CSE 527
Lectures ~12-13

Markov Models and Hidden Markov Models

Markov & Hidden Markov Models

- Reference: Durbin, Eddy, Krogh and Mitchison, "Biological Sequence Analysis" Cambridge, 1998

Independence

- A key issue: All models we've talked about so far assume *independence* of nucleotides in different positions - definitely unrealistic.

Example: "CpG Islands"

- CpG - 2 adjacent nucs, same strand (not Watson-Crick pair)
- C of CpG is often *methylated* (in Eukaryotes)
- Methyl-C mutates to T relatively easily
- Net: CpG is less common than expected genome-wide: f(CpG) < f(C)*f(G)
- BUT in promoter (& other) regions, CpG remain unmethylated, so CpG → TpG less likely there: makes "CpG Islands"; often mark gene-rich regions

CpG Islands

- CpG Islands
  - More CpG than elsewhere
  - More C & G than elsewhere, too
  - Typical length: few 100 to few 1000 bp
- Questions
  - Given short sequence (say 200 bp), is it a CpG island or not?
  - Given long sequence (say, 10-100kb), find CpG islands in it?

Markov Chains

A sequence $x_1, x_2, \ldots$ of random variables is a *k-th order Markov chain* if, for all *i*:

$$P(x_i \mid x_1, x_2, \ldots, x_{i-1}) = P(x_i \mid x_{i-k}, x_{i-k+1}, \ldots, x_{i-1})$$

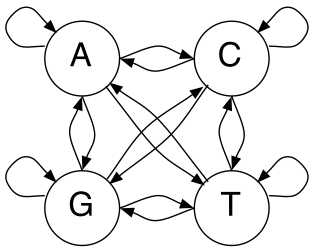
i.e., i^{th} value is independent of all but the previous *k* values

- Example 1: Uniform random ACGT
- Example 2: Weight matrix model
- Example 3: ACGT, but ↓ Pr(G following C)

} 0th order (Examples 1–2)

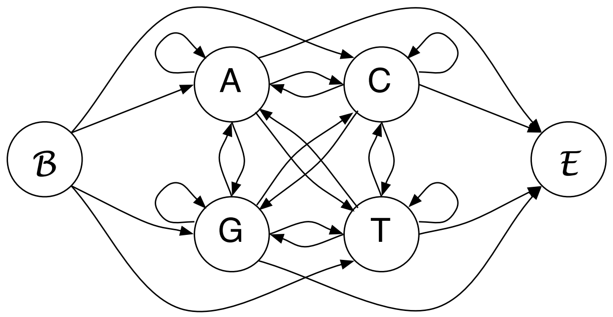
} 1st order (Example 3)

A Markov Model (1st order)



States: A,C,G,T
Emissions: corresponding letter
Transitions: $a_{st} = P(x_i = t \mid x_{i-1} = s)$ ⟵ 1st order

A Markov Model (1st order)



States: A,C,G,T
Emissions: corresponding letter
Transitions: $a_{st} = P(x_i = t \mid x_{i-1} = s)$
Begin/End states

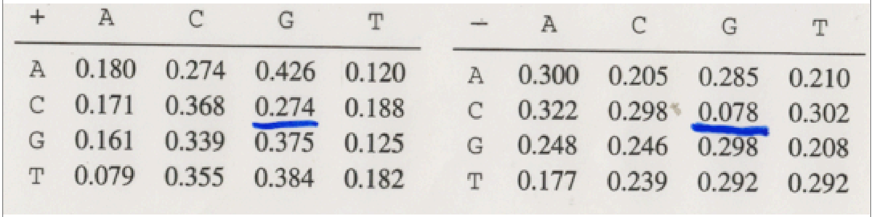
Pr of emitting sequence *x*

$$
\begin{aligned}
x &= x_1\, x_2\, \ldots\, x_n \\
P(x) &= P(x_1, x_2, \ldots, x_n) \\
&= P(x_1) \cdot P(x_2 \mid x_1) \cdots P(x_n \mid x_{n-1}, \ldots, x_1) \\
&= P(x_1) \cdot P(x_2 \mid x_1) \cdots P(x_n \mid x_{n-1}) \\
&= P(x_1) \prod_{i=1}^{n-1} a_{x_i, x_{i+1}} \\
&= \prod_{i=0}^{n-1} a_{x_i, x_{i+1}} \qquad \text{(with Begin state)}
\end{aligned}
$$

Training

Max likelihood estimates for transition probabilities are just the frequencies of transitions when emitting the training sequences

E.g., from 48 CpG islands in 60k bp:



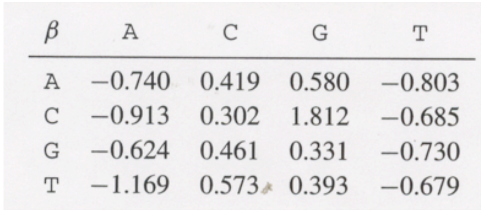
+	A	C	G	T
A	0.180	0.274	0.426	0.120
C	0.171	0.368	0.274	0.188
G	0.161	0.339	0.375	0.125
T	0.079	0.355	0.384	0.182

