Markov chains and hidden Markov models

Sophie Schbath

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Introduction

Markov chains and hidden Markov chains, two probabilistic models used for very different pruposes in bioinformatics, and particularly in sequence analysis.

Markov chains are not really used to model a biological sequence ; they are rather used to set a background model for random sequences mimicing some features of an observed sequence, allowing to detect exceptional events (thanks to *p*-values/*e*-values).
$\longrightarrow$ Part 1 with an illustration on DNA motifs

Conversely, **hidden Markov chains** will often be used to model the alternation of predefined states along a process (a sequence, an alignment, a signal, etc.)
$\longrightarrow$ Part 2

Part 1 : Markov chains

Definition

A Markov chain (by default of order 1) is a sequence of random variables

$$X_1, X_2, X_3, \cdots, X_n, \cdots$$

which are generally **not independent** from each other.

More particularly, each random variable X_i depends on the previous variable X_{i-1} :

$$\mathbb{P}(X_i = b \mid X_1, X_2, \ldots, X_{i-1}) = \mathbb{P}(X_i = b \mid X_{i-1}).$$

In this lesson, we will only consider the case where the X_i's take value in a finite set - say alphabet $\mathcal{A}$, for instance $\mathcal{A} = \{\mathtt{a}, \mathtt{c}, \mathtt{g}, \mathtt{t}\}$.

Transition matrix

Given the value of X_{i-1}, for instance $X_{i-1} = a$, the next random variable X_i takes its value according to the following transition probabilities :

$$\pi(a, b) = \mathbb{P}(X_i = b \mid X_{i-1} = a), a, b \in \mathcal{A}.$$

The matrix $\Pi = (\pi(a, b))_{a,b \in \mathcal{A}}$ is called the **transition matrix** of the Markov chain.

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Example : $\mathcal{A} = \{\mathtt{a}, \mathtt{c}, \mathtt{g}, \mathtt{t}\}$,

$$\Pi = \begin{pmatrix} 0.1 & 0.4 & 0.2 & 0.3 \\ 0.5 & 0 & 0.25 & 0.25 \\ 0.3 & 0.2 & 0.2 & 0.3 \\ 0.25 & 0.2 & 0.35 & 0.2 \end{pmatrix}$$

$$\begin{aligned} \mathbb{P}(X_i = \mathtt{c} \mid X_{i-1} = \mathtt{c}) &= 0 \\ \mathbb{P}(X_i = \mathtt{a} \mid X_{i-1} = \mathtt{t}) &= 0.25 \qquad \text{etc.} \end{aligned}$$

Note that $\sum_{b \in \mathcal{A}} \pi(a, b) = 1, \quad \forall a \in \mathcal{A}$.

Initial distribution

The distribution of the first random variable X_1 is given by the **initial distribution** of the Markov chain, say ν :

$$\nu(b) := \mathbb{P}(X_1 = b), \forall b \in \mathcal{A}.$$

Parameters $\theta = (\Pi, \nu)$ are sufficient to generate/simulate a Markov chain :

1. simulate X_1 from ν : $\mathbb{P}(X_1 = b) = \nu(b)$,
2. simulate X_2 from Π : $\mathbb{P}(X_2 = b \mid X_1) = \pi(X_1, b)$,
3. simulate X_3 from Π : $\mathbb{P}(X_3 = b \mid X_2) = \pi(X_2, b)$, etc.

Stationary distribution

In a general Markov chain $(X_i)_i$ with parameters $\theta = (\Pi, \nu)$, the random variables X_i's are not identically distributed. Indeed,

- Distribution of X_1 : $\mathbb{P}(X_1 = b) = \nu(b)$
- Distribution of X_2 : $\mathbb{P}(X_2 = b) = (\nu.\Pi)(b)$
- Distribution of X_3 : $\mathbb{P}(X_3 = b) = (\nu.\Pi^2)(b)$
- etc.

$\rightarrow$ Exercice : prove that the distribution of X_i is $\nu.\Pi^{i-1}$

However, a theorem says that there exists a distribution μ, such that :

$$\mathbb{P}(X_i = b) \longrightarrow \mu(b), \text{ as } i \rightarrow \infty.$$

μ is called the **stationary distribution** of the Markov chain and satisfies $\mu = \mu.\Pi$

Stationary Markov chain

The **stationary Markov chain** with transition matrix Π is the Markov chain with parameters $\theta = (\Pi, \mu)$, i.e. its initial distribution is set to the stationary distribution.

By doing so, **all random variables X_i are identically distributed with distribution** μ (indeed, $\mu.\Pi^{i-1} = \mu$).

In most applications of Markov models, the stationary framework is considered.

Probability of pattern occurrences

Let $X_1 X_2 X_3 \cdots X_n \cdots$ be a stationary Markov chain with transition matrix Π on the alphabet $\mathcal{A}$; μ denotes the stationary distribution.

