1} \left[nk \sum_{b \neq a} w_b y_a y_b + w_a \left(nk y_a^2 + y_a \right) \right] \\ &= y_a + \frac{nk}{nk+1} y_a \left(w_a - \sum_{b=1}^{m} w_b y_b \right) .\end{aligned}$$

In the expressions above $P_\mathrm{D}(\boldsymbol{q}|\boldsymbol{\alpha})$ is the Dirichlet distribution, with concentration parameters $\boldsymbol{\alpha}$ given by $\boldsymbol{n}k$ in our case. Note that if $w_m \neq 0$, the last line should instead read

$$\left\langle y_a' \right\rangle = y_a + \frac{nk}{nk+1} y_a \left(w_a - w_m - \sum_{b=1}^{m} w_b y_b \right) .$$

Thus, we obtain (with $w_m = 0$)

$$\left\langle y_a' - y_a \right\rangle = \left\langle \Delta y_a \right\rangle = \frac{nk}{nk+1} y_a \left(w_a - \sum_{b=1}^{m} w_b y_b \right) .$$

Following a similar approach, we can compute the second moments. First, we consider

$$\begin{aligned}\left\langle \left(y_a'\right)^2 \right\rangle &= \left\langle \left(\frac{n_a'}{\sum_{b=1}^{m} n_b'} \right)^2 \right\rangle \\ &= \left(\prod_{b=1}^{m} \int_0^\infty d\lambda_b \, P_\Gamma\left(\lambda_b | n_b k, R(1+w_b)\right) \right) \sum_{n'=0}^{\infty} P_\mathrm{P}\left(n' | \lambda\right) \sum_{\boldsymbol{n}' : \sum_{c=1}^{m} n_c' = n'} P_\mathrm{M}\left(\boldsymbol{n}' \middle| n', \frac{\boldsymbol{\lambda}}{\lambda} \right) \left(\frac{n_a'}{n'} \right)^2 \\ &= \left(\prod_{b=1}^{m} \int_0^\infty d\lambda_b \, P_\Gamma\left(\lambda_b | n_b k, R(1+w_b)\right) \right) \sum_{n'=0}^{\infty} P_\mathrm{P}\left(n' | \lambda\right) \left[\left(\frac{\lambda_a}{\lambda} \right)^2 + \frac{1}{n'} \frac{\lambda_a}{\lambda} \left(1 - \frac{\lambda_a}{\lambda} \right) \right] \\ &\approx \left(\prod_{b=1}^{m} \int_0^\infty d\lambda_b \, P_\Gamma\left(\lambda_b | n_b k, R\right) \right) \left[1 - \sum_{c=1}^{m} k w_c \left(n_c - \frac{\lambda_c}{R} \right) \right] \left[\left(\frac{\lambda_a}{\lambda} \right)^2 + \frac{1}{\lambda} \frac{\lambda_a}{\lambda} \left(1 - \frac{\lambda_a}{\lambda} \right) \right] \\ &= \int_0^\infty d\lambda \, P_\Gamma\left(\lambda | nk, R\right) \left(\prod_{b=1}^{m-1} \int dq_b \right) P_\mathrm{D}\left(\boldsymbol{q} | \boldsymbol{n} k\right) \left[1 - \sum_{c=1}^{m} k w_c \left(n_c - \frac{\lambda q_c}{R} \right) \right] \left[q_a^2 + \frac{q_a (1 - q_a)}{\lambda} \right] .\end{aligned}$$

