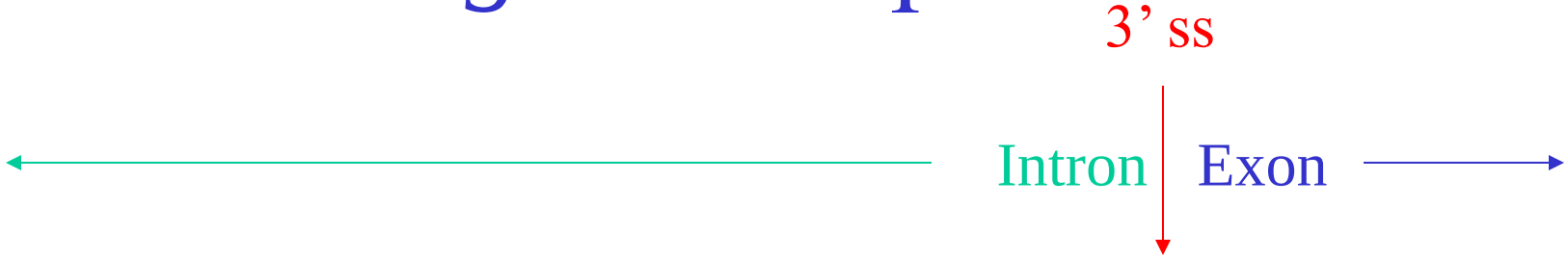
Today's Lecture

- Review:
  - Site models
  - Likelihood ratios & weight matrices
    - (Hypothesis testing & Neyman-Pearson lemma)
- Score distributions
- Limitations of site models
  - Gaps
  - Failure of independence assumption

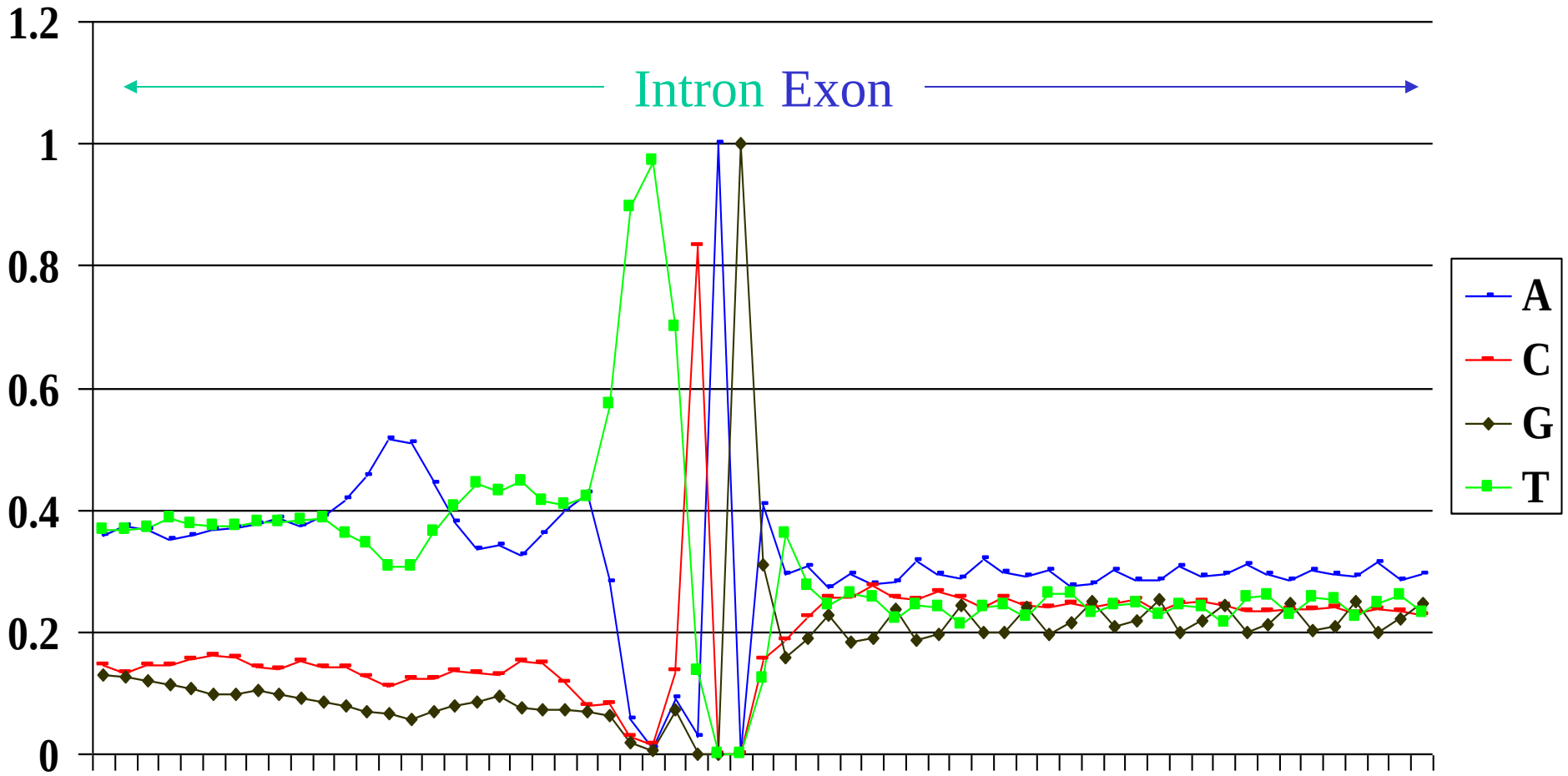
- Assumptions:
  - different examples of site can be aligned *without gaps* (indels) such that tend to have same residues in same positions
  - drop equal freq assumption: allow *position-specific freqs*
  - retain *independence* assumption (for now)