Let $w_1 w_2 \cdots w_\ell$ be a sequence of ℓ letters from $\mathcal{A}$ (a "word"), for instance `gttacg`.

The probability to observe such word in the random sequence, say starting at position i, can be easily calculated thanks to the Bayes formula :

$$\mathbb{P}(X_i X_{i+1} \cdots X_{i+\ell-1} = w_1 w_2 \cdots w_\ell) = \mu(w_1) \prod_{j=1}^{\ell-1} \pi(w_j, w_{j+1})$$

for instance

$$\mathbb{P}(\texttt{gttacg} \text{ at position } i) = \mu(\texttt{g})\pi(\texttt{g},\texttt{t})\pi(\texttt{t},\texttt{t})\pi(\texttt{t},\texttt{a})\pi(\texttt{a},\texttt{c})\pi(\texttt{c},\texttt{g})$$

Exercice : prove the above equation.

Probabilist view : The most probably sequence ?

When the Markov model is known, i.e. the transition matrix Π is given, it is then possible to look for the observed sequence of length n the most likely to be drawn.
For that, one just has to maximise the **likelihood**

$$\mathbb{P}(X_1 X_2 \cdots X_n = x_1 x_2 \cdots x_n)$$

over all possible observed sequences $x_1 x_2 \cdots x_n \in \mathcal{A}^n$.

Remember :

$$\begin{aligned}
&\mathbb{P}(X_1 X_2 \cdots X_n = x_1 x_2 \cdots x_n) \\
&= \mu(x_1)\pi(x_1, x_2) \times \cdots \times \pi(x_{n-1}, x_n) \\
&= \mu(x_1) \prod_{a,b \in \mathcal{A}} \big(\pi(a,b)\big)^{N^{\text{obs}}(ab)}
\end{aligned}$$

where $N^{\text{obs}}(ab)$ is the count of the 2-letter word ab in $x_1 x_2 \cdots x_n$.

Statistician view : The best transition matrix ?

In statistics, the observed sequence is given and one looks for the parameters (Π) which maximize the likelihood

$$L(\Pi) = \mu(x_1) \prod_{a,b \in \mathcal{A}} \big(\pi(a,b)\big)^{N^{\text{obs}}(ab)}$$

This is the well known **maximum likelihood estimation** procedure.

In our case, the (log-)likelihood is a function of $|\mathcal{A}| \times (|\mathcal{A}| - 1)$ free parameters, and resolving the partial derivatives $= 0$ leads to :

$$\widehat{\pi}(a,b) = \frac{N^{\text{obs}}(ab)}{N^{\text{obs}}(a+)}, \forall a, b \in \mathcal{A}.$$

Similarly, the stationary distribution will be estimated by

$$\widehat{\mu}(a) = \frac{N^{\text{obs}}(a)}{n}.$$

Interpretation of the MLE

Let $N(ab)$ be the number of occurrences of the 2-letter word ab in the Markov chain $X_1X_2X_3\cdots X_n$ with transition matrix Π.
We can show that

$$\mathbb{E}N(ab) = (n-1)\mathbb{P}(ab \text{ at position } i) = (n-1)\mu(a)\pi(a,b)$$

By using the MLE's $\widehat{\pi}(a,b) = \frac{N^{\text{obs}}(ab)}{N^{\text{obs}}(a+)}$ and $\widehat{\mu}(a) = \frac{N^{\text{obs}}(a)}{n}$, we have

$$\widehat{\mathbb{E}N(ab)} = \frac{n-1}{n}\frac{N^{\text{obs}}(a)}{N^{\text{obs}}(a+)}N^{\text{obs}}(ab) \simeq N^{\text{obs}}(ab).$$

In average, the random sequences would have the same 2-letter word composition than the observed sequence.

Conclusion : The Markov model of order 1 (denoted by **M1) allows to fit the counts of all 2-letter words** of the observed sequence.

Generalization to Mm, m-order Markov model

Definition : In a m-order Markov chain, the Markov property is

$$\mathbb{P}(X_i = b \mid X_1, X_2, \ldots, X_{i-1}) = \mathbb{P}(X_i = b \mid X_{i-m}, \ldots, X_{i-1}).$$

The initial/stationary distribution is given by

$$\mu(a_1 a_2 \cdots a_m) := \mathbb{P}(X_1 = a_1, \ldots, X_m = a_m)$$

The MLE of the parameters are

$$\begin{aligned} \widehat{\pi}(a_1 a_2 \cdots a_m, a_{m+1}) &= \frac{N(a_1 a_2 \cdots a_m a_{m+1})}{N(a_1 a_2 \cdots a_m +)} \\ \widehat{\mu}(a_1 a_2 \cdots a_m) &= \frac{N(a_1 a_2 \cdots a_m)}{n - m + 1} \end{aligned}$$

Conclusion : The model **Mm fits the counts of all $(m+1)$-letter words** of the observed sequence.