−	A	C	G	T
A	0.300	0.205	0.285	0.210
C	0.322	0.298	0.078	0.302
G	0.248	0.246	0.298	0.208
T	0.177	0.239	0.292	0.292

Discrimination/ Classification

Log likelihood ratio of CpG model vs background model

$$S(x) = \log \frac{P(x|\text{model}+)}{P(x|\text{model}-)} = \sum_{i=1}^{L} \log \frac{a^{+}_{x_{i-1}x_i}}{a^{-}_{x_{i-1}x_i}} = \sum_{i=1}^{L} \beta_{x_{i-1}x_i}$$



β	A	C	G	T
A	−0.740	0.419	0.580	−0.803
C	−0.913	0.302	1.812	−0.685
G	−0.624	0.461	0.331	−0.730
T	−1.169	0.573	0.393	−0.679

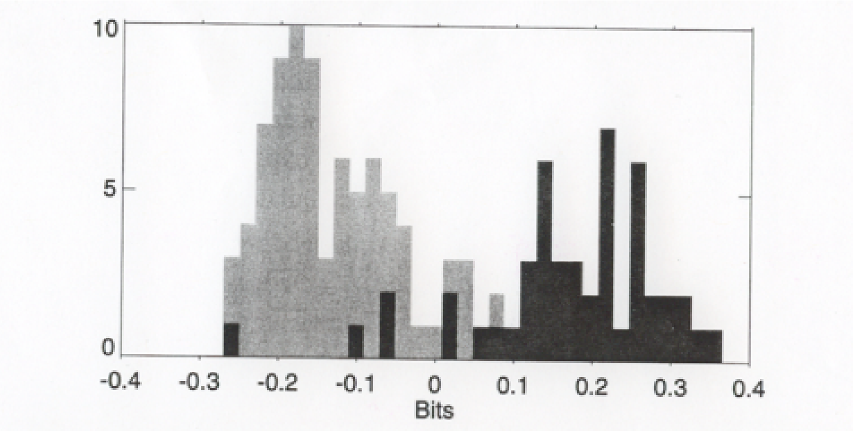


Figure 3.2 *The histogram of the length-normalised scores for all the sequences. CpG islands are shown with dark grey and non-CpG with light grey.*

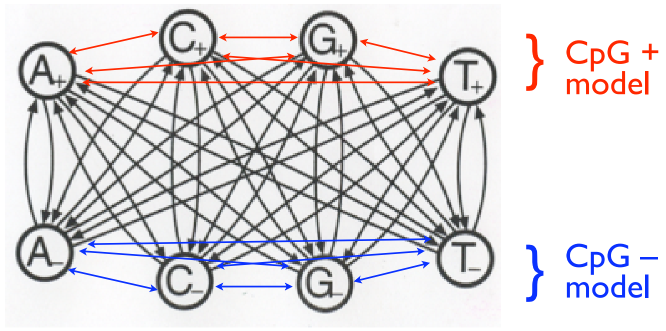
Questions

Q1: Given a *short* sequence, is it more likely from feature model or background model? Above

Q2: Given a *long* sequence, where are the features in it (if any)

- Approach 1: score 100 bp (e.g.) windows
  - Pro: simple
  - Con: arbitrary, fixed length, inflexible
- Approach 2: *combine* +/- models.

Combined Model



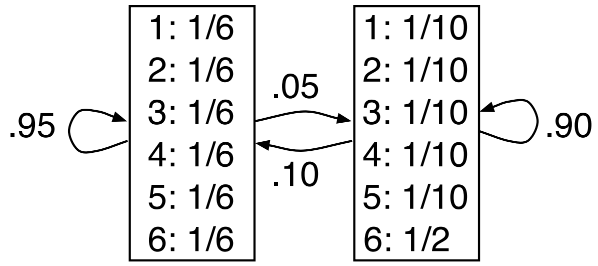
Emphasis is "Which hidden state(s)?" not "Which model?"

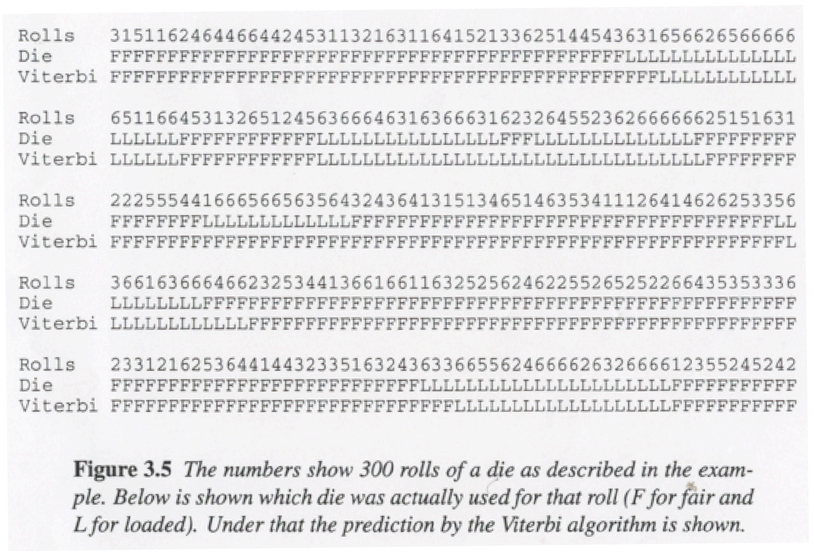
Hidden Markov Models (HMMs)

