

1 Deciphering the code of viral-host adaptation
2 through maximum entropy models

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12 **Abstract**

13 Understanding how the genome of a virus evolves depending on the host it
14 infects is an important question that challenges our knowledge about several
15 mechanisms of host-pathogen interactions, including mutational signatures,
16 innate immunity, and codon optimization. A key facet of this general topic
17 is the study of viral genome evolution after a host-jumping event, a topic
18 which has experienced a surge in interest due to the fight against emerging
19 pathogens such as SARS-CoV-2. In this work, we tackle this question by in-
20 troducing a new method to learn Maximum Entropy Nucleotide Bias models
21 (MENB) reflecting single, di- and tri- nucleotide usage, which can be trained
22 from viral sequences that infect a given host. We show that both the viral
23 family and the host leave a fingerprint in nucleotide usages which MENB
24 models decode. When the task is to classify both the host and the viral fam-
25 ily for a sequence of unknown viral origin MENB models outperform state
26 of the art methods based on deep neural networks. We further demonstrate
27 the generative properties of the proposed framework, presenting an example
28 where we change the nucleotide composition of the 1918 H1N1 Influenza
29 A sequence without changing its protein sequence, while manipulating the
30 nucleotide usage, by diminishing its CpG content. Finally we consider two

31 well-known cases of zoonotic jumps, for the H1N1 Influenza A and for the
32 SARS-CoV-2 viruses, and show that our method can be used to track the
33 adaptation to the new host and to shed light on the more relevant selective
34 pressures which have acted on motif usage during this process. Our work
35 has wide-ranging applications, including integration into metagenomic stud-
36 ies to identify hosts for diverse viruses, surveillance of emerging pathogens,
37 prediction of synonymous mutations that effect immunogenicity during viral
38 evolution in a new host, and the estimation of putative evolutionary ages for
39 viral sequences in similar scenarios. Additionally, the computational frame-
40 work introduced here can be used to assist vaccine design by tuning motif
41 usage with fine-grained control.

42 **Author summary**

43 In our research, we delved into the fascinating world of viruses and their
44 genetic changes when they jump from one host to another, a critical topic
45 in the study of emerging pathogens. We developed a novel computational
46 method to capture how viruses change the nucleotide usage of their genes
47 when they infect different hosts. We found that viruses from various families
48 have unique strategies for tuning their nucleotide usage when they infect the
49 same host. Our model could accurately pinpoint which host a viral sequence
50 came from, even when the sequence was vastly different from the ones we
51 trained on. We demonstrated the power of our method by altering the nu-
52 cleotide usage of an RNA sequence without affecting the protein it encodes,
53 providing a proof-of-concept of a method that can be used to design better
54 RNA vaccines or to fine-tune other nucleic acid-based therapies. Moreover
55 the framework we introduce can help tracking emerging pathogens, predict-
56 ing synonymous mutations in the adaptation to a new host and estimating
57 how long viral sequences have been evolving in it. Overall, our work sheds
58 light on the intricate interactions between viruses and their hosts.

59 **1 Introduction**

60 The recent COVID-19 pandemic inspired the scientific community to investi-
61 gate zoonotic transmission of viruses [Parrish et al., 2008, Andersen et al., 2020]
62 and the subsequent evolutionary dynamics of viral adaptation to a new
63 host. Several experimental [Starr et al., 2020, Moulana et al., 2022] and
64 computational [Rodriguez-Rivas et al., 2022, Tubiana et al., 2022] investi-
65 gations pointed out the impact of amino-acid mutations in the spike glyco-
66 protein and their effects on its interaction with the human ACE2 receptor,
67 which conferred a fitness advantage and resulted in selective sweeps of new
68 variants [Kang et al., 2021, Lee et al., 2022].

69 Another fundamental question is identifying Pathogen-Associated Molec-
70 ular Patterns (PAMPs) in a viral sequence [Akira and Hemmi, 2003] and
71 predicting how the virus changed those patterns to adapt to the human
72 environment and to alter innate immune recognition and response. This
73 topic had been previously explored for the H1N1 strain of the 1918 H1N1
74 influenza pandemic. In this context it has been shown that the viral genome
75 evolved in a predictable way to lose CpG motifs (a cytosine followed by a
76 guanine in the 5'-to-3' sense) after entering its human host from an avian
77 reservoir [Greenbaum et al., 2008, Greenbaum et al., 2014]. This observa-

78 tion, together with the fact that most human-infecting viruses have a low
79 abundance of CpG motifs, was followed by the identification of the CpG-
80 dependent receptor specificity of the human Zinc-finger Antiviral Protein
81 (ZAP, coded by ZC3HAV1 gene) [Gao et al., 2002, Takata et al., 2017], im-
82 plying such approaches can identify recognition sites by host anti-viral re-
83 striction factors. Similar analyses for the early evolution of SARS-CoV-
84 2 have been carried out [Di Gioacchino et al., 2021, Kumar et al., 2022],
85 showing a similar pressure to reduce CpG motifs in CpG-rich regions of
86 the viral genome. Finally, understanding and controlling the impact of a
87 foreign RNA sequence on the stimulation of the innate immune response
88 has an important application in DNA and RNA vaccine design in order
89 to avoid over-stimulating the host innate reaction to nucleic acids in the
90 vaccine [Zhang et al., 2023], while also optimizing for features such as codon
91 bias [Pardi et al., 2018].

92 These questions are facets of the fundamental problem of determining
93 how the interaction of a virus with its host is imprinted upon evolving viral
94 genomes. This topic has been considered in several contexts [Hall et al., 2013,
95 Bloom et al., 2023], demonstrating that viruses of the same family accumu-
96 late mutations to use similar nucleotide patterns when they evolve in inter-
97 action with a specific host. This idea has been in turn the cornerstone of
98 a fruitful series of works aimed at determining the host of a virus from its
99 genome. Remarkably, it has been shown that methods that do not resort
100 to sequence alignment perform, for this specific task, comparably well with
101 alignment-based methods [Li and Sun, 2018]. These methods typically rely
102 on using machine learning based on the frequencies of k -mers (subsequences
103 of length k) up to a given length k_{\max} , either alone [Tang et al., 2015,
104 Brierley and Fowler, 2021], together with other features such as physical-
105 chemical properties of amino-acids [Young et al., 2020], or using a hybrid
106 method that integrates alignment-based features [Babayan et al., 2018]. Re-
107 cently, techniques based on deep neural networks have been suggested to
108 solve the task of finding the correct host of a given virus, completely by-
109 passing the choice of the features used for a model [Mock et al., 2020]. While
110 most of these methods can give remarkable classification performances, there
111 is a pressing need for techniques that are effective at the classification task
112 while remaining at the same time simple to use and interpretable. The latter
113 point is particularly important to increase our molecular understanding of
114 the evolutionary processes that a virus undergoes after an host switch, which
115 can then be targeted by a antiviral therapies during a zoonotic transmission.

116 In this work, we address all these issues by taking a novel approach:
117 we build a maximum entropy model whose parameters are inferred to cap-

118 ture short-range (up to 3-mers) nucleotide usage patterns in viral genome
119 sequences. Maximum-entropy models have been already used in several con-
120 texts, such as for protein sequences [Morcos et al., 2011, Cocco et al., 2018,
121 Mayer et al., 2022], neuronal spiking activity [Tavoni et al., 2017, Ferrari et al., 2017]
122 and social dynamics [Bialek et al., 2012, Chen et al., 2022], demonstrating
123 the effectiveness and flexibility of this approach. In the context of viral
124 evolution and identification of PAMPS in RNA sequences the approach
125 introduced here extends the selective force model previously introduced
126 [Greenbaum et al., 2014, Tanne et al., 2015, Di Gioacchino et al., 2021] which
127 reproduced the motif usage of a particular k -mers only: CpG dinucleotide
128 and other individual motifs. In analogy to k -mer based methods our model
129 does not require any alignment or annotation of the genetic sequence under
130 analysis. We show our technique is simple but extremely effective to tackle
131 the host classification task, resulting in performances comparable with deep
132 neural network models or, in the more challenging setting where no phylo-
133 genetic information is available, superior in its discrimination capability.

134 2 Results

135 2.1 MENB: a model for host and viral origin classification

Our unsupervised learning model, MENB, infers parameters associated for each k -mer up to $k = 3$ and defines a probability distribution on viral sequences (of a fixed length), in such a way that the expected k -mer frequencies from this distribution match with those observed in the training data. As shown in Methods Sec. 5.1.1, this results in the following probability distribution for a viral sequence \mathbf{s} :

$$p(\mathbf{s}) \propto \exp \left(\sum_{a \in \mathcal{S}} f_a^{(1)} n_a(\mathbf{s}) + \sum_{ab \in \mathcal{S}} f_{ab}^{(2)} n_{ab}(\mathbf{s}) + \sum_{abc \in \mathcal{S}} f_{abc}^{(3)} n_{abc}(\mathbf{s}) \right),$$

136 where \mathcal{S} is the set of nucleotides, $n_m(\mathbf{s})$ is the number of times the mo-
137 tif m is present in \mathbf{s} , and the parameters indicated by f are the “forces”
138 [Greenbaum et al., 2014] to be inferred from the training data.

139 To train our model we collected viral sequences from the BV-BRC database
140 [Olson et al., 2022], and filtered the data for sequences of three host classes:
141 human, avian and swine viruses. We required at least 150 (different) viral
142 genomes for each host class, and this left us with 4 viral families: *Coro-*
143 *naviridae*, *Flaviviridae*, *Picornaviridae*, and *Orthomyxoviridae* (focusing on
144 Influenza A alone). We stress that such number of sequences is in principle

145 not necessary to train our models: a single sequence (of sufficient length)
146 is enough, provided that the number of motifs observed in that sequence is
147 representative. To avoid biases in choosing this reference sequence, however,
148 we decided to train the models on sets of 100 sequences (the remaining se-
149 quences are used as test set). We then test the model in the task of host
150 classification from a viral sequence. We consider three strategies to assign
151 an host to a given viral sequence. In the simplest one, called “MENB-H”, for
152 each host h we grouped together the sequences belonging to different viral
153 families and trained a single MENB model that approximates the probabil-
154 ity $p(s|h)$. Given a new sequence s , we can therefore estimate the probability
155 of it coming from host h using Bayes formula $p(h|s) \propto p(s|h)p(h)$, where
156 $p(h)$ is a prior that we will consider uniform over the host distribution.