Bernoulli model = M0

The Bernoulli model where the random variables X_i's are independent and identically distributed with distribution μ is a particular case of the Markov model.
It would correspond to the following transition matrix in which all lines equal the stationary distribution :

$$\Pi = \begin{pmatrix} \mu(\mathtt{a}) & \mu(\mathtt{c}) & \mu(\mathtt{g}) & \mu(\mathtt{t}) \\ \mu(\mathtt{a}) & \mu(\mathtt{c}) & \mu(\mathtt{g}) & \mu(\mathtt{t}) \\ \mu(\mathtt{a}) & \mu(\mathtt{c}) & \mu(\mathtt{g}) & \mu(\mathtt{t}) \\ \mu(\mathtt{a}) & \mu(\mathtt{c}) & \mu(\mathtt{g}) & \mu(\mathtt{t}) \end{pmatrix}.$$

The Bernoulli model will be denoted by M0 in the remainder (order 0).

Model **M0 fits the letter composition** of the observed sequence.

Illustration : DNA motifs with unexpected frequencies

The Chi example in *E. coli*

Observation : The octamer `gctggtgg` occurs 762 times in the *Escherichia coli* genome (leading strands, $n = 4\,638\,858$).
Is it expected or (significantly) unexpected ?
$\Rightarrow$ **What should we expect ?** To what to compare ?

- random sequences of length $n = 4\,638\,858$ (model M00)
- + same letter composition than *E. coli* genome (model M0)
- + same 2-letter word composition than *E. coli* genome (M1),
- etc.
- + same 7-letter word composition than *E. coli* genome (M6)

Two/three steps :

1. choose a sequence model and estimate the parameters
2. compute the estimated expected count $\widehat{\mathbb{E}N}$ of the motif
3. compute the *p*-value $\mathbb{P}(N \geq 762)$ to assess significance

Influence of the model

Results for `gctggtgg` occurring 762 times in *E. coli* genome.

model	fit	expected	score	*p*-value	rank
M00	length	70.783			
M0	bases	85.944	72.9	$< 10^{-323}$	3
M1	dimers	84.943	73.5	$< 10^{-323}$	1
M2	3-mers	206.791	38.8	$< 10^{-323}$	1
M3	4-mers	355.508	22.0	$1.4\,10^{-107}$	5
M4	5-mers	355.312	22.9	$2.3\,10^{-116}$	2
M5	6-mers	420.867	19.7	$1.0\,10^{-86}$	1
M6	7-mers	610.114	10.6	$1.5\,10^{-26}$	3

Influence of the model (2)

Expected counts (**top 5** **top 50**)

		gctggtgg 762 occ.	ggcgctgg 828 occ.	ccggccta 71 occ.
M0	bases	**85.944**	**85.524**	70.445
M1	2-mers	**84.943**	**125.919**	48.173
M2	3-mers	**206.791**	**255.638**	35.830
M3	4-mers	**355.508**	**441.226**	**14.697**
M4	5-mers	**355.312**	**392.252**	**15.341**
M5	6-mers	**420.867**	**633.453**	**27.761**
M6	7-mers	**610.114**	812.339	**25.777**