States:	$1, 2, 3, \ldots$
Paths:	sequences of states $\pi = (\pi_1, \pi_2, \ldots)$
Transitions:	$a_{k,l} \ = P(\pi_i = l \mid \pi_{i-1} = k)$
Emissions:	$e_k(b) = P(x_i = b \mid \pi_i = k)$
Observed data:	emission sequence
Hidden data:	state/transition sequence

The Occasionally Dishonest Casino

1 fair die, 1 "loaded" die, occasionally swapped





```
Rolls   315116246446644245311321631164152133625144543631656626566666
Die     FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLLLLL
Viterbi FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLL

Rolls   651166453132651245636664631636663162326455236266666625151631
Die     LLLLLLFFFFFFFFFFFFLLLLLLLLLLLLLLLLFFFLLLLLLLLLLLLLLFFFFFFFFF
Viterbi LLLLLLFFFFFFFFFFFFLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLFFFFFFFF

Rolls   222555441666566563564324364131513465146353411126414626253356
Die     FFFFFFFFLLLLLLLLLLLLLFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFLL
Viterbi FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFL

Rolls   366163666466232534413661661163252562462255265252266435353336
Die     LLLLLLLLFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
Viterbi LLLLLLLLLLLLFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF

Rolls   233121625364414432335163243633665562466662632666612355245242
Die     FFFFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLLLLLLLLLLLLFFFFFFFFFFF
Viterbi FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLLLLLLLLLFFFFFFFFFFF
```

Figure 3.5 *The numbers show 300 rolls of a die as described in the example. Below is shown which die was actually used for that roll (F for fair and L for loaded). Under that the prediction by the Viterbi algorithm is shown.*

Inferring hidden stuff

Joint probability of a given path π & emission sequence *x*:

$$P(x,\pi) = a_{0,\pi_1} \prod_{i=1}^{n} e_{\pi_i}(x_i) \cdot a_{\pi_i,\pi_{i+1}}$$

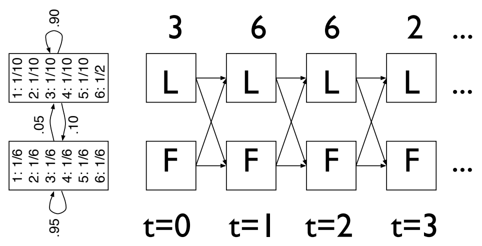
But π is hidden; what to do? Some alternatives:

1. Most probable single path
   $$\pi^* = \arg\max_{\pi} P(x,\pi)$$
2. Sequence of most probable states
   $$\hat{\pi}_i = \arg\max_{k} P(\pi_i = k \mid x)$$
3. ...

The Viterbi Algorithm: The most probable path

- Viterbi finds: $\pi^* = \arg\max_{\pi} P(x,\pi)$
- Possibly there are 10^{99} paths of prob 10^{-99}
- More commonly, one path dominates others. (If not, other approaches may be preferable.)
- Key problem: exponentially many paths π

Unrolling an HMM

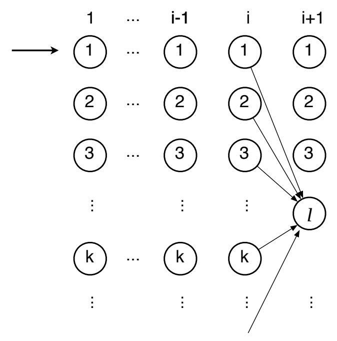


- Conceptually, sometimes convenient
- Note exponentially many paths

Viterbi

$v_l(i) =$ probability of the most probable path emitting $x_1, x_2, \ldots, x_i$ and ending in state l

$$v_l(i+1) = e_l(x_{i+1}) \cdot \max_k (v_k(i)\, a_{k,l})$$



Initialize:

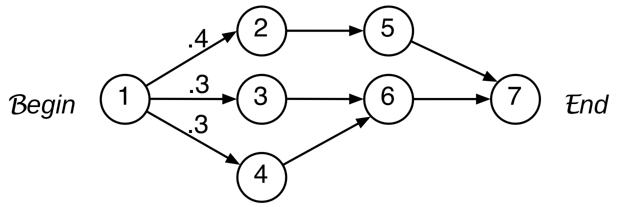
$$v_l(0) = \begin{cases} 1 & \text{if } l = Begin \text{ state} \\ 0 & \text{otherwise} \end{cases}$$

Viterbi Traceback

- Above finds *probability* of best path
- To find the path itself, trace *backward* to the state *k* attaining the max at each stage

Is Viterbi "best"?