157 To introduce a more complex strategy we start by training a set of MENB
158 models $p(s|h, v)$ at fixed viral family v and host h . As in the previous case,
159 we can then obtain the probability of a sequence to be associated to a host-
160 virus, (h, v) , pair as $p(h, v|s) \propto p(s|h, v)p(h, v)$. If we know the viral origin
161 (v_0) of the test sequence we can limit ourselves to compare models trained
162 for that family on different host, a strategy that we name “MENB-H|V”,
163 and by assuming an uniform prior $p(h, v_0)$ we obtain $p(h|s, v_0) \propto p(s|h, v_0)$.

164 If, on the contrary, we ignore the viral family of the sequence we can then
165 sum over the different viral families to have a probability a virus is associated
166 with a given host, a strategy that we will call “MENB-H,V”. By assuming
167 again a uniform prior we obtain $p(h|s) \propto \sum_v p(s|h, v)$. Remarkably, for all
168 viral genomes analyzed in this work, there is a unique term that contributes
169 much more than all the others to the above summation. Hence we can
170 associate to a viral sequence a specific host as the most likely origin, and
171 likewise guess the viral family from the term that mostly contributes to the
172 probability of that host.

173 The results of the host classification task on test viral sequences, after
174 having trained the models using the three strategies (“MENB-H”, “MENB-
175 H,V”, “MENB-H|V”) discussed are displayed in in Fig. 1A. We first notice
176 that the viral agnostic models, MENB-H, has a low accuracy: the accu-
177 racy averaged over the viral families is about 51%, (blue dashed line), only
178 marginally better than random guessing (33%, black dashed line), with per-
179 formances comparable to random guessing for *Coronaviridae* and *Orthomyx-*
180 *oviridae*. Similar results have been observed elsewhere [Mock et al., 2020].
181 A possible explanation for the failure of this viral-agnostic host inference
182 strategy is that viral genomes are highly constrained (for instance, they
183 need to code for multiple, sometimes overlapping protein sequences while
184 interacting with viral proteins for encapsulation), hence not free to evolve to

185 change their nucleotide usage in a way that depends uniquely upon the host.
186 Such explanation is confirmed by the improved performances obtained when
187 learning viral-families dependent models for each hosts (“MENB-V,H”), and
188 marginalizing over viral families to find the most probable host. “MENB-
189 V,H” gives an average performance in classification of (85%, orange bars
190 in Fig. 1A). Moreover when comparing (Fig. 1B) the values of v that give
191 the largest contribution to the sum with the real viral families. We find an
192 average accuracy of about 97%, confirming that the “MENB-V,H” strategy
193 is able to predict, with a very good accuracy, both the host and the viral
194 family of a new sequence.

195 **2.2 Comparison of MENB with other approaches**

196 Given the performance of MENB models for the host classification task, a
197 natural question is how it compares with other state-of-the-art approaches.
198 To answer this, we considered VIDHOP [Mock et al., 2020], a deep-neural
199 network designed specifically for this task which can be obtained from a pub-
200 lic code repository re-trained by any user. The authors in [Mock et al., 2020]
201 noticed that their algorithm could not generalize to viruses of different fam-
202 ilies, so they designed VIDHOP to work at fixed viral family. As we demon-
203 strated, MENB can in principle work without information about the viral
204 family of the target sequence, but to make the comparison fairer we modi-
205 fied our approach to use MENB models to assess the host of viral sequences
206 at fixed viral family: we considered as hosts directly the $\arg \max_h p(h, v|s)$,
207 where the correct viral family v is used instead of summing on all possible
208 families. We retrained VIDHOP and MENB on the same sequences, and
209 compared their performances. As expected from the higher complexity (in
210 terms of number of learnable parameters) of VIDHOP, its performances are
211 better than MENB in most cases and in particular for *Coronaviridae*, while
212 being very similar for *Orthomyxoviridae*, as shown in Fig. 1A (green and red
213 bars). On the other hand, VIDHOP requires many more resources (in terms
214 of time and computational power) with respect to MENB (for instance, for
215 each viral family VIDHOP requires about 1 hour on a 56-core CPU, while
216 MENB requires less than 5 minutes on 3 cores).

217 We then wanted to confirm that the host classification results we ob-
218 tained with MENB models are actually related to viral adaptation to their
219 hosts, and not caused by spurious effects such as phylogenetic correlations
220 that lead to strong similarity of sequences in the training and test set.
221 We therefore designed a more difficult classification task based on out-of-
222 distribution data points: we trained our model on a part of the viral se-

223 quences (the first half for *Coronaviridae*, *Flaviviridae* and *Picornaviridae*,
224 and on all segments but PB2 for *Orthomyxoviridae*), and used it to deter-
225 mine the host from the other part of the sequences. In this way the classifi-
226 cation is performed on sequences that are completely different (in terms of
227 edit distance) from those used during training, but as shown in Fig. 1C and
228 D, the model can still determine quite precisely the viral family of the test
229 part of the sequences (the average accuracy is about 89%), and performs
230 much better than a random classifier in determining the host (the average
231 accuracy is about 67%), although the performances are degraded with re-
232 spect to those obtained with full sequences. Remarkably, in this test MENB
233 performs sensibly better than VIDHOP, whose results are only marginally
234 superior than those of a random classifier (black dashed line in the plot).
235 It is therefore reasonable to expect that the extremely good performance of
236 VIDHOP on full sequences relies on the large similarity between training
237 and test sequences, even if cross-validation during training is used to select
238 the best model on a validation dataset.

239 In general, the performance of MENB models derive from the differences
240 between the probability distributions over viral sequences that each model
241 learns. In Fig. 2 we show the symmetrized Kullback-Leibler (KL) divergence
242 (for a definition, see Methods Sec. 5.1.3) between each pair of distributions.
243 Remarkably, models trained on viruses infecting the same host encode far
244 more different probability distributions than models trained on viruses of
245 the same family, suggesting that the nucleotide usage is more driven by
246 phylogenetic correlations than by host adaptation. This is compatible with
247 the much greater performances of the MENB models in discriminating viral
248 families rather than hosts, and the smaller divergences within viral families
249 ultimately justify the choice of using the “MENB-H,V” strategy. Moreover,
250 we notice that *Orthomyxoviridae* viruses have smaller differences between
251 hosts with respect to other viral families, probably because of their tendency
252 to commonly undergo reassortments with segments of viruses adapted to
253 different hosts.

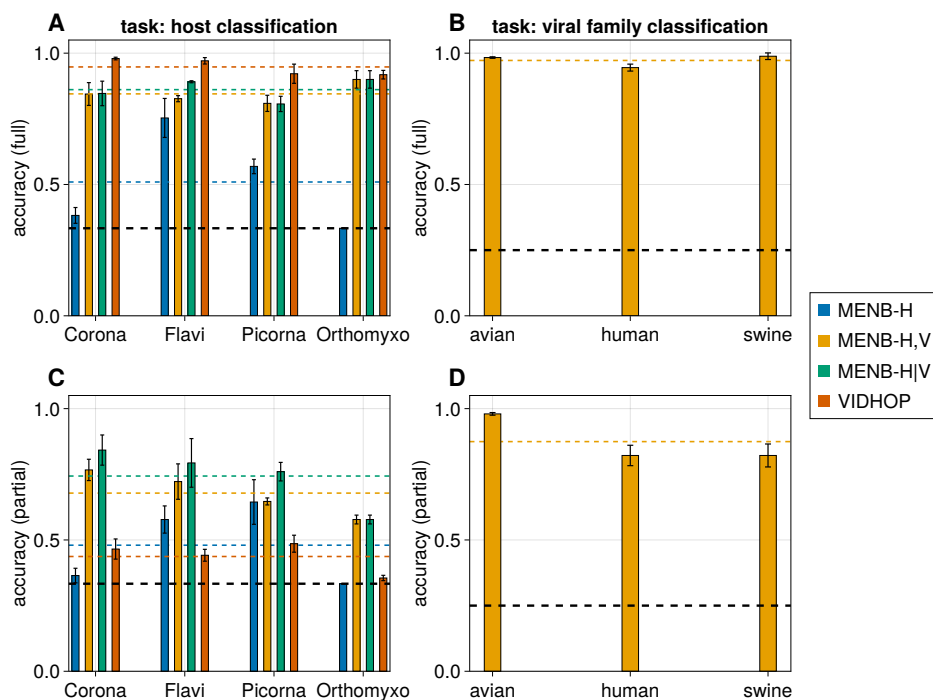


Figure 1: **MENB models can predict host and viral family of viral genomes.** A: accuracy of MENB models trained on all viruses with the same host (blue bars) and on all virus-host pairs (orange bars) on the host classification task on the test set of viral genomes; green bars are obtained using the same models used for the orange bar, but using only the correct viral family of the target viral genome, and red bars are the accuracy of the host classification task in this same setting with the VIDHOP algorithm. B: accuracy of MENB models trained on all virus-host pairs in determining the correct viral family for the target test genome as the one that mostly contributes in the host classification. C: same as A, but the training is done on the first half of the genome (for *Coronaviridae*, *Flaviviridae*, *Picornaviridae*) or on all segments but PB2 (for *Orthomyxoviridae*), and the test is done on the remaining part of the sequence. D: same as B, with the same task as described in C.

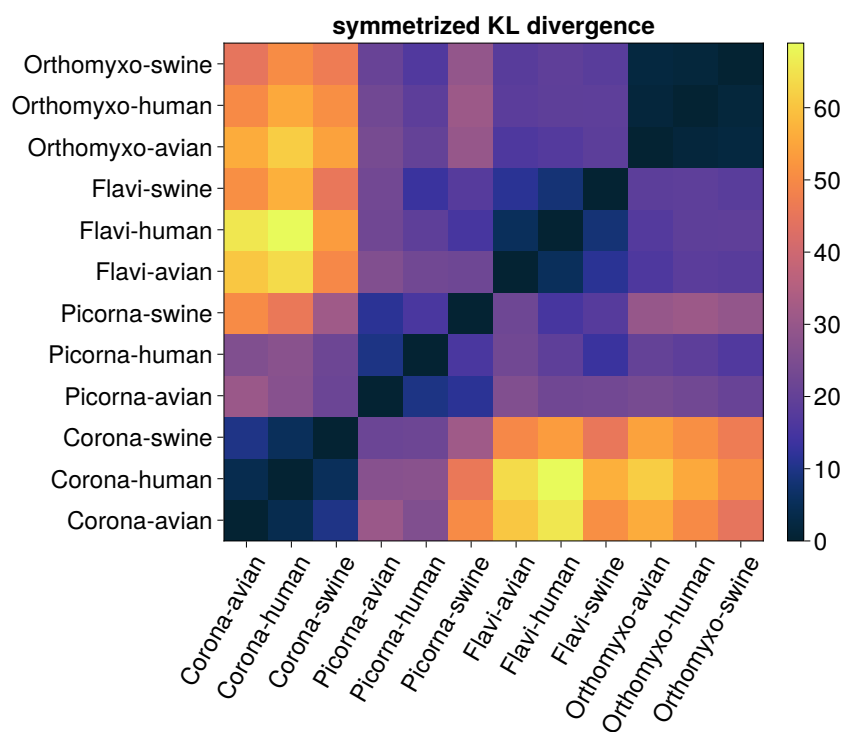


Figure 2: **Viruses infecting the same host use nucleotides in different ways.** Symmetrized Kullback-Leibler divergences between all (full) MENB model pairs considered in this work. The divergence is computed with respect to sequences having an arbitrary length of 1000 nucleotides, see Methods Sec. 5.1.3.