Over-represented $\not\Leftrightarrow$ significantly over-represented

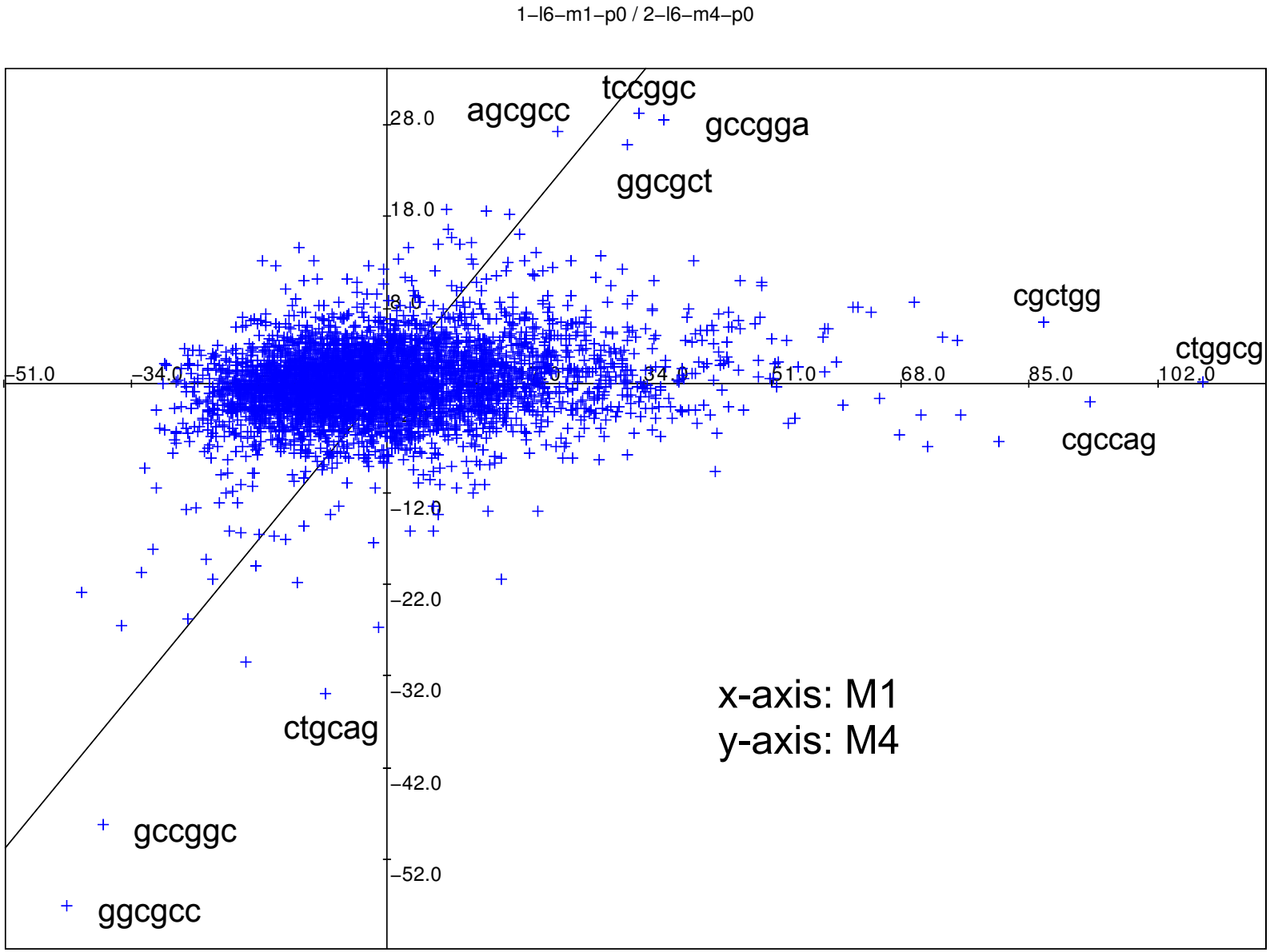
frequent $\not\Leftrightarrow$ significantly over-represented

Influence of the model (3)

The higher the order m of the Markov chain :

- the better fit to the observed sequence (in terms of kmer composition)
- the less significantly unexpected words (see next figure)

Influence of the model (4)



Influence of the model (5)

The higher the order m of the Markov chain :

- the better fit to the observed sequence (in terms of kmer composition)
- the less significantly unexpected words (see next figure)

but

- the higher the number of parameters to estimate ($\rightarrow$ needs a long sequence)
- for a particular word, the expected count is not necessarily closer to the observed count

$\Rightarrow$ **Choose the model according to the amount of data available and to the biological information you want to take into account to compute "what to expect ?"**

Part 2 : Hidden Markov models (HMM)

Limitation of Markov chain models

Markov chains from Part 1 are **homogeneous**, i.e. the transition probabilities $\pi(a, b)$ do not depend on the positions in the sequence. Depending on the application, this can be a strong assumption.

Here are some examples :

1. there exists an intrinsic 3-periodicity in coding sequences (codons)
2. compositional biases exist between exons/introns/intergenic regions due to selection pressure
3. compositional biases (%GC) exists in eukaryotes (isochores)
4. compositional biaises exist between variable/conserved regions in virus

Limitation of Markov chain models (2)

3-periodicity in coding sequences can be easily taken into account by using 3 transition matrices, one for each position inside codons.

Theoretically, one could consider an **heterogenous Markov chain** with transition matrices Π_i, $i = 1, \ldots, n$, but their estimation is usually problematic.

If the sequence of the different states (eg. exons/introns/intergenic) is known, one can use a transition matrix per state (eg. Π_{exon}, Π_{intron}, $\Pi_{\text{intergenic}}$), ...
... but generally the segmentation is part of the problem (eg. gene prediction)

$\Longrightarrow$ Hidden Markov models

Definition of an HMM

Two random processes :

1. An unobservable ("hidden") process which represents the alternating states $S_1 S_2 S_3 \cdots S_n$, $S_i \in \mathcal{S}$ and is a stationary **Markov chain of order 1**. Parameters will be denoted by Π_s and μ_s.
2. An observable process $X_1 X_2 X_3 \cdots X_n$, $X_i \in \mathcal{A}$ for which the distribution of each variable X_i ($\mathbb{P}(X_i = \cdot)$) depends on the state S_i. No more precision of these distributions.