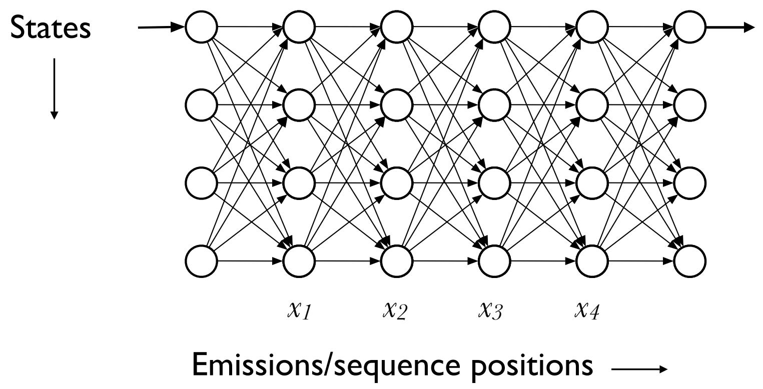
Viterbi finds $\pi^* = \arg\max_\pi P(x, \pi)$



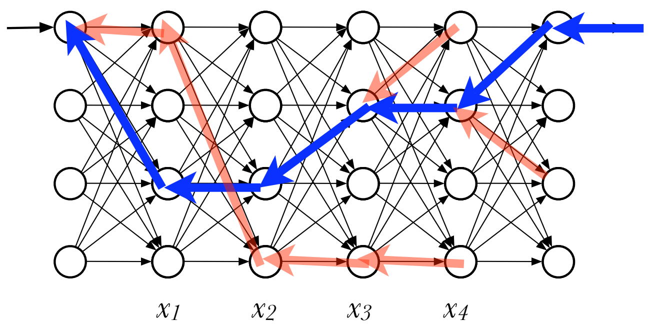
Most probable (Viterbi) path goes through 5, but most probable state at 2nd step is 6

(Viterbi is not the only interesting answer.)

An HMM (unrolled)



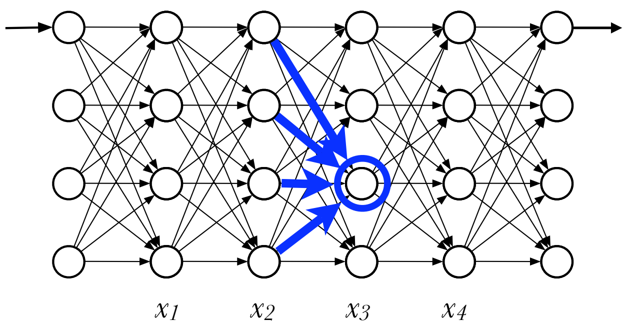
Viterbi: best path to each state



$$v_l(i+1) = e_l(x_{i+1}) \cdot \max_k (v_k(i)\, a_{k,l})$$

The Forward Algorithm

For each state/time, want *total* probability of all paths leading to it, with given emissions



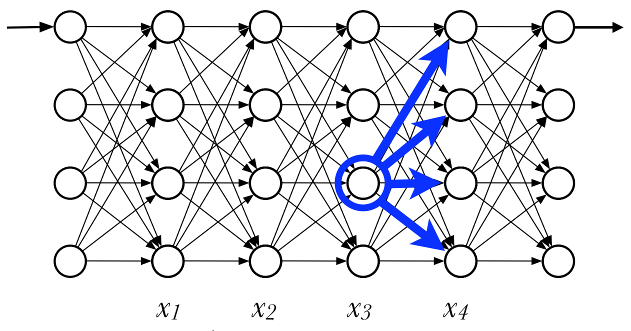
$$f_k(i) = P(x_1 \ldots x_i,\ \pi_i = k)$$

$$f_l(i+1) = e_l(x_{i+1}) \sum_k f_k(i) a_{k,l}$$

$$P(x) = \sum_\pi P(x,\pi) = \sum_k f_k(n) a_{k,0}$$

The Backward Algorithm

Similar: for each state/ time, want total probability of all paths from it, with given emissions, conditional on that state.



$$b_k(i) \triangleq P(x_{i+1} \cdots x_n \mid \pi_i = k)$$

$$b_k(i) = \sum_l a_{k,l}\, e_l(x_{i+1})\, b_l(i+1)$$

$$b_k(n) = a_{k,0}$$

In state *k* at step *i* ?

$$P(x,\ \pi_i = k)$$

$$= P(x_1,\ldots,x_i,\ \pi_i = k) \cdot P(x_{i+1},\ldots,x_n \mid x_1,\ldots,x_i,\ \pi_i = k)$$

$$= P(x_1,\ldots,x_i,\ \pi_i = k) \cdot P(x_{i+1},\ldots,x_n \mid \pi_i = k)$$

$$= f_k(i) \cdot b_k(i)$$

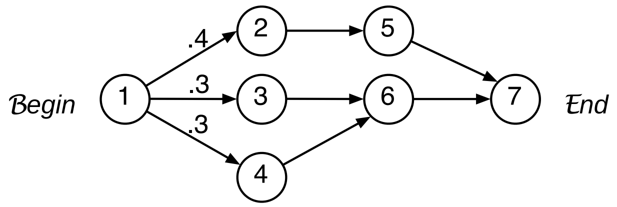
$$P(\pi_i = k \mid x) = \frac{P(x,\pi_i = k)}{P(x)} = \frac{f_k(i) \cdot b_k(i)}{P(x)}$$

Posterior Decoding, I

Alternative 1: what's the most likely state at step *i*?