In going from the third to the fourth line above, we have made the approximation that

$$\left\langle \frac{1}{n'} \right\rangle_{P_{\rm P}(n'|\lambda)} \approx \frac{1}{\lambda},$$

which is valid for $\lambda \gtrsim 1$. Similarly,

$$\begin{aligned}
\langle y'_a y'_b \rangle &= \left\langle \frac{n'_a n'_b}{\left(\sum_{c=1}^m n'_c\right)^2} \right\rangle \\
&= \int_0^\infty \left(\prod_{c=1}^m d\lambda_c \, P_\Gamma\left(\lambda_c | n_c k, R(1+w_c)\right) \right) \sum_{n'=0}^\infty P_{\rm P}\left(n'|\lambda\right) \left(1 - \frac{1}{n'}\right) \frac{\lambda_a \lambda_b}{\lambda^2} \\
&\approx \int_0^\infty \left(\prod_{c=1}^m d\lambda_c \, P_\Gamma\left(\lambda_c | n_c k, R\right) \right) \left[1 - \sum_{d=1}^m k w_d \left(n_d - \frac{\lambda_d}{R} \right) \right] \left(1 - \frac{1}{\lambda}\right) \frac{\lambda_a \lambda_b}{\lambda^2} \\
&= \int_0^\infty d\lambda \, P_\Gamma\left(\lambda | nk, R\right) \left(\prod_{c=1}^{m-1} \int dq_c \right) P_{\rm D}\left(\boldsymbol{q} | \boldsymbol{n} k\right) \left[1 - \sum_{d=1}^m k w_d \left(n_d - \frac{\lambda q_d}{R} \right) \right] \left(1 - \frac{1}{\lambda}\right) q_a q_b \,.
\end{aligned}$$

Simplifying the expressions above is tedious but straightforward. The following results are helpful:

$$\begin{aligned}
\int_0^\infty d\lambda \, P_\Gamma\left(\lambda | nk, R\right) \lambda &= nR, \\
\int_0^\infty d\lambda \, P_\Gamma\left(\lambda | nk, R\right) \frac{1}{\lambda} &= \frac{k/R}{nk-1}, \\
\left(\prod_{c=1}^{m-1} \int dq_c \right) P_{\rm D}\left(\boldsymbol{q} | \boldsymbol{n} k\right) q_a q_b &= \frac{nk}{nk+1} y_a y_b, \\
\left(\prod_{b=1}^{m-1} \int dq_b \right) P_{\rm D}\left(\boldsymbol{q} | \boldsymbol{n} k\right) q_a^2 &= y_a^2 + \frac{y_a(1-y_a)}{nk+1} = \frac{nk}{nk+1} y_a^2 + \frac{1}{nk+1} y_a, \\
\left(\prod_{c=1}^{m-1} \int dq_c \right) P_{\rm D}\left(\boldsymbol{q} | \boldsymbol{n} k\right) q_a^2 q_b &= \left(y_a^2 + \frac{y_a(1-y_a)}{nk+1} \right) \frac{nk}{nk+2} y_b, \\
\left(\prod_{b=1}^{m-1} \int dq_b \right) P_{\rm D}\left(\boldsymbol{q} | \boldsymbol{n} k\right) q_a^3 &= \left(y_a^2 + \frac{y_a(1-y_a)}{nk+1} \right) \frac{nky_a + 2}{nk+2}.
\end{aligned}$$

Here we have frequently used $n_a = n y_a$ to simplify expressions.

With the above results, simplifying expressions for the second moments, we finally find

$$\left\langle \left(\Delta y_a\right)^2 \right\rangle = \left[\frac{1}{nk+1} + \frac{nk}{nk+1} \frac{k/R}{nk-1} \right] y_a \left(1 - y_a\right) + \mathcal{O}\left(1/n^2\right),$$

and

$$\langle \Delta y_a \Delta y_b \rangle = - \left[\frac{1}{nk+1} + \frac{nk}{nk+1} \frac{k/R}{nk-1} \right] y_a y_b + \mathcal{O}\left(1/n^2\right),$$

where we have assumed that the w_a are $\mathcal{O}(1/n)$, as in the Wright-Fisher model with weak selection. We have thus found that the first and second moments of frequency changes in our multi-variant epidemiological model have the same frequency dependence as those in the multispecies Wright-Fisher model, but with different scaling. The first moment ('drift') is multiplied by a factor of $nk/(nk+1)$, and the second moment ('diffusion') by

$$\frac{1}{nk+1} + \frac{nk}{nk+1} \frac{k/R}{nk-1}.$$

These prefactors match with the Wright-Fisher model exactly when $k \to \infty$ (i.e., a pure Poisson distribution for the number of new cases per infected individual) and $R = 1$.