Example 1

- $\mathbf{X} = X_1 X_2 \cdots X_n$ is a random sequence on $\mathcal{A} = \{\mathtt{a}, \mathtt{c}, \mathtt{g}, \mathtt{t}\}$.
- $\mathcal{S} = \{\text{exon, intron, intergenic }\}$ and $\mathbf{S} = S_1 S_2 \cdots S_n$ models the gene structure along the sequence $\mathbf{X}$
- $\mathbf{S}$ is a 1-order Markov chain on $\mathcal{S}$, its transition matrix Π_S is of dimension 3×3, for instance

$$\Pi_S = \begin{pmatrix} 0.8 & 0.15 & 0.05 \\ 0.1 & 0.9 & 0 \\ 0.05 & 0 & 0.95 \end{pmatrix}.$$

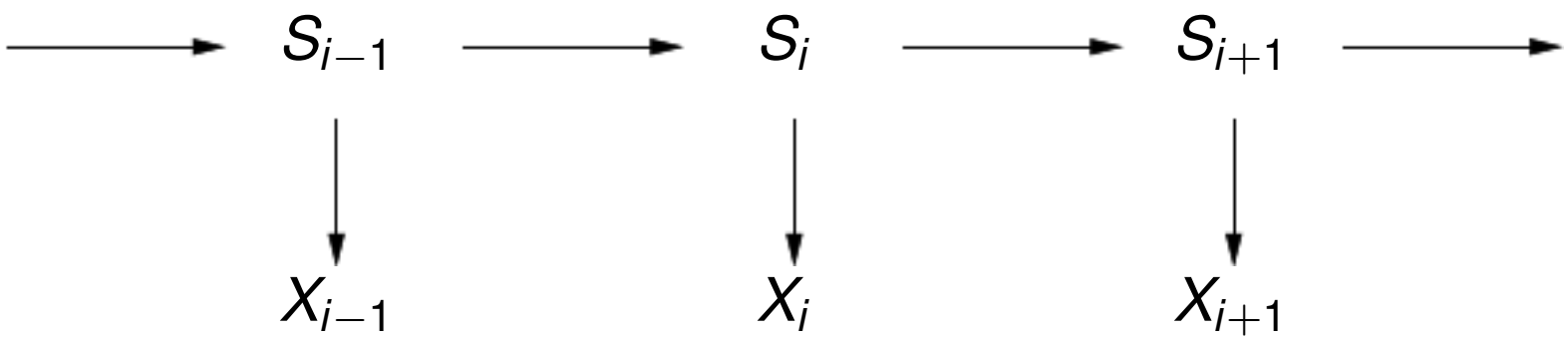
- Conditionnaly on $\mathbf{S}$, the letters X_i's are independent and distributed according to either p_{exon} or p_{intron} or $p_{\text{intergenic}}$:

$$p_u(b) = \mathbb{P}(X_i = b \mid S_i = u), \quad b \in \mathcal{A}, \quad u \in \mathcal{S}$$

for instance
$p_{\text{exon}} = (.2, .3, .3, .2)$, $p_{\text{intron}} = (.3, .2, .2, .3)$, $p_{\text{intergenic}} = (\frac{1}{4}, \frac{1}{4}, \frac{1}{4}, \frac{1}{4})$

Example 1 : dependence graph

The dependence graph of the previous HMM model is :



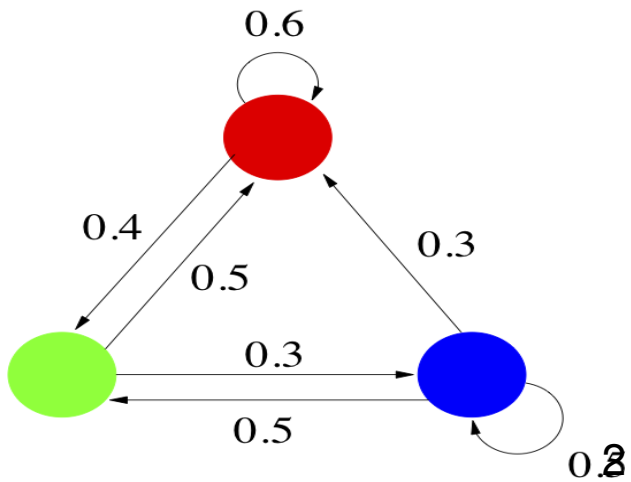
- **S** is a 1-order Markov chain on $\mathcal{S}$
- Conditionnaly on **S**, the letters X_i's are independent and $\mathbb{P}(X_i = b \mid S_i = u) = p_u(b)$.

Graphical representation of an HMM

The underlying structure of an HMM is usually represented by a graph in which nodes are the states and edges indicate non null transition probabilities between states.