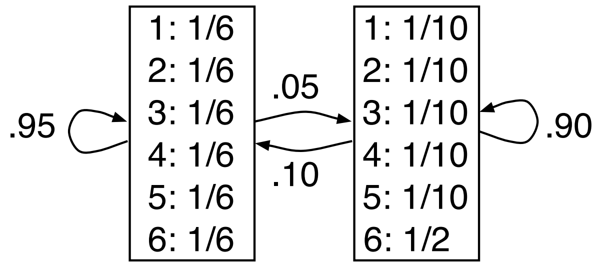
$$\hat{\pi}_i = \arg\max_k P(\pi_i = k \mid x)$$

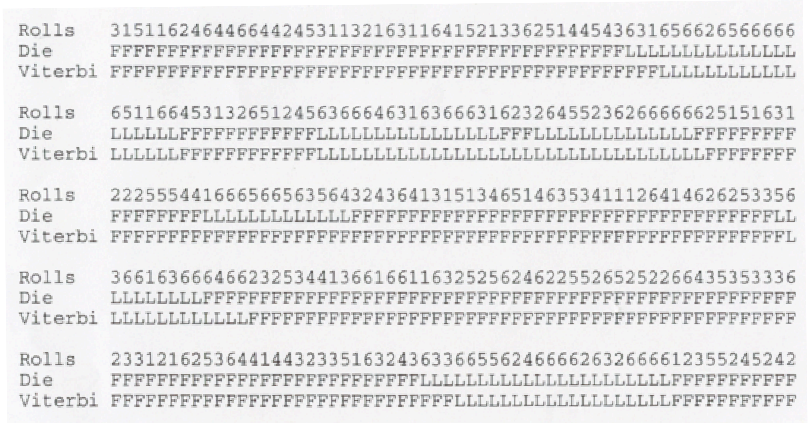
Note: the sequence of most likely states ≠ the most likely sequence of states. May not even be legal!



The Occasionally Dishonest Casino

1 fair die, 1 “loaded” die, occasionally swapped





```
Rolls   315116246446644245311321631164152133625144543631656626566666
Die     FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLLLLL
Viterbi FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLL

Rolls   651166453132651245636664631636663162326455236266666625151631
Die     LLLLLLFFFFFFFFFFFFLLLLLLLLLLLLLLLLFFFLLLLLLLLLLLLLLFFFFFFFFF
Viterbi LLLLLLFFFFFFFFFFFFLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLFFFFFFFF

Rolls   222555441666566563564324364131513465146353411126414626253356
Die     FFFFFFFFLLLLLLLLLLLLLFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFLL
Viterbi FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFL

Rolls   366163666466232534413661661163252562462255265252266435353336
Die     LLLLLLLLFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
Viterbi LLLLLLLLLLLLFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF

Rolls   233121625364414432335163243633665562466662632666612355245242
Die     FFFFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLLLLLLLLLLLLFFFFFFFFFFF
Viterbi FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLLLLLLLLLFFFFFFFFFFF
```

Figure 3.5 *The numbers show 300 rolls of a die as described in the example. Below is shown which die was actually used for that roll (F for fair and L for loaded). Under that the prediction by the Viterbi algorithm is shown.*

Posterior Decoding

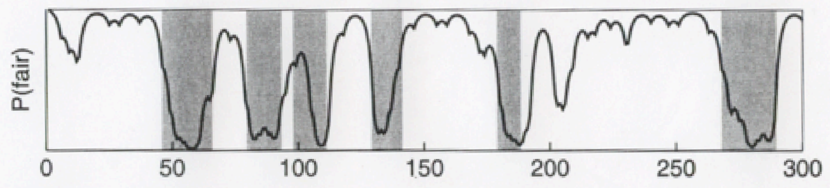


Figure 3.6 *The posterior probability of being in the state corresponding to the fair die in the casino example. The x axis shows the number of the roll. The shaded areas show when the roll was generated by the loaded die.*

Posterior Decoding, II

Alternative 1: what's most likely state at step *i* ?

$$\hat{\pi}_i = \arg\max_k P(\pi_i = k \mid x)$$

Alternative 2: given some function *g(k)* on states, what's its expectation. E.g., what's probability of "+" model in CpG HMM (*g(k)*=1 iff *k* is "+" state)?

$$G(i \mid x) = \sum_k P(\pi_i = k \mid x) \cdot g(k)$$

CpG Islands again

Data: 41 human sequences, totaling 60kbp, including 48 CpG islands of about 1kbp each