254 **2.3 Generative power of MENB models**

255 In Fig. 3 we focus on the human *Orthomyxoviridae* viral sequences and we
256 show that the MENB model reproduces, as expected, the 1-, 2- and 3-mer
257 statistics of the training set (Suppl. Fig. 5). Moreover, it generalizes to new
258 sequences in the test set, which are not used for the training, when full
259 genomes are used (Fig. 3A). These performances are only slightly degraded
260 when a fraction of each genome is used in the training test and the test set
261 contains new sequences and the unseen part of the genome (Fig. 3C), further
262 showing how nucleotide usage biases encompass the full viral sequences and
263 can be learned from a fraction of them.

264 The MENB models are trained to reproduce the frequency of 1-, 2-, and
265 3-mers observed in the training dataset; we next investigated how well these
266 models reproduce higher order statistics. To do so, we sampled sequences
267 from the probability distribution encoded by MENB models (using a stan-
268 dard Metropolis–Hastings algorithm) and compared to the 4-mer frequencies
269 observed in these sampled sequences with those of the training dataset. In
270 Fig. 3B,D we show that MENB model almost perfectly capture the 4-mer
271 statistics.

272 In Fig. 4 we further show how we can leverage MENB models together
273 with the Metropolis–Hastings sampling algorithm to change the nucleotide
274 usage of a protein-coding sequence, while keeping fixed its amino-acid se-
275 quence. As an illustration, we considered the PB2 coding region of the 1918
276 H1N1 strain and wanted to reduce its number of PAMP associated CpG
277 motifs [Greenbaum et al., 2008, Greenbaum et al., 2014].

278 We thus synthetically evolved the 1918 sequence by the Metropolis–Hasting
279 dynamics and using the MENB the force parameters inferred from the 1918
280 sequence apart from f_{CpG} which we fixed to $f_{CpG} = -1.9$. Such f_{CpG} value
281 is close to the average value in the human genome and is sensibly lower than
282 the one in the original H1N1 strain ($f_{CpG} = -0.6$). The original amino-acid
283 content of the 1918 sequence was kept by accepting only synonymous muta-
284 tions in the Metropolis–Hasting sampling dynamics [Chatenay et al., 2017].
285 As shown in Fig. 4 this resulted in a global change of the nucleotide content
286 of the sequence, where CpG dinucleotides and CpG-containing 3-mers are
287 mostly affected, while other dimers and trimers are generally conserved, see
288 Suppl. Fig. 8. Generation of synthetic sequences under fixed constraint on
289 other motifs can be analogously carried on by changing the corresponding
290 forces.

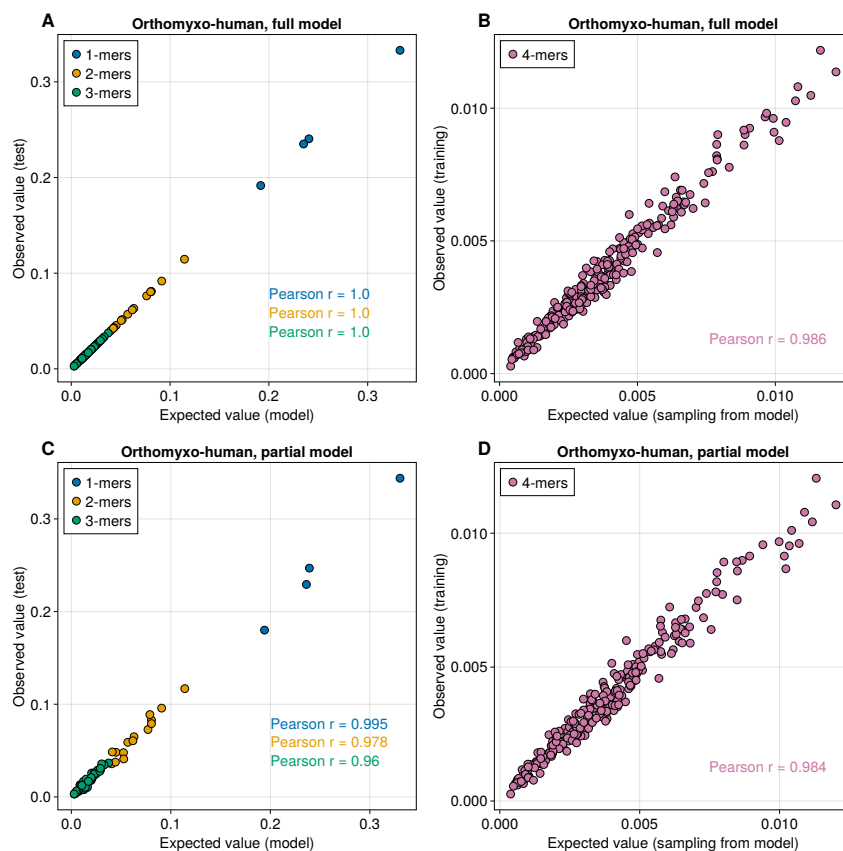


Figure 3: MENB models generalize well to test sequences and higher-order motifs. A: Frequency of nucleotides, 2-mers and 3-mers observed in the test set of full human *Orthomyxoviridae* sequences versus the value obtained analytically from the inferred MENB model. B: Same as A for the MENB model trained on human *Orthomyxoviridae* sequences without the segment coding for PB2. C: Frequency of 4-mers observed in the training set of full human *Orthomyxoviridae* sequences versus the value obtained from sequenced sampled from the inferred MENB model. D: Same as C for the MENB model trained on human *Orthomyxoviridae* sequences without the segment coding for PB2.

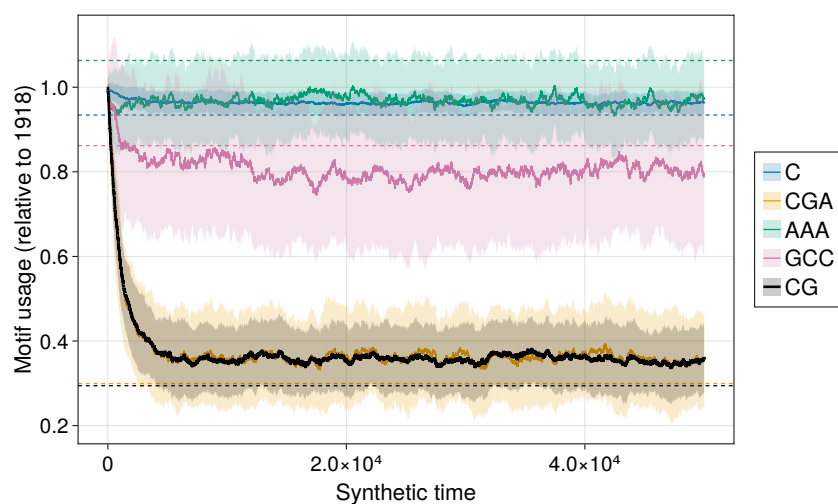


Figure 4: **MENB models can be used to design new sequences coding for the same proteins and with different nucleotide usage.** Motif usage (relative to the original sequence) during synthetic evolution of the PB2 coding sequence from 1918 H1N1 strain under a MENB model that enforces a lower usage of CpG dinucleotides. Solid lines are obtained as averages of 100 independent evolutions, and shaded areas denote one standard deviation. Dashed lines denote the expected motifs usage without the constrain to code for the PB2 protein of the 1918 H1N1 strain.

291 **2.4 Viruses adapt to their host after hosts jumps: Applica-** 292 **tions to H1N1 influenza and SARS-CoV-2**

293 We demonstrated that our model can infer the host of viral sequences from
294 their nucleotide statistics alone. Here we show the model describes the
295 evolution of a viral strain after a host jump. We start with the case of
296 1918 H1N1 influenza pandemics: we collected all PB2 segments available
297 in our dataset associated to the H1N1 strain up to 2008. It is commonly
298 accepted that the pandemics originated with a jump from avian to human
299 hosts [Taubenberger et al., 2005]. To compare the two hosts we will use in
300 our analysis the human and the avian model trained, for each host, on all
301 the segments of influenza viruses excluding PB2 to avoid potential overfit-
302 ting. Before assigning sequences to their host, we built a phylogenetic tree,
303 on a random subsample of up to 20 sequences per year, using Nextstrain
304 [Hadfield et al., 2018]. Fig. 5A shows the log-probability difference between
305 the influenza-human and influenza-avian MEMB models at fixed viral family
306 as a function of time since 1918. The log-probability difference allows classifi-
307 cation of the host, similarly to the host classification task with MENB-H,V in
308 Fig.1 from the sequences sampled over time but also from the reconstructed
309 roots along the phylogenetic tree. We observe that the maximum-entropy
310 model is misled in the assessment of the host of the 1918 PB2 segments
311 (left side of Fig. 5A), which is wrongly classified as an avian virus, while
312 being sampled in humans. This miscassification is a clear signature of the
313 host jump which had just occurred and originated the 1918 pandemic. The
314 classification changes with time: as the virus evolve in contact with the hu-
315 man host, the model assigns to it higher log-probability differences, giving
316 equal scores to human and avian origin around 1950. For more recent sam-
317 ples the model is more and more confident about the human classification.
318 Quite remarkably, the log-probability score introduced here works as a sort
319 of “molecular clock”, by steadily increasing as the virus adapts to the new
320 host. Similar results are obtained also by a simple model only reflecting the
321 nucleotide usage or also including the CpG forces [Greenbaum et al., 2014]
322 (Suppl. Fig. 4), although in these cases the difference of log-probability be-
323 tween the two models is less pronounced, confirming that host adaptation
324 takes place at different order on motif’s usage.

325 As a final application of our MENB models, we turned to the SARS-
326 CoV-2 virus. We wanted to check if we can see hints of host adaptation as
327 for the 1918 H1N1 virus. This case is different from H1N1 as the origi-
328 nal host of SARS-CoV-2 is currently unknown and subject of scientific
329 debate [Andersen et al., 2020]; we have therefore assumed that the origi-

330 nal Wuhan sequence is representative of the (unknown) previous host and
331 build its MEMB model from this unique sequence, while building the model
332 for SARS-Cov-2 in human host from the sequences collected during the re-
333 cent pandemic waves and collected in Nextstrain [Hadfield et al., 2018]. We
334 stress that although in principle our method could be used to investigate the
335 most likely origin of SARS-CoV-2, this would require *Coronaviridae* data of
336 other species (such as pangolins and bats), but current data is biased towards
337 sequences similar to the human SARS-CoV-2 and hence not representative
338 of the original host.