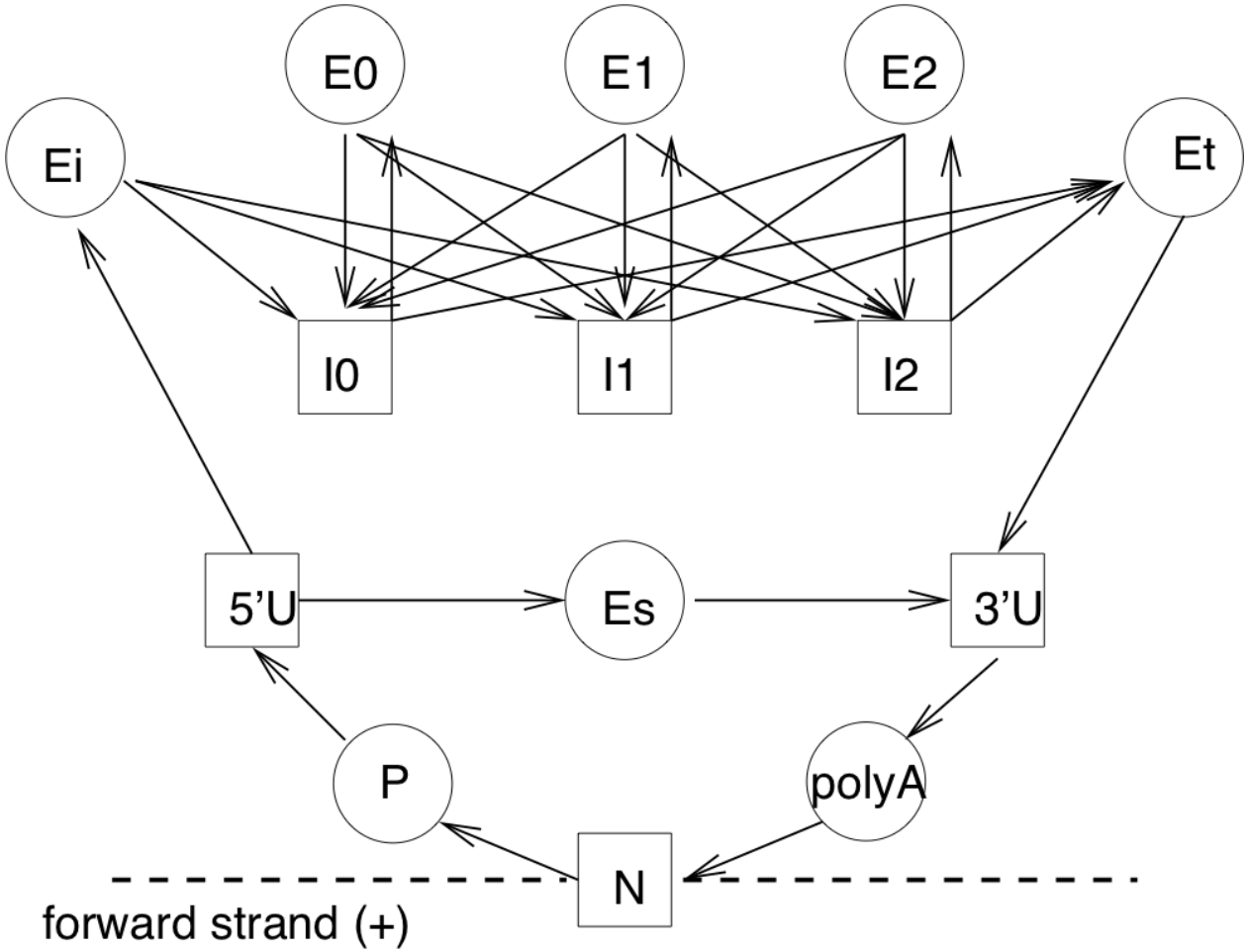
For instance, the graph below would represent an HMM with 3 states and the following transition matrix

$$\Pi_S = \begin{pmatrix} \mathbf{0.6} & 0.4 & 0 \\ 0.5 & \mathbf{0} & 0.5 \\ 0.3 & 0.5 & \mathbf{0.2} \end{pmatrix}$$