	Post-process:
• Viterbi:	
Found 46 of 48	46/48
plus 121 false positives	67 false pos
• Posterior Decoding:	
same 2 false negatives	46/48
plus 236 false positives	83 false pos

(merge within 500; discard < 500)

COMBI Seminar

Dr. William Noble
"Identifying remote protein homologs by network propagation"

Wednesday, November 16, 2005
1:30-2:30pm
HSB K-069

Training

- Given model topology & training sequences, learn transition and emission probabilities
- If π *known*, then MLE is just frequency observed in training data

$$\begin{aligned} a_{k,l} &= \frac{\text{count of } k \to l \text{ transitions}}{\text{count of } k \to \text{anywhere transitions}} \quad \longleftarrow \\ e_k(b) &= \ldots \end{aligned}$$

+ pseudocounts?

- If π *hidden*, then use EM:
  given π, estimate θ; given θ estimate π. $\Big\}$ 2 ways

Viterbi Training

given π, estimate θ; given θ estimate π

- Make initial estimates of parameters θ
- Find Viterbi path π for each training sequence
- Count transitions/emissions on those paths, getting new θ
- Repeat

- *Not* rigorously optimizing desired likelihood, but still useful & commonly used. (Arguably good if you're doing Viterbi decoding.)

Baum-Welch Training

given θ, estimate π ensemble; then re-estimate θ

$$P(\pi_i = k,\ \pi_{i+1} = l \mid x, \theta)$$

$$= \frac{f_k(i \mid \theta)\ a_{k,l}\ e_l(x_{i+1})\ b_l(i+1 \mid \theta)}{P(x \mid \theta)}$$

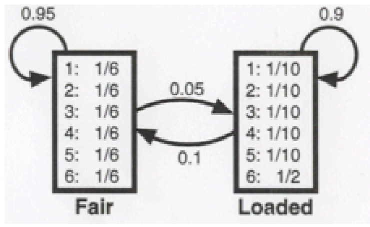
Estimated $\#$ of $k \rightarrow l$ transitions $\hat{A}_{k,l}$

$$= \sum\nolimits_{\text{training seqs } x^j} \sum\nolimits_i P(\pi_i = k,\ \pi_{i+1} = l \mid x^j, \theta)$$

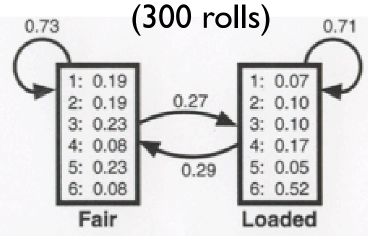
New estimate $\hat{a}_{k,l} = \dfrac{\hat{A}_{k,l}}{\sum_l \hat{A}_{k,l}}$

Emissions: similar

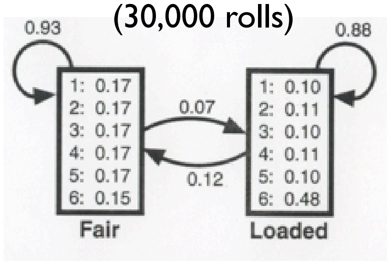
True Model



Learned Model (300 rolls)



Learned Model (30,000 rolls)



Log-odds per roll

True model	0.101 bits
300-roll est.	0.097 bits
30k-roll est.	0.100 Bits

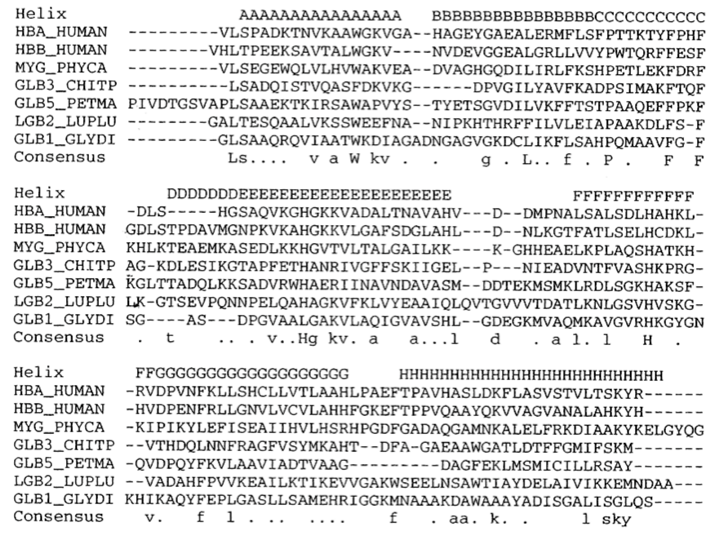
(NB: overfitting)

HMM Summary

- Viterbi – best single path (max of products)
- Forward – Sum over all paths (sum of products)
- Backward – similar
- Baum-Welch – Training via EM and forward/backward (aka the forward/backward algorithm)
- Viterbi training – also EM, but Viterbi-based