2.4. Derivation of the selection coefficient estimator

The derivation in this section closely follows that given in ref.[116]. It is well known that a WF process can be approximated by a continuous-time continuous-frequency diffusion process in the large n limit. In the continuous-time limit the time variable t has units of n generations, with one generation in discrete time taking $\tau = 1/n$ continuous time units. The selection coefficients w_a are assumed to scale with n such that $w_a = \tilde{w}_a/n$, where $\tilde{w}_a$ is a parameter independent of the population size n. In the limit of large population size, our generalized super-spreading model can, like the WF process, be approximated by a diffusion process, where the transition probability density ϕ is the solution to the Fokker-Planck equation

$$\frac{\partial \phi}{\partial t} = \left[-\sum_{a=1}^{M} \frac{\partial}{\partial x_a} \boldsymbol{d}(\boldsymbol{y}(t)) + \sum_{a=1}^{M}\sum_{b=1}^{M} \frac{\partial}{\partial y_a}\frac{\partial}{\partial y_b} C_{ab}(\boldsymbol{y}(t)) \right] \phi \,,$$

where M is the number of distinct genotypes, $\boldsymbol{y}$ is the genotype frequency vector, $\boldsymbol{d}$ is the drift vector, and C is the diffusion matrix. Here we ignore recombination and mutation, since these are comparatively small and therefore unlikely to significantly affect estimates of changes in viral transmission (though these can be included and the solution remains tractable). The drift and diffusion have entries given by,

$$\begin{aligned}
\tilde{d}_a(\boldsymbol{y}(t)) &= \lim_{n\to\infty} n \langle \Delta y_a \rangle \\
&= \lim_{n\to\infty} \frac{nk}{nk+1} y_a(t) \left(w_a - \sum_{b=1}^{M} w_b y_b(t) \right) \\
&= y_a(t) \left(\tilde{w}_a - \sum_{b=1}^{M} \tilde{w}_b y_b(t) \right) , \\
\tilde{C}_{ab}(\boldsymbol{y}(t)) &= \frac{1}{2} \lim_{n\to\infty} n \langle \Delta y_a \Delta y_b \rangle \\
&= \frac{1}{2}\left[\frac{1}{k} + \frac{1}{R}\right] \begin{cases} y_a(t)\,(1-y_a(t)) & a = b \\ -y_a(t) y_b(t) & a \neq b \,. \end{cases}
\end{aligned}$$

For genotype frequencies observed at times t and $t+\tau\Delta t$ (i.e., over Δt generations), and for small $\tau\Delta t$, the Fokker-Planck equation can be converted into a path integral approximation for the transition probability density (see ref.[116] for a rigorous derivation)

$$\begin{aligned}
&\phi(\boldsymbol{y}(t+\tau\Delta t)|\boldsymbol{y}(t)) \\
&\approx \frac{\exp\left\{ -\frac{4n}{\Delta t} \sum_{a=1}^{M}\sum_{b=1}^{M} \left[y_a(t+\tau\Delta t) - y_a(t) - \tilde{d}_a(\boldsymbol{y}(t))\tau\Delta t \right] \left(\tilde{C}^{-1}(y_a(t)) \right)_{ab} \left[y_b(t+\tau\Delta t) - y_b(t) - \tilde{d}_b(\boldsymbol{y}(t))\tau\Delta t \right] \right\}}{(4\pi\tau\Delta t)^{M/2} \sqrt{\det\left(\tilde{C}(\boldsymbol{y}(t))\right)}} \,.
\end{aligned}$$