339 The log-probability difference between the two models is plotted in Fig. 6
340 as a function of time for the first 1100 days from the start of the 2020 pan-
341 demic. It shows a slow but steady adaptation to human nucleotide usage
342 (black line, whose slope is significantly different from 0 with a p-value of
343 10^{-9}). Quite surprisingly, the slope of the fitting line is larger for sequences
344 collected in the last 6 months (data downloaded on June 30th, 2023), sug-
345 gesting an increase of the adaptation speed in the Omicron 23A variant
346 that appeared in early January 2023 and rapidly took over the entire SARS-
347 CoV-2 global population. In the above analysis we have taken into account
348 a number of limitations and delicate points that we discuss here. First, the
349 SARS-CoV-2 sequence data is heavily biased, both geographically (a large
350 fraction of the sequences are collected in a small number of countries) and
351 temporarily (the rate of sequence collection increased steadily in the first
352 months of the pandemics). Second, as discussed above, we have used the
353 single Wuhan sequence to infer the model for the unknown virus transmit-
354 ting host. Third, the time over which the adaptation to the human host has
355 been sampled is much smaller than that of the H1N1 strain, on such a short
356 time scale adaption driven by non-synonymous mutations with clear fitness
357 advantages could result in a confounding signal.

358 To address the first issue we used a curated dataset of sequences collected
359 by Nextstrain [Hadfield et al., 2018] to build the model of SARS-CoV-2 in
360 the human host: in this dataset sequences are subsampled to reduce biases
361 from different geographic regions and time periods, and most of the se-
362 quences are collected in the last 6 months. As for the second issue, although
363 a MEMB model can be trained with a single sequence, in this case the motif
364 frequencies are less representative of the virus-host pair under analysis, so
365 additional caution must be used in this case in interpreting the results ob-
366 tained. Indeed, by construction the initial log-likelihood associated to the
367 “Wuhan” host will be higher than the one for human *Coronaviridae*. More-
368 over the “Wuhan” host is likely not to be the human [Andersen et al., 2020],
369 but using specific viral sequences that has been collected in bats or pangolins

370 (that have been suggested to be the reservoir of SARS-CoV-2 ancestors)
371 due to their similarity with the Wuhan sequence would give very similar re-
372 sults. Regarding the third problem outlined before, there is no way to deal
373 with it other than collecting sequences for longer times, but the questions
374 of whether some early signals of host adaptation can be spotted with the
375 genomes observed so far is still well-posed.

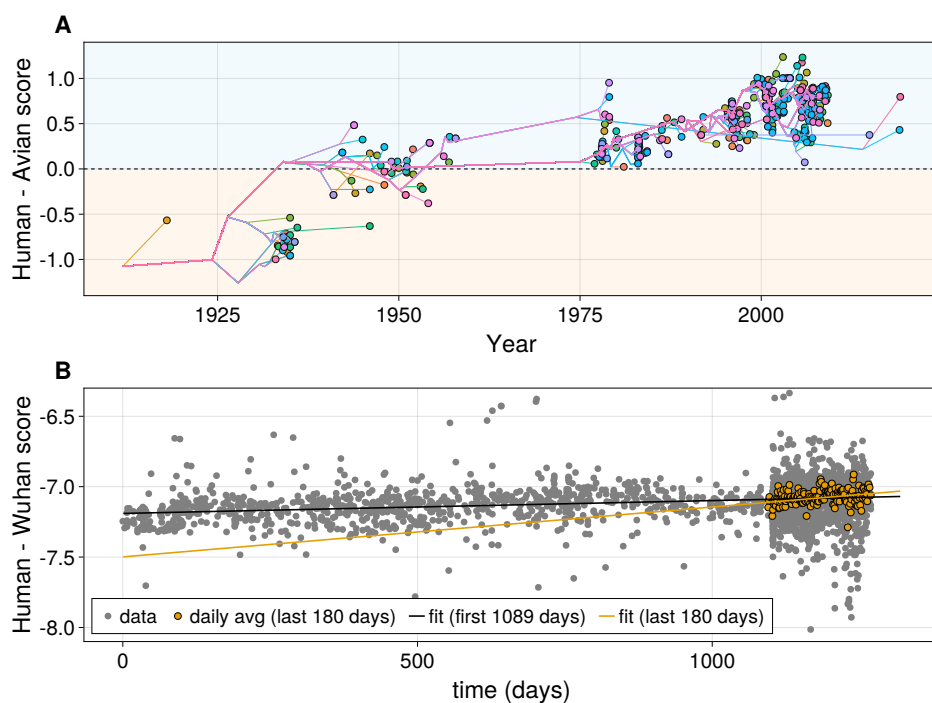


Figure 5: MENB models can be used to quantify host adaptation dynamics after host jumps. A: Scatter plot of loglikelihood differences of the MENB *Orthomyxoviridae* human and avian models versus time of H1N1 Influenza A sequences. The colored lines are the reconstructed paths of the inferred phylogenetic tree that connect the root to each leaf (observed sequence), and the score versus inferred time is plotted also for the internal node (inferred) sequences. B: Scatter plot of loglikelihood differences of the MENB *Coronaviridae* model versus a MENB model trained on the original Wuhan SARS-CoV-2 sequence versus time from December 26th 2019. The black line is a linear fit on the first 1089 days (slope: $9 \cdot 10^{-5}$, p-value: 10^{-9}), the orange line is a linear fit of the last 180 days (slope: $3.5 \cdot 10^{-4}$, p-value: 10^{-7}). To ease the visualization of the increasing trend of the score difference in the last 180 days, daily averages of the score differences are plotted as orange points.

376 2.5 MEMB models' parameters reflect biologically-relevant 377 features

378 The MEMB models offer the advantage to have a relatively low number
379 of learnable parameters and that each of them is related to the usage of
380 the corresponding motif. Such models are therefore ideal candidates for
381 interpretation, that in turn can be useful to accumulate insight into potential
382 roles in molecular biology of motifs, for instance associated to the recognition
383 by the host innate immune system.

384 To showcase this we considered two models trained on the PB2 seg-
385 ments of *Orthomyxoviridae* viruses: one ("H1N1 1918") has been trained on
386 the sequence collected in 1918, the other ("H1N1 2007") has been trained
387 on 26 sequences collected in 2007. In Fig. 6A we show the entire param-
388 eter profile of the two models. Parameters different from zero reflect the
389 presence of selective forces which push up or down the number of the cor-
390 responding motif with respect to sequences generated uniformly at random.
391 Considering the fact that the 1918 strain was likely of avian origin, the first
392 interesting remark is an overall similarity of the force profile in the two cases,
393 especially for nucleotides and dimers, which indicate that many of the force
394 parameters did not significantly change during the adaptation to the hu-
395 man host. The two dinucleotides with the largest negative forces are the
396 CpG, reflecting the well-known avoidance of CpG motifs, followed by UpA,
397 another known avoided motif that is supposed to have a role in codon ef-
398 ficiency [Tulloch et al., 2014, Atkinson et al., 2014]. Moreover, the force in
399 UpG motif is large and positive, likely due to the C>U and A>G mutational
400 processes on, respectively, CpG and UpA motifs. This observation points
401 out an important concept that is commonly overlooked in k-mer analyses of
402 genetic sequences: the lack of one or more motif is necessarily compensated
403 by an increase in abundance of other motifs, and vice-versa. In our frame-
404 work this is deeply connected to the *gauge choices* that have to be taken due
405 to conservation of probabilities at single, di and tri-nucleotide levels and are
406 discussed in more details in Methods Sec. 5.1.2.

407 The differences in the parameter profile of In Fig. 6A disclose the selective
408 pressures on the nucleotide biases, dimers and trimers driving the evolution
409 of the viral sequence in the adaptation to the new host. The most striking
410 differences between the 1918 and the 2007 viruses are the further decreases
411 in the CpG force, as well as CGU motifs decrease, from a value around zero
412 in 1918 to a negative value in 2007. An opposite evolution is observed for
413 the GpG force increasing from zero to a positive value and for the CGG force
414 which relaxes from a negative value toward zero in the 2007 (see also Supp.

415 Fig.9). The decrease in *CpG* forces confirms previous findings and what
416 obtained with a simpler model containing only the CpG force, moreover the
417 different behavior for the tri-nucleotide mirrors the context dependence of
418 the CpG loss [Greenbaum et al., 2008, Greenbaum et al., 2014].

419 A more rigorous way to study the evolution of the forces is to find the
420 key parameters to discriminate the models inferred from the 1918 and 2007
421 sequences. This problem can be addressed within the framework of inte-
422 grated gradients [Sundararajan et al., 2017]: We compute the symmetrized
423 KL divergence between the two MENB models as the sum of attributions,
424 i. e. integrated gradients with respect to each parameter (more details about
425 the procedure are given in Methods Sec. 5.1.3; see Suppl. Fig. 7 for the
426 comparison of symmetrized versus non-symmetrized KL divergences). In
427 Fig. 6B we show the values for the top-20 attributions to the symmetryzed
428 KL divergence: consistently with the forces differences, we find that the
429 largest attribution is on CpG dinucleotide, and several 3-nucleotides mo-
430 tifs containing CpG (CGA, CGU, CGG, CCG) are present. The GpG and
431 GpA and UpA dinucleotides and several related trinucleotides (TGG, GGC,
432 GGC, CGG, GAG, TAC, TAG) have a large attribution too.

433 Once the inference of parameters is performed we can analytically com-
434 pute the expected number of 1-, 2-, and 3-nucleotide motifs in a viral se-
435 quence according to the MENB models (see Methods Sec. 5.1.3), which (as
436 shown in Fig. 5) should reproduce, by model construction, the motif fre-
437 quencies in the data, as previously shown in Fig. 8A. It is interesting to
438 compare the force attributions in flu evolution to the relative difference in
439 motif frequency Fig. 6C as, due to network effects, they are only marginally
440 related. Nucleotide or dinucleotide usage can, for instance, be driven also
441 by the di-nucleotide and tri-nucleotide forces. In agreement with the force
442 attributions, the CpG dinucleotide shows, among all dinucleotides resulting
443 in human-adapted H1N1 strains, the largest relative decrease in 2007 with
444 respect to 1918. Moreover we observe more UA and AA nucleotides with
445 respect to the 1918 strain. As for 3-mers, the signal is dominated by de-
446 crease in usage of specific CpG-containing motifs, although for instance an
447 increase of TAC motifs is observed (Fig. 6B). It is important to notice that
448 relative changes of 3-mers cannot be compared immediately with those of
449 2-mers, due to the fact that there are 64 different 3-mers and 16 2-mers and
450 so individual 3-mers are in general rarer than individual 2-mers and largest
451 changes are to be expected.