HMMs in Action: Pfam

- Proteins fall into *families*, both across & within species
    - Ex: Globins, GPCRs, Zinc Fingers, Leucine zippers,...
- Identifying family is very useful, suggests function, etc.
- So, *search* & *alignment* are both important
- One very successful approach: *profile HMMs*



```
Helix                  AAAAAAAAAAAAAAAA    BBBBBBBBBBBBBBBBCCCCCCCCCCC
HBA_HUMAN   ---------VLSPADKTNVKAAWGKVGA--HAGEYGAEALERMFLSFPTTKTYFPHF
HBB_HUMAN   --------VHLTPEEKSAVTALWGKV----NVDEVGGEALGRLLVVYPWTQRFFESF
MYG_PHYCA   ---------VLSEGEWQLVLHVWAKVEA--DVAGHGQDILIRLFKSHPETLEKFDRF
GLB3_CHITP  ----------LSADQISTVQASFDKVKG------DPVGILYAVFKADPSIMAKFTQF
GLB5_PETMA  PIVDTGSVAPLSAAEKTKIRSAWAPVYS--TYETSGVDILVKFFTSTPAAQEFFPKF
LGB2_LUPLU  --------GALTESQAALVKSSWEEFNA--NIPKHTHRFFILVLEIAPAAKDLFS-F
GLB1_GLYDI  ---------GLSAAQRQVIAATWKDIAGADNGAGVGKDCLIKFLSAHPQMAAVFG-F
Consensus             Ls....  v a W kv .  .    g . L.. f . P .   F  F

Helix           DDDDDDDEEEEEEEEEEEEEEEEEEEEE              FFFFFFFFFFFF
HBA_HUMAN   -DLS-----HGSAQVKGHGKKVADALTNAVAHV---D--DMPNALSALSDLHAHKL-
HBB_HUMAN   GDLSTPDAVMGNPKVKAHGKKVLGAFSDGLAHL---D--NLKGTFATLSELHCDKL-
MYG_PHYCA   KHLKTEAEMKASEDLKKHGVTVLTALGAILKK----K-GHHEAELKPLAQSHATKH-
GLB3_CHITP  AG-KDLESIKGTAPFETHANRIVGFFSKIIGEL--P---NIEADVNTFVASHKPRG-
GLB5_PETMA  KGLTTADQLKKSADVRWHAERIINAVNDAVASM--DDTEKMSMKLRDLSGKHAKSF-
LGB2_LUPLU  LK-GTSEVPQNNPELQAHAGKVFKLVYEAAIQLQVTGVVVTDATLKNLGSVHVSKG-
GLB1_GLYDI  SG----AS---DPGVAALGAKVLAQIGVAVSHL--GDEGKMVAQMKAVGVRHKGYGN
Consensus    .  t    .. . v..Hg kv. a   a...l   d   . a l. l   H  .

Helix        FFGGGGGGGGGGGGGGGGGG     HHHHHHHHHHHHHHHHHHHHHHHHHH
HBA_HUMAN   -RVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYR------
HBB_HUMAN   -HVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH------
MYG_PHYCA   -KIPIKYLEFISEAIIHVLHSRHPGDFGADAQGAMNKALELFRKDIAAKYKELGYQG
GLB3_CHITP  --VTHDQLNNFRAGFVSYMKAHT--DFA-GAEAAWGATLDTFFGMIFSKM-------
GLB5_PETMA  -QVDPQYFKVLAAVIADTVAAG---------DAGFEKLMSMICILLRSAY-------
LGB2_LUPLU  --VADAHFPVVKEAILKTIKEVVGAKWSEELNSAWTIAYDELAIVIKKEMNDAA---
GLB1_GLYDI  KHIKAQYFEPLGASLLSAMEHRIGGKMNAAAKDAWAAAYADISGALISGLQS-----
Consensus     v.    f  l . ..   ....    f    . aa. k. .      l sky
```

Alignment of 7 globins. A-H mark 8 alpha helices.
Consensus line: upper case = 6/7, lower = 4/7, dot=3/7.
Could we have a profile (aka weight matrix) w/ indels?

Profile Hmm Structure

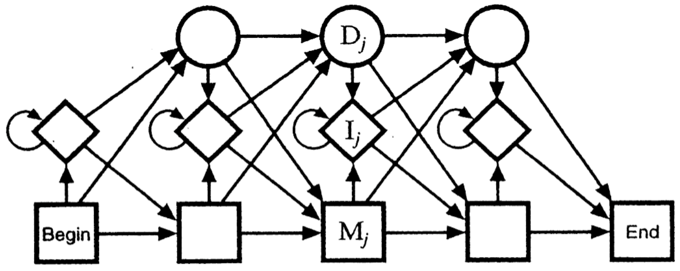
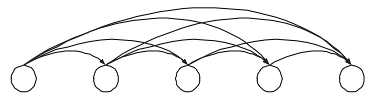


Figure 5.2 *The transition structure of a profile HMM.*

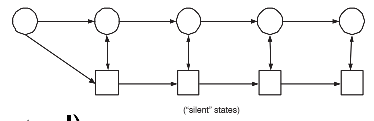
Mj:	Match states (20 emission probabilities)
Ij:	Insert states (Background emission probabilities)
Dj:	Delete states (silent - no emission)

Silent States

- Example: chain of states, can skip some



- Problem: many parameters.
- A solution: chain of "silent" states fewer parameters (but less detailed control)



- Algorithms: basically the same.