From this result, and recalling $\tau = 1/n$, the transition probability from time t_m to t_{m+1} of the original branching process (for large $n/\Delta t$) can be approximated by

$$\begin{aligned}
&P(\boldsymbol{y}(t_{m+1})|\boldsymbol{y}(t_m)) \\
&\approx \phi(\boldsymbol{y}(t_{m+1})|\boldsymbol{y}(t_m)) \prod_{a=1}^{M} dy_a(t_{m+1}) \\
&= \frac{\exp\left\{ -\frac{n}{2} \sum_{a=1}^{M}\sum_{b=1}^{M} \left[\frac{y_a(t_{m+1}) - y_a(t_m)}{\Delta t_m} - d_a(\boldsymbol{y}(t_m)) \right] \left(C^{-1}(y_a(t_m)) \right)_{ab} \left[\frac{y_b(t_{m+1}) - y_b(t_m)}{\Delta t_m} - d_b(\boldsymbol{y}(t_m)) \right] \right\}}{(2\pi\Delta t_m/n)^{M/2} \sqrt{\det\left(C(\boldsymbol{y}(t_m))\right)}} \prod_{a=1}^{M} dy_a(t_{m+1}) \,,
\end{aligned}$$

where we write the re-scaled drift vector as $d_a = \tilde{d}_a\tau$, the re-scaled diffusion matrix as $C_{ab} = 2\tilde{C}_{ab}$, and $\Delta t_m = t_{m+1} - t_m$. Since we aim to infer selection coefficients for the SNVs, it is more convenient to work with the allele frequencies x_i instead of the genotype frequencies y_a. The allele frequency at site i is given by

$$x_i(t_m) = \sum_{a=1}^{M} g_i^a y_a(t_m) \,,$$

where g_i^a is a 1 if there there is a mutant allele at site i on genome a and zero if there is not. Similarly, if the selection coefficient for the genotype a is w_a and the allele level selection coefficient for allele j is s_j, then they are related by:

$$w_a = \sum_{j=1}^{L} g_j^a s_j \,,$$

where L is the length of the genome.

The allele level drift and diffusion terms will be linear combinations of the genotype level drift and diffusion, just as with the frequencies and the selection coefficients. The drift vector for the allele frequencies can be transformed by

$$\begin{aligned} d_i\left(\boldsymbol{x}(t_m)\right) &= \sum_{a=1}^{M} g_i^a d_a\left(\boldsymbol{y}(t_m)\right) \\ &= \sum_{a=1}^{M} g_i^a y_a(t_m)\left(w_a - \sum_{b=1}^{M} w_b y_b(t_m)\right) \\ &= x_i(t_m)(1-x_i(t_m))s_i + \sum_{j=1, j\neq i}^{L} \left(x_{ij}(t_m) - x_i(t_m)x_j(t_m)\right) s_j \,. \end{aligned}$$

This can be used, along with the transition probability density for genomes, in order to find an approximation for the mutant allele transition probability density:

$$\begin{aligned} &P\left(\boldsymbol{x}(t_{m+1})|\boldsymbol{x}(t_m)\right) \\ &\approx \frac{\exp\left\{-\frac{n}{2}\sum_{i=1}^{L}\sum_{j=1}^{L}\left[\frac{x_i(t_{m+1})-x_i(t_m)}{\Delta t_m} - d_i(\boldsymbol{x}(t_m))\right]\left(C^{-1}(\boldsymbol{x}(t_m))\right)_{ij}\left[\frac{x_j(t_{m+1})-x_j(t_m)}{\Delta t_m} - d_j(\boldsymbol{x}(t_m))\right]\right\}}{(2\pi\Delta t_m/n)^{L/2}\sqrt{\det\left(C(\boldsymbol{x}(t_m))\right)}} \prod_{i=1}^{L} dx_i(t_{m+1}) \,, \end{aligned}$$

where here the diffusion C is derived similarly to the drift $\boldsymbol{d}$ and has entries

$$C_{ij}(\boldsymbol{x}(t_m)) = \left[\frac{1}{k} + \frac{1}{R}\right]\left(x_{ij}(t_m) - x_i(t_m)x_j(t_m)\right) \,.$$