452 We next discuss the force comparison in the context of virus and host
453 classification, from MENB models inferred from the ensemble of sequences
454 for a fixed viral family and host, to bring out similarities and difference in

455 motif usage through the force parameters. The overall similarity of force
456 profiles is again apparent, see Suppl. Fig. 1, reflecting a direct cross contam-
457 ination and adaptation through zoonotic transmissions or the presence of
458 similar molecular mechanisms driving the adaptation of the viral sequences
459 to the host. Compatibly with Fig. 2 largest differences are present among
460 viruses than among hosts. The attributions and differences in motif usage
461 depends quite strongly on both viral family and pair of host analyzed, as
462 shown in Supp. Fig. 3 and Supp. Fig. 2, further underlying the peculiarities
463 of each viral family and host and the importance of inferring MENB models
464 for each viral family and host independently.

465 **3 Discussion**

466 We demonstrate our maximum-entropy approach can successfully be used
467 to predict from a sequence its viral origin and host based on conditional
468 probabilities and Bayes rule. Consistently with some recent empirical ob-
469 servations [Mock et al., 2020], we show viral sequences adapt to the host
470 nucleotide usage under specific viral-family depending constraints. In the
471 host-classification task, our interpretable MENB algorithm has competi-
472 tive performance with state-of-the-art approaches based on deep neural
473 networks, despite being far simpler in terms of number of learnable pa-
474 rameters. As expected by classical bias-variance trade-off considerations
475 [Posani et al., 2022], our methods is less subject to the specific details of
476 the training data, and shows remarkable out-of-distribution generalization
477 properties. This can be of direct applicability in practical cases, such as
478 when a new viral subfamily is discovered which possesses a genome different
479 enough from those used in the training set. This scenario is likely to become
480 more and more relevant in the near future, as new viral sequences continue
481 to be discovered [Tisza et al., 2020, Edgar et al., 2022].

482 Our framework can predict the viral genome evolution in a new host, as
483 the log-probability difference in the new host with respect to the previous
484 host increases in time and measures how well the sequence has adapted to its
485 new host environment. This is clearly shown for the H1N1 Influenza for for
486 which we have 100 years of sampled sequences at our disposal; we see a sim-
487 ilar trend of host adaptation for the SARS-CoV-2 pandemics as well, which
488 hass accelerated with the expansion of new variants [Di Gioacchino et al., 2021,
489 Kumar et al., 2022].

490 An important open question is whether the adaptation to the host that
491 we observe directly provides a fitness advantage to the viruses, or if it is a

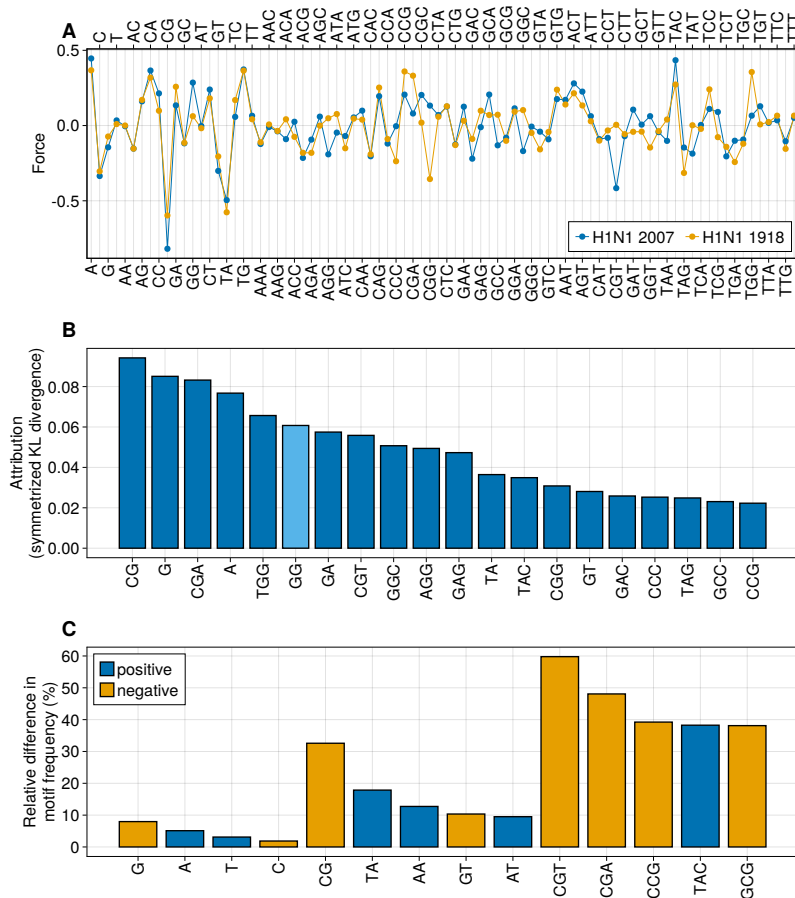


Figure 6: **The learned parameters of MENB models can be directly visualized and interpreted.** A: Plot of each of the 84 parameters (forces) learned by MENB models trained on all segments but PB2 of H1N1 Influenza A strains collected in 2007 (blue) and of the 1918 strain (orange). B: Attributions computed with the method of integrated gradients (Methods Sec. 5.1.3) for the symmetrized Kullback-Leibler divergence between the MENB models used in panel A. To allow for an easier visualization only the 20 parameters with the highest contribution (in absolute value) to the symmetrized KL divergence are shown. Light blue bars denote negative attributions. C: Relative difference in expected motif frequencies between the MENB models used in panel A (Methods Sec. 5.1.3). Only the 5 top differences (in absolute value) are plotted for 2-mers and 3-mers. Blue (orange) bars correspond to positive (negative) differences.

492 neutral consequence of the viral evolution within a new environment. Ar-
493 guments for both possibilities exist: for instance, viruses can reduce their
494 CpG content after infection in an host that uses CpG-recognizing antiviral
495 mechanisms (as ZAP in humans) [Shaw et al., 2021], which is likely an adap-
496 tation that provides a fitness advantage. On the other hand, the interferon-
497 inducible antiviral protein APOBEC A3G in humans causes hypermutations
498 on cytosines [Chemudupati et al., 2019] and as such it decreases the C con-
499 tent in viral genomes. In this case it is possible that the observed mutations
500 are those that fix in the viral population without destroying the viral life
501 cycle, and so can have null or (extremely) weak replicative fitness effects.
502 The two effects can also coexist and emerge from sequence data on different
503 time scales of the viral evolution. The analysis of the attributions on the
504 early evolution SARS-CoV2 in Supp. Fig (6), shows that among the little
505 changes observed on the overall force parameters, the attributions contain-
506 ing C and U and their repetition (UUU, CCC) are the largest one. These
507 results are consistent with previous analysis showing the large diminution of
508 C occurrences [Hodcroft, 2021] and the presence of local pressures on the
509 CpG, on specific regions of the genome. In particular, large CpG diminu-
510 tion has been observed in the N protein open reading frame which occupies
511 a small region in the genome but is one of the most abundant transcript in
512 the cytoplasm [Di Gioacchino et al., 2021].

513 The work described here has several potential applications. The fast and
514 flexible host detection algorithm introduced here can easily be integrated
515 within metagenomics studies to infer the host of viruses, even if it is quite dif-
516 ferent from the sequences used to train the algorithm. Moreover, recent stud-
517 ies have pointed out viral mimicry by some repeats in the human genome,
518 and our group has suggested to use a MENB model to identify similar-
519 ities between genomic regions and viral families [Šulc et al., 2023]. Secondly,
520 MENB models can be broadly used to study emerging pathogens and their
521 adaptation to new hosts, as a support in surveillance studies. Moreover the
522 modeling at the nucleotide level is necessary to capture some features of vi-
523 ral evolution which should further combined research within the inference of
524 epistatic fitness landscapes of viral genomes that including in a single model
525 synonymous and non-synonymous mutations, as the synonymous mutations
526 may well have fitness costs [Neher and Shraiman, 2011, Zeng et al., 2021].
527 Finally, thanks to their generative properties underlined here, MENB models
528 are ideal candidate for the optimization in RNA vaccine design for efficiency
529 and minimizing rejection due to immunogenicity [Pardi et al., 2018]. By
530 predicting how viruses adapt to their new host we can better understand
531 mechanisms that drive their adaptation and design intervention

532 4 Acknowledgement

533 This work was supported by grant ANR-19 Decrypted CE30-0021-01, and
534 by the European Union’s Horizon 2020 research and innovation programme
535 under the Marie Skłodowska-Curie grant agreement No 101026293.

536 5 Methods

537 5.1 The maximum entropy nucleotide bias model

538 In this section, we will first give a maximum-entropy derivation of the MENB
539 model as given in Eq. (2.1). This will clarify why some of the parameters
540 can be arbitrarily fixed as they are redundant (gauge choice) and we will
541 discuss the specific choices in this regard made here. Finally we will describe
542 how all the computations involving the MENB model used in this paper can
543 be performed exactly and efficiently building on classical statistical-physics
544 methods.