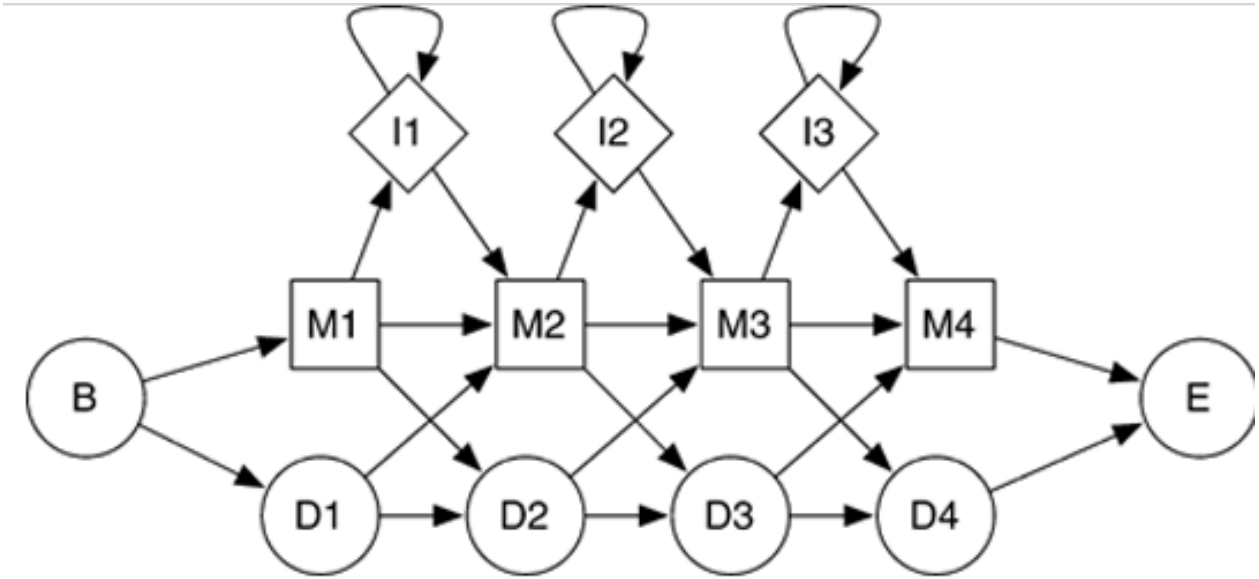


Example 2 : gene prediction

Architecture of the Genscan model for gene prediction



Example 3 : profile HMM for multiple alignment



from HMMER home page `https://bibiserv.cebitec.uni-bielefeld.de/sadr2/databasesearch/hmmer/index.html`

Segmentation property

Time spent in a given state $u \in \mathcal{S}$ is geometrically distributed :

$$\mathbb{P}(\text{time } t \text{ in state } u) = \Big(\pi_S(u,u)\Big)^{t-1}\big(1-\pi_S(u,u)\big)$$

meaning that the expected length of segments in state u is $\frac{1}{1-\pi_S(u,u)}$.

This can be a modeling limitation.

Estimation/segmentation

Given an observed sequence $x_1 x_2 \cdots x_n$, the aim is double :

1. estimate the parameters $\theta = (\Pi_S, p_u)$ for each state $u \in \mathcal{S}$ (**estimation step**)
2. recover all the hidden states $s_1 s_2 \cdots s_n$ (**segmentation step**)

For that, there are two possible approaches :

- a supervised approach, which requires a learning set, that is another observed sequence $\mathbf{x}'$ already segmented,
- an unsupervised approach.

Supervised approach

Two steps :

1. step 1 : **estimation** of $\theta = (\Pi_S, p_u)$ from a segmented sequence $\mathbf{x}'$
   $\rightarrow$ maximum likelihood method leading to $\widehat{\theta}$
2. step 2 : **segmentation** of $\mathbf{x}$ given the parameters $\widehat{\theta}$
   $\rightarrow$ Viterbi algorithm leading to states $s_1^* s_2^* \cdots s_n^*$
   (or *forward-backward* algorithm leading to $\mathbb{P}(S_i = u \mid \mathbf{X} = \mathbf{x}; \widehat{\theta})$)

In the remainder, we consider the basic HMM where the X_i's are independent conditionnaly on the hidden states.

Supervised approach : estimation step (MLE)

Here we assume that we observe the segmentation $\mathbf{s} = s_1 s_2 \cdots s_n$.

The likelihood is then $\mathbb{P}(\mathbf{X} = x_1 x_2 \cdots x_n \mid \mathbf{s}; \theta) =$

$$
\begin{aligned}
&= \mu_S(s_1)\pi_S(s_1, s_2)\ldots\pi_S(s_{n-1}, s_n) \times p_{s_1}(x_1)\ldots p_{s_n}(x_n) \\
&= \mu_S(s_1) \prod_{u,v\in\mathcal{S}} \pi_S(u,v)^{N(uv)} \times \prod_{u\in\mathcal{S}} \prod_{a\in\mathcal{A}} p_u(a)^{N(u,a)}
\end{aligned}
$$

where $N(uv)$ is the count of uv in $\mathbf{s}$ and $N(u, a)$ is the number of letter a in state u.