A path integral then gives the probability of observing a trajectory of allele frequencies $(\boldsymbol{x}(t_1), \boldsymbol{x}(t_2), ..., \boldsymbol{x}(t_{T-1}))$, and is given by

$$P\left(\left(\boldsymbol{x}(t_m)\right)_{m=1}^{T} \middle| \boldsymbol{x}(t_0)\right) = \prod_{m=0}^{T-1} P\left(\boldsymbol{x}(t_{m+1})|\boldsymbol{x}(t_m)\right) \,.$$

Bayesian analysis can then be used to show that the posterior probability of the selection coefficients $\boldsymbol{s} = (s_1, s_2, ..., s_L)$ given an observed frequency path $\boldsymbol{x}(t_0), \boldsymbol{x}(t_1), ..., \boldsymbol{x}(t_{T-1})$ is

$$P\left(\boldsymbol{s}\middle|\left(\boldsymbol{x}(t_m)\right)_{m=0}^{T}\right) \propto P\left(\left(\boldsymbol{x}(t_m)\right)_{m=1}^{T}\middle|\boldsymbol{x}(t_0)\right) \times P_{\mathrm{Prior}}(\boldsymbol{s}) \,, \tag{S2}$$

where we use a Gaussian prior distribution with zero mean and adjustable covariance determined by the parameter γ, which is the precision.

For the inferred coefficients, we take those that maximize the posterior probability. They can be analytically found by a simple application of the Euler-Lagrange equations to (S2) and are given by

$$\hat{\boldsymbol{s}} = \left[\gamma I + \sum_m n\frac{k^2R^2}{(R+k)^2}C(t_m)\right]^{-1}\left[\sum_m \frac{nkR}{k+R}\left(\Delta\boldsymbol{x}(t_m)\right)\right] \,. \tag{S3}$$

2.5. Extension to multiple regions

In the SARS-CoV-2 pandemic, and in real disease outbreaks in general, there are frequently multiple different outbreaks in different regions that develop largely or entirely independently of one another. In order to find the best estimate for the selection coefficients using the data from multiple regions, the estimator can be generalized to find the maximum a posteriori estimate for the selection coefficients given the time series of allele frequencies in each of the regions. If the probability for a specific

path in a specific region r is given by $P\left((\boldsymbol{x}_r(t_{r,m}))_{m=1}^{T_r}|\boldsymbol{x}_r(t_{r,0})\right)$, where x_r is the allele frequency vector in region r, then the joint probability of the specific paths in all of the regions is simply the product of the individual region probabilities:

$$P\left((\boldsymbol{x}_1(t_{1,m}))_{m=1}^{T_1},...,(\boldsymbol{x}_Q(t_{Q,m}))_{m=1}^{T_Q}\big|\{\boldsymbol{x}_r(t_{r,0})\}_{r=1}^{Q}\right)=\prod_{r=1}^{Q}P\left((\boldsymbol{x}_r(t_{r,m}))_{m=1}^{T_r}|\boldsymbol{x}_r(t_{r,0})\right),$$

where Q is the number of different regions. Since this is a product of exponential functions, the log posterior will be the sum of the exponents and the regularization. This can be maximized with respect to the selection coefficient vector $\boldsymbol{s}$ as before and leads to the estimator:

$$\hat{\boldsymbol{s}}=\left[\gamma I+\sum_r\sum_{t_{r,m}}\frac{n_r k_r^2 R_r^2}{(k_r+R_r)^2}C_r(t_{r,m})\right]^{-1}\left[\sum_r\sum_{t_{r,m}}\frac{k_r n_r R_r}{k_r+R_r}\Delta\boldsymbol{x}_r(t_{r,m})\right]. \tag{S4}$$