How Profile HMM's used

- Search
  - Forward or Viterbi
  - Scoring
    - Log likelihood (length adjusted)
    - Log odds vs background
    - Z scores from either
- Alignment
  - Viterbi

} next slides

Likelihood vs Odds Scores

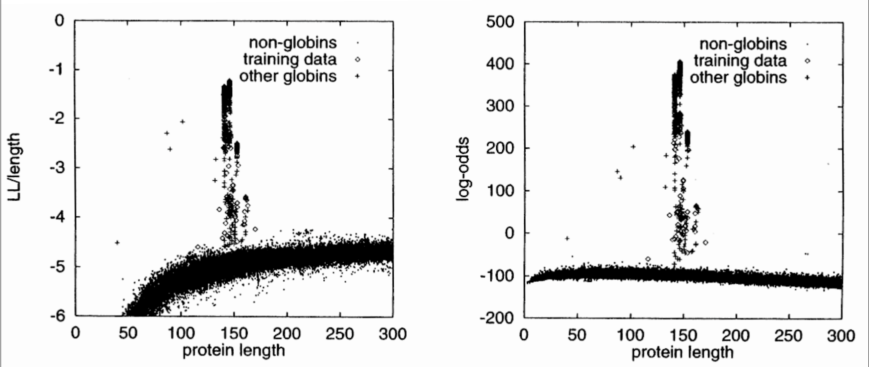


Figure 5.5 *To the left the length-normalized LL score is shown as a function of sequence length. The right plot shows the same for the log-odds score.*

Z-Scores

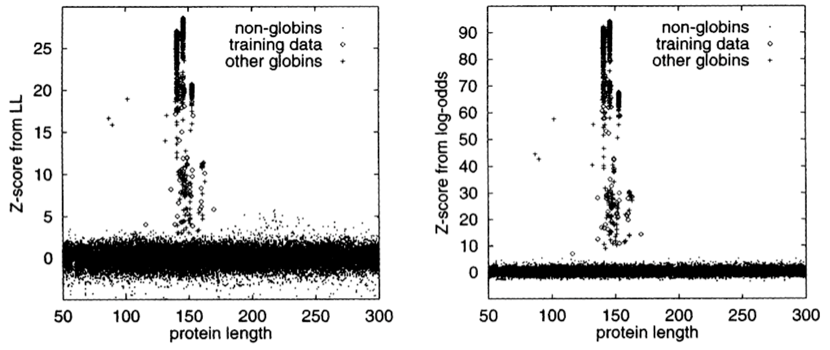


Figure 5.6 *The Z-score calculated from the LL scores (left) and the log-odds (right).*

Pfam Model Building

- Hand-curated "seed" multiple alignments
- Train profile HMM from seed alignment
- Hand-chosen score threshold(s)
- Automatic classification/alignment of all other protein sequences
- 7973 families in Rfam 18.0, 8/2005

  (covers ~75% of proteins)

Model building refinements

- Pseudocounts (count = 0 common when training with 20 aa's)

$$e_i(a) = \frac{C_{i,a} + A \cdot q_a}{\sum_a C_{i,a} + A}, \quad A \sim 20, \; q_a = \text{background}$$

(~50 training sequences)

- Pseudocount "mixtures", e.g. separate pseudocount vectors for various contexts (hydrophobic regions, buried regions,...)

(~10-20 training sequences)

More refinements

- Weighting: may need to down weight highly similar sequences to reflect phylogenetic or sampling biases, etc.
- Match/insert assignment: Simple threshold, e.g. "> 50% gap ⇒ insert", may be suboptimal.

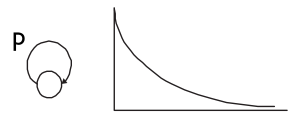
  Can use forward-algorithm-like dynamic programming to compute max *a posteriori* assignment.

Model structure

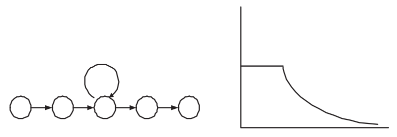
- Define it as well as you can.
- In principle, you can allow all transitions and hope to learn their probabilities from data, but it usually works poorly – too many local optima

Duration Modeling

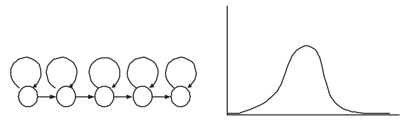
- Self-loop duration: geometric $p^n(1-p)$



- min, then geometric



- "negative binomial"



- More general: possible (but slower)

Numerical Issues

- Products of many probabilities $\rightarrow$ 0
- For Viterbi: just add logs
- For forward/backward: also work with logs, but you need sums of products, so need "log-of-sum-of-product-of-exp-of-logs", e.g., by table/interpolation
- Keep high precision and perhaps scale factor
- Working with log-odds also helps.