The likelihood maximization gives the following estimated parameters :

$$
\begin{aligned}
\widehat{\pi}_S(u,v) &= \frac{N(uv)}{N(u+)} \\
\widehat{p}_u(a) &= \frac{N(u,a)}{N(u)}
\end{aligned}
$$

Supervised approach : segmentation step (Viterbi)

Given $\mathbf{x} = (x_1, x_2, \ldots, x_n)$ and θ, we look for $(s_1^*, s_2^*, \ldots, s_n^*)$ which maximize

$$\mathbb{P}(S_1 = s_1, \ldots, S_n = s_n \,|\, \mathbf{X} = \mathbf{x}; \theta)$$

or (Bayes formula)

$$\mathbb{P}(X_1 = x_1, \ldots, X_n = x_n, S_1 = s_1, \ldots, S_n = s_n \,; \theta)$$

Supervised approach : segmentation step (Viterbi)

Given $\mathbf{x} = (x_1, x_2, \ldots, x_n)$ and θ, we look for $(s_1^*, s_2^*, \ldots, s_n^*)$ which maximize

$$\mathbb{P}(S_1 = s_1, \ldots, S_n = s_n \,|\, \mathbf{X} = \mathbf{x}; \theta)$$

or (Bayes formula)

$$\mathbb{P}(X_1 = x_1, \ldots, X_n = x_n, S_1 = s_1, \ldots, S_n = s_n \,; \theta)$$

Let $\mathbb{P}^* = \max_{s_1,\ldots,s_n} \mathbb{P}(X_1 = x_1, \ldots, X_n = x_n, S_1 = s_1, \ldots, S_n = s_n \,; \theta)$.

$$\mathbb{P}^* = \max_{v} \underbrace{\max_{s_1,\ldots,s_{n-1}} \mathbb{P}(X_1 = x_1, \ldots, X_n = x_n, S_1 = s_1, \ldots, S_{n-1} = s_{n-1}, S_n = v \,; \theta)}_{Z_n(v)}$$

$$s_n^* = \arg\max_v Z_n(v)$$

Viterbi algorithm (2)

$$\mathbb{P}^* = \max_{v} \underbrace{\max_{s_1,\ldots,s_{n-1}} \mathbb{P}(X_1 = x_1, \ldots, X_n = x_n, S_1 = s_1, \ldots, S_{n-1} = s_{n-1}, S_n = v\,;\theta)}_{Z_n(v)}$$

$$s_n^* = \arg\max_v Z_n(v)$$

Forward recursive formula to compute $Z_i(v)$:

$$\begin{cases} Z_1(v) &= \mathbb{P}(X_1 = x_1, S_1 = v) = \mu_S(v) p_v(x_1) \\ Z_i(v) &= \max_u \Big(Z_{i-1}(u) \pi_S(u,v) \Big) p_v(x_i) \end{cases}$$

Backward recursion for s_i^* :

$$s_{i-1}^* = \arg\max_u (Z_{i-1}(u) \pi_S(u, s_i^*))$$

Unsupervised approach

If the segmentation **S** is unknown, the likelihood $\mathbb{P}(\mathbf{X} = x_1, x_2, \ldots, x_n\,; \theta)$ is not tractable.
$\Rightarrow$ EM algorithm to approximate the MLE.

Expectation-Maximization is an iterative algorithm such that the likelihood increases at each step :

- start with an initial value $\theta^{(0)}$
- iteration k, $k = 1, 2 \ldots$
  - **step E** : compute $\mathbb{P}(S_i^{(k)} = u \mid \mathbf{X} = \mathbf{x}, \theta^{(k-1)})$, $i = 1, \ldots, n$, $u \in \mathcal{S}$ (*Forward-Backward* algorithm)
  - **step M** : compute $\theta^{(k)}$ given the distribution of $\mathbf{S}^{(k)}$ by maximizing $\mathbb{E}_S[\log \mathbb{P}(\mathbf{X}, \mathbf{S}^{(k)}\,; \theta^{(k-1)}) \mid \mathbf{X}]$
- stop when $|\log \mathbb{P}(\mathbf{X} = \mathbf{x}\,; \theta^{(k+1)}) - \log \mathbb{P}(\mathbf{X} = \mathbf{x}\,; \theta^{(k)})| < \varepsilon$ ou $k > M$

EM : M step

Maximization step :

$$\pi_s^{(k)}(u,v) = \frac{\sum_i \mathbb{P}(S_i^{(k)} = u, S_{i+1}^{(k)} = v \mid \mathbf{X} = \mathbf{x}\,; \theta^{(k-1)})}{\sum_i \mathbb{P}(S_i^{(k)} = u \mid \mathbf{X} = \mathbf{x}\,; \theta^{(k-1)})}$$

$$p_u^{(k)}(a) = \frac{\sum_i \mathbf{1}\{X_i = a\}\mathbb{P}(S_i^{(k)} = u \mid \mathbf{X} = \mathbf{x}\,; \theta^{(k-1)})}{\sum_i \mathbb{P}(S_i^{(k)} = u \mid \mathbf{X} = \mathbf{x}\,; \theta^{(k-1)})}$$

For more details