545 5.1.1 Maximum entropy justification

546 Consider an set of sequences observed (data), we want to find a probabil-
547 ity distribution on the sequence space (model) such that: (i) the observed
548 frequencies of nucleotides, 2-mers and 3-mers in the data match those ex-
549 pected by sampling sequences according to the model, and (ii) the entropy
550 $-\sum_{\mathbf{s}} p(\mathbf{s}) \log p(\mathbf{s})$ is maximized. Therefore the MENB model probability
551 distribution maximizes the following quantity

$$\begin{aligned} & - \sum_{\mathbf{s}} p(\mathbf{s}) \log p(\mathbf{s}) + \sum_{a \in \mathcal{S}} f_a^{(1)} \left(\langle n_a(\mathbf{s}) \rangle - n_a^{obs} \right) \\ & + \sum_{ab \in \mathcal{S}} f_{ab}^{(2)} \left(\langle n_{ab}(\mathbf{s}) \rangle - n_{ab}^{obs} \right) + \sum_{abc \in \mathcal{S}} f_{abc}^{(3)} \left(\langle n_{abc}(\mathbf{s}) \rangle - n_{abc}^{obs} \right) \end{aligned} \quad (1)$$

552 over $p(\mathbf{s})$ and the Lagrange multipliers $f_a^{(1)}$, $f_{ab}^{(2)}$ and $f_{abc}^{(3)}$. Here $\langle f(\mathbf{s}) \rangle =$
553 $\sum_{\mathbf{s}} p(\mathbf{s}) f(\mathbf{s})$, and quantities with the *obs* superscript are averages com-
554 puted on the data sequences. By taking the functional derivative with
555 respect $p(\mathbf{s})$, we obtain the functional form given in Eq. (2.1), where the
556 Lagrange multipliers, that we also call force parameters, need to be fixed so
557 that the observed frequencies of nucleotides, 2-mers and 3-mers in the data
558 match those expected by sampling sequences according to the model. Foll-
559 wing [Greenbaum et al., 2014], this parameter inference can be performed

560 by computing the partition function

$$Z = \sum_{\mathbf{s} \in \mathcal{S}^L} \exp \left(\sum_{a \in \mathcal{S}} f_a^{(1)} n_a(\mathbf{s}) + \sum_{ab \in \mathcal{S}} f_{ab}^{(2)} n_{ab}(\mathbf{s}) + \sum_{abc \in \mathcal{S}} f_{abc}^{(3)} n_{abc}(\mathbf{s}) \right) \quad (2)$$

561 that normalizes the probability distribution in Eq. (2.1) and using it to es-
562 timate the quantities $\langle n_a(\mathbf{s}) \rangle$, $\langle n_{ab}(\mathbf{s}) \rangle$, $\langle n_{abc}(\mathbf{s}) \rangle$. Finally, a root-finding
563 algorithm such as the Newton–Raphson method can be used to find the cor-
564 rect values for the parameters. Optionally the observed quantities n_a^{obs} , n_{ab}^{obs}
565 and n_{abc}^{obs} can be regularized by adding pseudocounts to avoid parameter di-
566 vergences or to give less weight to the sequence details during the inference.

567 5.1.2 Gauge choices for MENB model

568 The MENB model specifies a probability distribution over sequences of
569 length L . As such, any change of parameters that does not change the prob-
570 ability of any sequence does not have any observable effect and it is called
571 a gauge degree of freedom. For instance, we can send $f_a^{(1)} \rightarrow f_a^{(1)} + K$ and,
572 for any value of K , this modification does not impact the probability of any
573 sequence as it can be readily showed using the fact that $\sum_{a \in \mathcal{S}} n_a(\mathbf{s}) = L$.
574 As a consequence, we are free to choose a value for K so that, for instance,
575 $f_T^{(1)} = 0$, or so that $\sum_{a \in \mathcal{S}} f_a^{(1)} = 0$.

576 The presence of gauge degrees of freedom stems from the fact that there
577 are many ways of choosing the 84 force parameters in Eq. (2.1) so that the
578 observed frequencies of nucleotides, 2-mers and 3-mers in the data match
579 those expected from the model. Indeed, although this requirement can be
580 written as a set of 84 equations, some of them are not independent because

581 of the following considerations:

$$\begin{aligned}
 \sum_{a \in \mathcal{S}} n_a(\mathbf{s}) &= L \\
 \sum_{ab \in \mathcal{S}} n_{ab}(\mathbf{s}) &\simeq L \\
 \sum_{a \in \mathcal{S}} n_{ax}(\mathbf{s}) &\simeq n_x, \quad \sum_{a \in \mathcal{S}} n_{xa}(\mathbf{s}) \simeq n_x \quad \forall x \in \mathcal{S} \\
 \sum_{abc \in \mathcal{S}} n_{abc}(\mathbf{s}) &\simeq L \\
 \sum_{ab \in \mathcal{S}} n_{abx}(\mathbf{s}) &\simeq n_x, \quad \sum_{ab \in \mathcal{S}} n_{axb}(\mathbf{s}) \simeq n_x \quad \sum_{ab \in \mathcal{S}} n_{xab}(\mathbf{s}) \simeq n_x \quad \forall x \in \mathcal{S} \\
 \sum_{a \in \mathcal{S}} n_{xya}(\mathbf{s}) &\simeq n_{xy}, \quad \sum_{a \in \mathcal{S}} n_{axy}(\mathbf{s}) \simeq n_{xy} \quad \forall x, y \in \mathcal{S}
 \end{aligned} \tag{3}$$

582 where the symbol \simeq means that the condition is respected in the large- L
583 limit, which is the relevant case for all sequences considered in this work.
584 This set of equations can be used to fix the gauge degrees of freedom (“choose
585 the gauge”), and we do so in this work by choosing a gauge where the
586 maximum number of parameters is set to zero, that we call lattice-gas gauge
587 (with a slight abuse of notation), or by choosing a gauge where there is no
588 arbitrary symmetry breaking among the model parameters, that we call
589 zero-sum gauge.

590 For the lattice-gas gauge, we decide to set to zero all forces of the form
591 $f_T^{(1)}$, $f_{Tx}^{(2)}$ $\forall x \in \mathcal{S}$, $f_{xT}^{(2)}$ $\forall x \in \mathcal{S}$, $f_{TTT}^{(3)}$, $f_{TTx}^{(3)}$ $\forall x \in \mathcal{S}$, $f_{TxT}^{(3)}$ $\forall x \in \mathcal{S}$,
592 $f_{Txy}^{(3)}$ $\forall x, y \in \mathcal{S}$, $f_{xyT}^{(3)}$ $\forall x, y \in \mathcal{S}$. Therefore non-zero T -containing forces only
593 have the form h_{xTy} with $x, y \in \mathcal{S}$. This means that the effective number of
594 free parameters to be inferred goes from 84 to 48.

595 The lattice-gas gauge is particularly useful to speed-up the inference
596 process and to avoid the Newton–Raphson method to fail to converge due
597 to flat directions in the parameter space, but it is not practical when looking
598 at the inferred parameters to interpret them. For this reason after inference
599 we use the zero-sum gauge, that is defined by the following set of equations

$$\begin{aligned}
 \sum_{a \in \mathcal{S}} f_a^{(1)} &= 0 \\
 \sum_{a \in \mathcal{S}} f_{xa}^{(2)} &= \sum_{a \in \mathcal{S}} f_{ax}^{(2)} = 0 \quad \forall x \in \mathcal{S} \\
 \sum_{a \in \mathcal{S}} f_{xya}^{(3)} &= \sum_{a \in \mathcal{S}} f_{axy}^{(3)} = 0 \quad \forall x, y \in \mathcal{S}.
 \end{aligned} \tag{4}$$

600 **5.1.3 Computation of the partition function and related quanti-**
 601 **ties**

602 An remarkable characteristic of the MENB model is that the partition func-
 603 tion Z given in Eq. (2) can be computed exactly in a time that scales linearly
 604 with the length of the sequence L using the so-called transfer matrix method,
 605 well-known in statistical physics. This method has been already described
 606 for a similar problem in [Greenbaum et al., 2014] (Supporting Information),
 607 and the only difference in this case is that the matrices also contain a term
 608 that accounts for the 3-body interaction.

609 Once the partition function of a MENB model is computed, we have
 610 immediate access to a wealth of relevant quantities. In particular, we can
 611 compute the expected number of ℓ -mers M as

$$\langle n_M(\mathbf{s}) \rangle = \frac{\partial}{\partial f_M^{(\ell)}} \log Z, \quad (5)$$

612 which is the main quantity used to produce Fig. 6B.

613 Another relevant quantity is the Kullback-Leibler divergence between
 614 two models, p_1 and p_2 . It can be written as

$$D_{KL}(p_1, p_2) = \sum_s p_1(s) \log \left(\frac{p_1(s)}{p_2(s)} \right) = \log Z_2 - \log Z_1 + \sum_s p_1(s) (E_2(s) - E_1(s)). \quad (6)$$

615 $\log Z_1$ and $\log Z_2$ can be computed exactly with the transfer matrix method,
 616 and to compute the last term on the r.h.s. of Eq. (6) we define

$$Z_{12}(\lambda) = \sum_s e^{-E_1(s) + \lambda(E_2(s) - E_1(s))}, \quad (7)$$

617 and we have

$$\sum_s p_1(s) (E_2(s) - E_1(s)) = \left. \frac{\partial}{\partial \lambda} \log Z_{12}(\lambda) \right|_{\lambda=0}. \quad (8)$$

618 From the KL divergence we can compute the attributions showed in Fig. 6C.
 619 Following [Sundararajan et al., 2017], we consider two MENB models de-
 620 fined by the force parameters \mathbf{f}_1 and \mathbf{f}_2 . We will use the notation $D_{KL}(\mathbf{f}_1, \mathbf{f}_2)$
 621 to denote the KL divergence between the models with parameter \mathbf{f}_1 and \mathbf{f}_2 .
 622 Thanks to the fundamental theorem of calculus for line integrals, and using
 623 $D_{KL}(\mathbf{f}_1, \mathbf{f}_1) = 0$, we get

$$D_{KL}(\mathbf{f}_1, \mathbf{f}_2) = \sum_i (f_{1,i} - f_{2,i}) \int_0^1 \nabla_i D_{KL}(\mathbf{f}_2 + t(\mathbf{f}_1 - \mathbf{f}_2), \mathbf{f}_2) dt. \quad (9)$$

624 The individual terms of the sum in this equations are the attribution plotted,
625 after rescaling for the total KL divergence, in Fig. 6C and Suppl. Fig. 3. As
626 a final remark, we notice that the attributions depends on the gauge used.
627 In this work we always computed attributions in the zero-sum gauge, and
628 we observe that if the parameters \mathbf{f}_1 and \mathbf{f}_2 are from models in the zero-sum
629 gauge, then Eqs. (4) still hold for $\mathbf{f}_1 + t(\mathbf{f}_1 - \mathbf{f}_2)$, and so the path of models
630 used in Eq. (9) preserve the zero-sum gauge.

631 5.2 Data and code availability

632 All sequence data has been collected from the BV-BRC database [Olson et al., 2022].
633 After discarding short viral sequences (length lower than 1000 bases), we se-
634 lected the pairs of host and viral family so that each viral family has at least
635 100 sequences annotated with each host chosen. We discarded Influenza A
636 sequences collected after 2009 as the database is dominated by strains of
637 the H1N1 “swine flu”, whose triple-reassortment origin [Garten et al., 2009]
638 and (likely) not perfect adaptation to humans is a confounding factor dur-
639 ing training. The resulting dataset, that is the starting point for all the
640 results presented here, is available at [https://zenodo.org/doi/10.5281/
641 zenodo.10050076](https://zenodo.org/doi/10.5281/zenodo.10050076). The SARS-CoV-2 data used for Fig. 5B can be down-
642 loaded at <https://nextstrain.org>. Notice, however, that this data is often
643 updated as it is focused on the last 6 months. To allow exact reproducibil-
644 ity of our results we uploaded the data we used (downloaded on June 30th
645 2023) together with the sequence data at [https://zenodo.org/doi/10.
646 5281/zenodo.10050076](https://zenodo.org/doi/10.5281/zenodo.10050076).