2.6. Simplification of the estimator

In real outbreaks the parameters k, R, and n are in general time-varying. In our simulations as well, R and n are time-varying (and k can be constant or time-varying). In order to accurately infer the selection coefficients according to Eq. (S3) or Eq. (S4), it would seem that we need to accurately infer the values of k, R, and N at every point in the time series. In practice, this would be extremely difficult. For general discussion about the effective reproduction number R and the basic reproduction number R_t as well as some attempts to infer this, see refs.[117–121]. In order to get an accurate estimate for k it is necessary to have pervasive contact tracing, so that the negative binomial distribution is well sampled, and there are other difficulties in inferring k as well[122–124]. Lastly, it can be difficult to estimate the number of new infections due to multiple factors, including the difference between the population that gets tested and the population that does not, test result inaccuracies, and delays between symptom onset, testing, and reporting[125,126].

We propose an alternative that lets us avoid these complications. The prefactor $nkR/(R+k)$, multiplies both the numerator and the denominator. Therefore, the only effect of the prefactor is to weight time points more heavily if the population size, the dispersion parameter, or the basic reproduction number, is larger. This makes sense in theory, because a larger n or k implies that there is less noise and the trajectories are more deterministic, while a larger R means that there are more new infections per generation and thus more data to use to infer the selection coefficients. This does hold with perfect information, that is, if all infected individuals are sampled at every time point. However, in practice, finite sampling is the source of significantly more noise than that due to a time-varying population size or dispersion, so weighting the time points based upon n, k, or R in fact leads to worse inference than assuming the parameters are constant in time and thus weighting the time points equally. However, in the special and unrealistic case of perfect sampling, using the actual parameters does lead to better inference than using constant parameters (see **Supplementary Fig. 10**). If the time points are weighted equally, then, provided that the regularization γ is scaled appropriately (and in general it must be determined by separate means, discussed below), the prefactors in the numerator and denominator cancel, and the estimator is independent of n, k, and R. Defining $\gamma'=\gamma nkR/(k+R)$ and $\bar{C}$ by

$$C=\left[\frac{nkR}{k+R}\right]\bar{C},$$

so that

$$\bar{C}_{ij}=\begin{cases}x_{ij}(t_m)-x_i(t_m)x_j(t_m) & i\neq j\\ x_i(t_m)\left(1-x_i(t_m)\right) & i=j\end{cases},$$

Eqs. (S3) and (S4) for the selection coefficients become, respectively

$$\hat{\boldsymbol{s}}=\left[\gamma' I+\sum_{t_m}\bar{C}(t_m)\right]^{-1}\left[\sum_{t_m}\Delta\boldsymbol{x}(t_m)\right],$$

$$\hat{\boldsymbol{s}}=\left[\gamma' I+\sum_r\sum_{t_{r,m}}\bar{C}_r(t_{r,m})\right]^{-1}\left[\sum_r\sum_{t_{r,m}}\Delta\boldsymbol{x}_r(t_{r,m})\right],$$

which are the same as the MPL estimators for the Wright-Fisher model except for the absence of a mutation term[116].

2.7. Covariance of the inferred selection coefficients

Since the posterior given in (S2) is a Gaussian distribution for the selection coefficients, the covariance matrix of the inferred selection coefficients can be easily found. For any Gaussian distributed random vector $\boldsymbol{z}$, the inverse of the covariance can be

calculated as the second derivative with respect to $\boldsymbol{z}$ of the negative log of the probability density function. That is, if we define