647 The code to infer models is written in Julia and publicly available in the
648 GitHub repository at [https://github.com/adigioacchino/MaxEntNucleotideBiases.
649 jl](https://github.com/adigioacchino/MaxEntNucleotideBiases.jl).

650 We trained our MENB models on 100 viral sequences randomly selected
651 from our dataset for each pair of host and viral family. We replicated
652 this sub-sample three times, observing quite small quantitative differences
653 based on the sequence choice (see error bars in Fig. 1). For the compar-
654 ison with VIDHOP presented in Fig. 1A, C we used exactly the same se-
655 quences. A Snakemake pipeline to train and test the MENB models on the
656 data used here is available at [https://github.com/adigioacchino/MENB_
657 snakemake](https://github.com/adigioacchino/MENB_snakemake).

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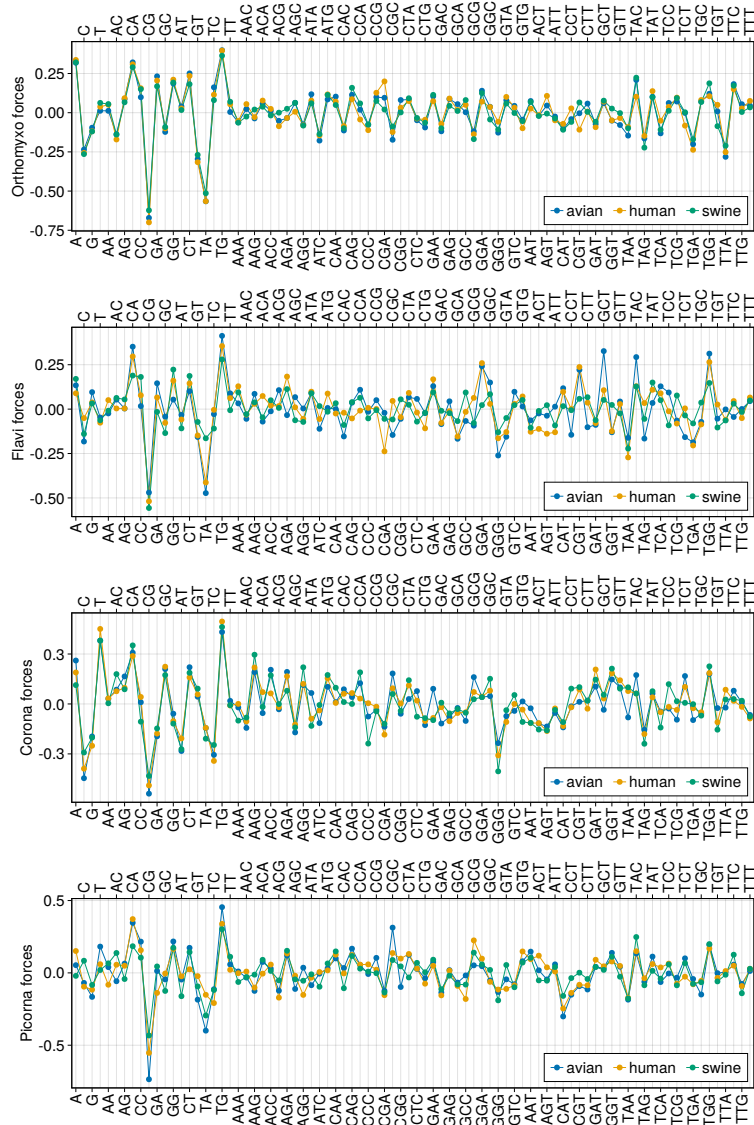
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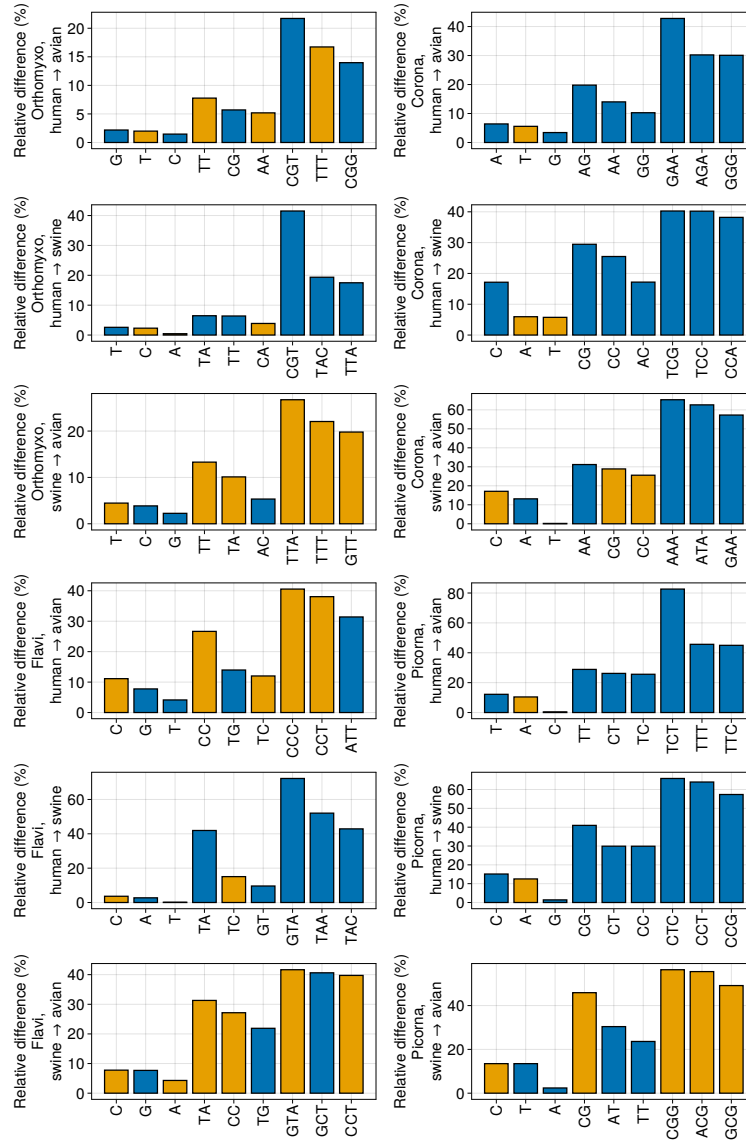
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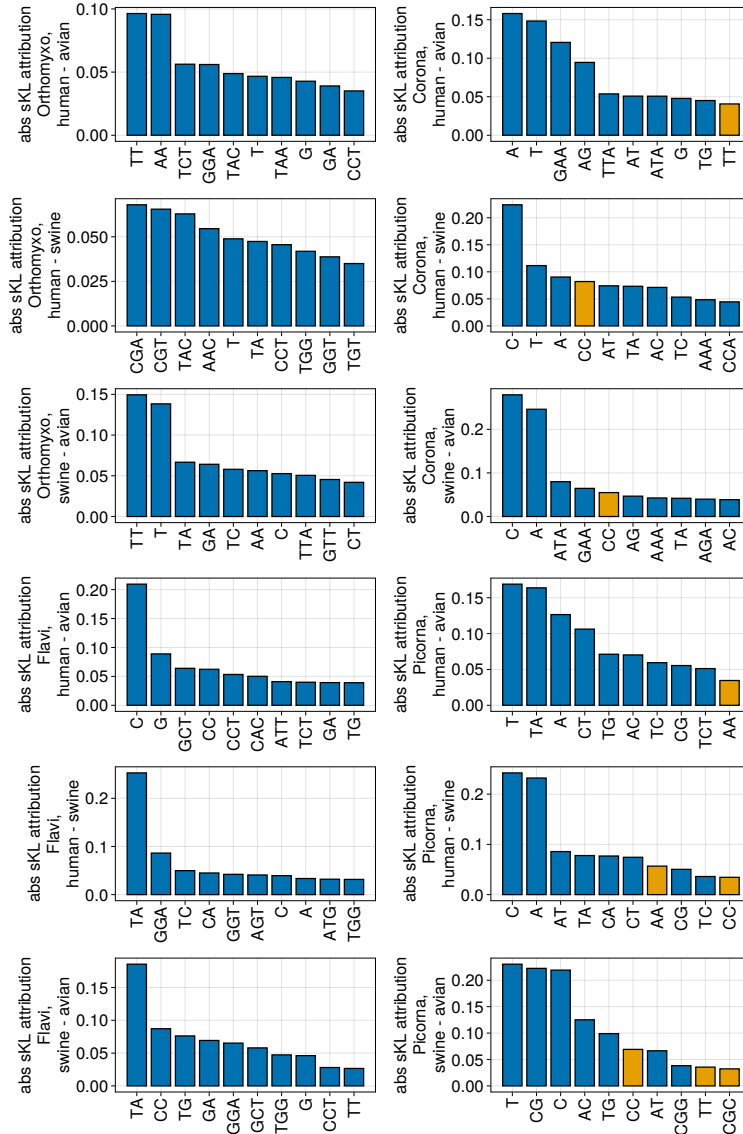
870 **6 Supplementary figures**



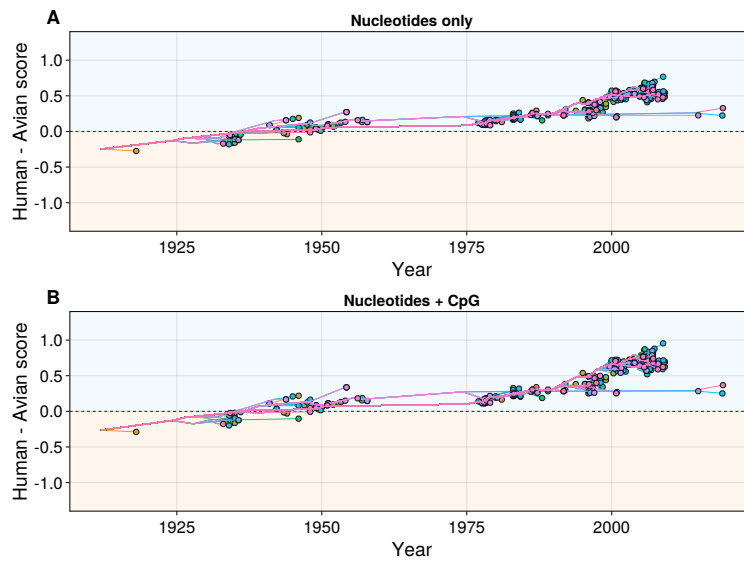
Suppl. Fig. 1: All forces shown for each model learned in this work.



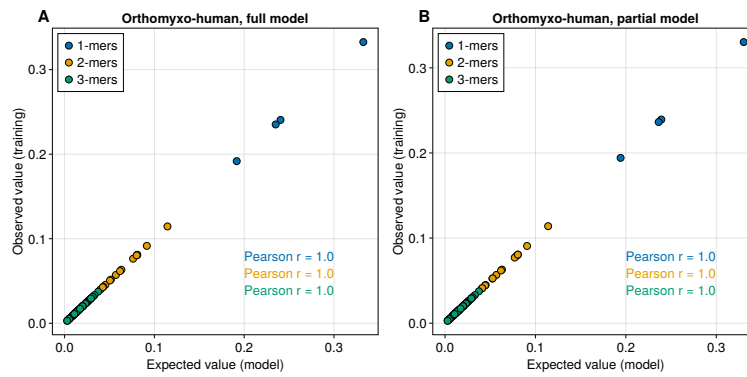
Suppl. Fig. 2: Relative difference in motif usage shown for each pair of hosts at given viral family. Blue bars correspond to increases in motif usage, and orange bars to decreases. Only the 3 highest differences (in absolute value) are shown for nucleotides, 2-mers and 3-mers.



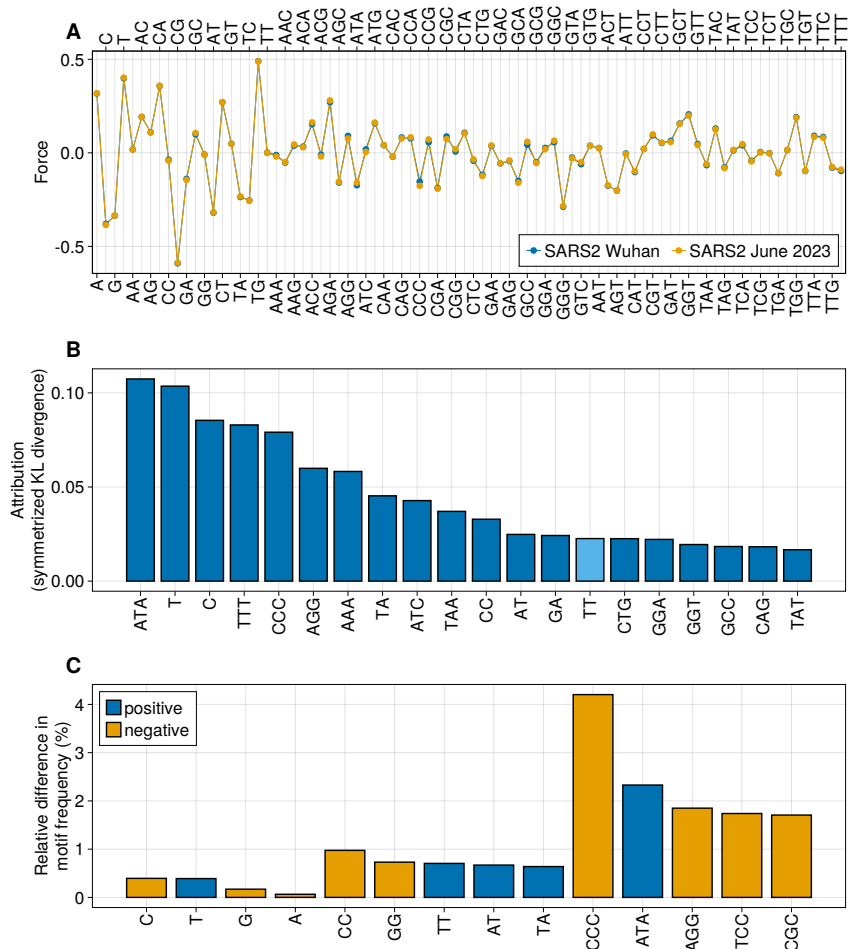
Suppl. Fig. 3: Attribution to symmetrized KL divergence shown for each pair of hosts at given viral family. Blue bars correspond to positive attributions, and orange bars to negative attributions. Only the 10 highest attributions (in absolute value) are shown.



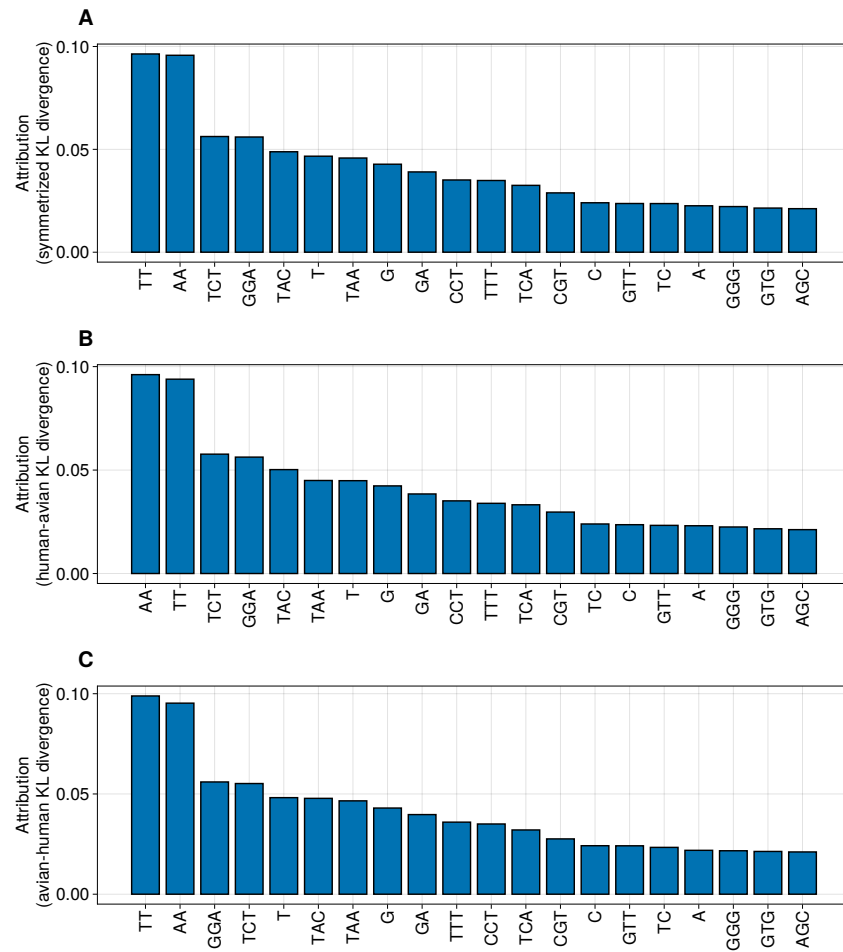
Suppl. Fig. 4: Loglikelihood differences of simplified MENB *Orthomyxoviridae* human and avian models versus time of H1N1 Influenza A sequences. In panel A a model with only nucleotide force inferred is used, and in panel B these forces are inferred together with the CpG force. The colored lines are the reconstructed paths of the inferred phylogenetic tree that connect the root to each leaf (observed sequence), and the score versus inferred time is plotted also for the internal node (inferred) sequences.



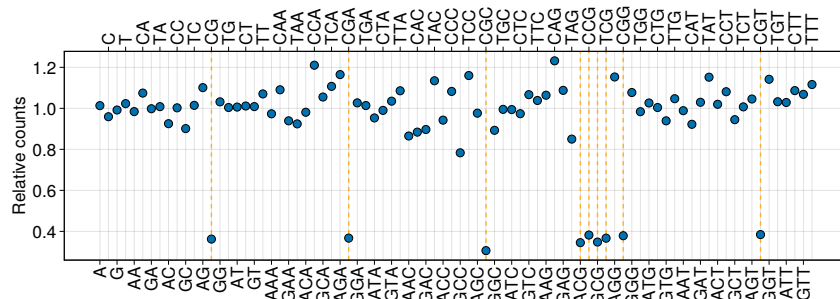
Suppl. Fig. 5: A: Frequency of nucleotides, 2-mers and 3-mers observed in the training set of full human *Orthomyxoviridae* sequences versus the value obtained analytically from the inferred MENB model. B: Same as A for the MENB model trained on human *Orthomyxoviridae* sequences without the segment coding for PB2. .



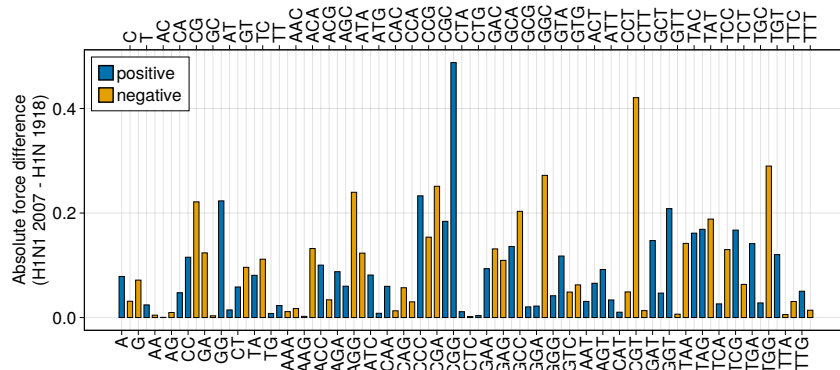
Suppl. Fig. 6: A: Plot of each of the 84 parameters (forces) learned by MENB models trained on the SARS-CoV-2 sequence collected in Wuhan in December 2019 (blue) and on sequences collected in June 2023 (orange). B: Relative difference in expected motif frequencies between the MENB models used in panel A (Methods Sec. 5.1.3). Only the 5 top differences (in absolute value) are plotted for 2-mers and 3-mers. Blue (orange) bars correspond to positive (negative) differences. C: Attributions computed with the method of integrated gradients (Methods Sec. 5.1.3) for the symmetrized Kullback-Leibler divergence between the MENB models used in panel A. To allow for an easier visualization only the 20 parameters with the highest contribution (in absolute value) to the symmetrized KL divergence are shown. Orange bars denote negative attributions.



Suppl. Fig. 7: Comparison between attribution to the symmetrized KL divergence between *Orthomyxoviridae* human and avian viruses (panel A), and the two non-symmetrized KL divergences that compose it (panels B, C).



Suppl. Fig. 8: Comparison between the number of motif observed in the 1918 H1N1 PB2 sequence and in PB2-coding sequence synthetically evolved to reduce their CpG number. A value of 1 means no change in motif abundance. CpG-containing motifs are highlighted with orange lines.



Suppl. Fig. 9: Comparison between the forces inferred on the 1918 and in 2007 H1N1 sequences. Blue/orange bars correspond to increased/decreased forces of 2007 sequences with respect to the 1918 sequence.