$$\begin{aligned} J &= -\ln\left[P\left(\boldsymbol{s}\middle|\left(\boldsymbol{x}(t_m)\right)_{m=0}^{T}\right)\right] \\ &= \frac{1}{2}\left[\gamma\boldsymbol{s}^2 + \sum_{m=0}^{T-1} n\left[\boldsymbol{x}(t_{m+1}) - \boldsymbol{x}(t_m) - \boldsymbol{d}(\boldsymbol{x}(t_m))\right]^T C^{-1}(\boldsymbol{x}(t_m))\left[\boldsymbol{x}(t_{m+1}) - \boldsymbol{x}(t_m) - \boldsymbol{d}(\boldsymbol{x}(t_m))\right]\right. \\ &\qquad \left. + \sum_{m=0}^{T-1}\left(L\ln\left(\frac{2\pi}{n}\right) + \ln(\det C)\right)\right], \end{aligned}$$

then the inverse of the covariance matrix of the parameters is given by the second derivative of J with respect to $\boldsymbol{s}$. The first derivative of J with respect to $\boldsymbol{s}$ gives

$$\frac{\partial J}{\partial \boldsymbol{s}} = \gamma\boldsymbol{s} - \sum_{m=0}^{T-1}\frac{nkR}{k+R} C C^{-1}\left[\boldsymbol{x}(t_{m+1}) - \boldsymbol{x}(t_m) - \boldsymbol{d}(\boldsymbol{x}(t_m))\right].$$

The second derivative, which is the inverse of the covariance of the selection coefficients $\boldsymbol{s}$, is

$$\frac{\partial^2 J}{\partial \boldsymbol{s}\partial \boldsymbol{s}^T} = \gamma + \sum_{m=0}^{T-1}\frac{nk^2R^2}{(k+R)^2} C(\boldsymbol{x}(t_m)).$$

This implies that the covariance of the inferred coefficients is given by

$$\Sigma = \left[\gamma I + \sum_{m=0}^{T-1}\frac{nk^2R^2}{(k+R)^2} C(\boldsymbol{x}(t_m))\right]^{-1}.$$

Using the definitions of γ' and $\bar{C}$ given above, in the case where the parameters n, k, and R are constant, this reduces to

$$\Sigma = \frac{k+R}{nkR}\left[\gamma' I + \sum_{m=0}^{T-1} C(\boldsymbol{x}(t_m))\right]^{-1}. \tag{S5}$$

Since $(k+R)/nkR$ is a decreasing function of k, this implies that the theoretical covariance decreases as the dispersion k becomes larger.

2.8. Covariance of inferred selection coefficients for a group of fully linked sites

The above analysis can be used to quantify the covariance between inferred coefficients for a group of SNVs that are fully linked, meaning that all of the SNVs in the group appear together on every sequence on which one of the SNVs appear. This is useful because it provides an estimate for the maximum covariance between linked SNVs. An analytical result is presented only for the special case where all of the SNVs under consideration are fully linked, though simulations indicate that the maximum value is not strongly dependent on other SNVs that are partially linked to the main group. The covariance matrix at any time for a group of fully linked SNVs has (i,j)th element given by $(C(t_m))_{ij} = \left[\frac{1}{k} + \frac{1}{R}\right] x_i(t_m)(1 - x_i(t_m))$ for any (i,j), since the frequencies $x_i(t_m)$ for all of the SNVs are identical. This implies that the second term in (S5) is a matrix with every entry identical. If we define the elements of the matrix

$$\sum_{m=0}^{T-1}\frac{nk^2R^2}{(k+R)^2} C_{ij}(\boldsymbol{x}(t_m)) \equiv \alpha,$$

the vector $\boldsymbol{u}$ as the vector of all 1's, and use the notation $(\cdot)^{\mathrm{T}}$ to denote transpose, then the covariance of the inferred coefficients can be written as

$$\Sigma_{\text{linked}} = \left[\gamma I + \alpha\boldsymbol{u}\boldsymbol{u}^{\mathrm{T}}\right]^{-1}.$$

Because of the simplicity of this form of the matrix, the inversion can be carried out explicitly using the Sherman-Morrison formula, which for an $n \times n$ matrix gives

$$\begin{aligned} \Sigma_{\text{linked}} &= \frac{1}{\gamma} I - \frac{\alpha\frac{1}{\gamma^2} I\boldsymbol{u}\boldsymbol{u}^{\mathrm{T}} I}{1 + \alpha\boldsymbol{u}^{\mathrm{T}} I\boldsymbol{u}\frac{1}{\gamma}} \\ &= \frac{1}{\gamma} I - \frac{1}{\frac{\gamma^2}{\alpha} + \gamma n}\boldsymbol{u}\boldsymbol{u}^{\mathrm{T}}. \end{aligned}$$

From this the correlation matrix can be easily calculated, and the off-diagonal elements represent the maximum correlation between n SNVs that are fully linked to one another. The off diagonal elements of the correlation matrix are given by

$$\rho_{i,j} = \frac{1}{1 - n - \frac{\gamma}{\alpha}}.$$

We analyzed sets of strongly linked mutations in the Alpha, Delta, and Omicron variants to test our ability to distinguish the independent selective effects of individual mutations. **Supplementary Figure 14** shows that, while many inferred selection coefficients are naturally correlated, this correlation is far from complete. Only in rare circumstances (e.g., the three nucleotide mutations comprising N:D3L in Alpha) are SNVs so strongly linked that their effects cannot be at least partially disentangled. [127,128]

3. Simulations

We tested the inference using simulations of disease spread. Specifically, we ran super-spreader simulations based on the model described above, which is an analog of the Wright-Fisher model where the sampling distribution for the number of new infections per infected individual is drawn from a negative binomial distribution instead of a pure Poisson distribution.

3.1. Description of simulations

We simulated disease spread as a branching process in which the number of individuals infected per currently infected individual is drawn from a negative binomial distribution whose shape is determined by the basic reproduction number R_0 (or the reproduction number, R, in a population that is not totally susceptible) and the dispersion parameter k. Because we sample in this way, the population size is not constant. However, if the population size is too small, then the population is extremely likely to die off stochastically, and if the population size is too large, then sampling from the negative binomial becomes too computationally expensive. In order to avoid both of these problems, once the population size is large enough R is adaptively adjusted so that the average reproduction number for the entire population will remain near 1, and the population size will oscillate around a fixed value. An explicit time-varying population size can also be used as input, and R will be adaptively adjusted to remain near the given curve. Constant values can be used for the dispersion k or k can vary as a function of time, perhaps representing different degrees of social distancing or lockdown measures at different times. Since different interventions implemented to prevent the spread of disease would likely affect the shape of the distribution of the number of individuals infected by a single infected individual, time-varying values for k and R can be used to reflect these effects.

3.2. Inference

The simulations are run for a number of generations and genomes are sampled from the population of infected individuals at different times using a multinomial sampling distribution. This sampled time series is then used to infer the selection coefficients using (S3). Alternatively, multiple simulations can be run and the joint inference of the selection coefficients can be made using (S4). We find that, given good enough sampling, a long enough time series, and sampling that occurs at a sufficient number of times, the selection coefficients can be inferred very accurately (**Fig. 1**). The quality of inference is significantly improved if multiple simulations are combined and if mutated sites show up in more than one of the simulations, even under less than ideal sampling conditions. Beneficial coefficients are typically inferred more accurately than deleterious ones, likely because deleterious SNVs frequently die off and therefore there is less data to use for inference.

The inference is robust to shortening the time-series or lowering the number of samples taken per generation, though obviously if either of these conditions is too extreme (or worse, both), the inference starts to break down. The negative effects of a short time-series or poor sampling can be somewhat made up for by using multiple simulations, which is analogous to using data from outbreaks in multiple regions. In addition, the diffusion approximation is only valid in the large n limit. However, we tested the inference for small population sizes and found that inference is accurate even if the population of newly infected individuals per serial interval is as low as a few hundred (**Fig. 1**).

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