Nucleotide Counts for 8192 *C. elegans* 3' Splice Sites



A	3276	3516	2313	476	67	757	240	8192	0	3359	2401	2514
C	970	648	664	236	129	1109	6830	0	0	1277	1533	1847
G	593	575	516	144	39	595	12	0	8192	2539	1301	1567
T	3353	3453	4699	7336	7957	5731	1110	0	0	1017	2957	2264
CONSENSUS	W	W	W	T	T	t	C	A	G	r	w	w
A	0.400	0.429	0.282	0.058	0.008	0.092	0.029	1.000	0.000	0.410	0.293	0.307
C	0.118	0.079	0.081	0.029	0.016	0.135	0.834	0.000	0.000	0.156	0.187	0.225
G	0.072	0.070	0.063	0.018	0.005	0.073	0.001	0.000	1.000	0.310	0.159	0.191
T	0.409	0.422	0.574	0.896	0.971	0.700	0.135	0.000	0.000	0.124	0.361	0.276

3’ Splice Sites – *C. elegans*



Probability Models for Sites (assuming independence!)

- For each position i, $1 \le i \le n$, let P_i be a prob dist'n on the alphabet of residues
  - e.g. constructed using counts at that position in a sample of sites.
  - $P_i(r)$ for each residue r is the probability that r occurs at position i in a sequence.
- Prob dist'n P on the space S of sequences of length n is defined by

$$P(s) = \prod_{1 \le i \le n} P_i(s_i)$$

where $s = s_1 s_2 \ldots s_n$

Zero Probabilities

- If $P_i(r) = 0$ for some i and r, then $P(s) = 0$ for some sequences.
  - may or may not be desirable
- If due to failure to observe residue because of small sample size,
  - should perform "small-sample correction" to change $P_i(r)$ to a small non-zero value.
  - usually done by adding 'pseudocounts' to each value in the counts matrix;
    - e.g. add 1 to each cell (has justification in Bayesian statistics)
  - Particularly an issue with proteins, due to larger alphabet size.
- If reflects real biological constraints
  - then leave as 0.
  - e.g. requirement for G at position +1 (first intronic base) in 5'ss

Likelihood Ratios

- The *likelihood* of a model M given an observation s is

$$L(M \mid s) = P(s \mid M)$$

  This is *not* the *probability* of the model! – (the sum over all models is not 1).

- The *likelihood ratio* (LR) of two models M_a and M_0 is given by

$$LR(M_a, M_0 \mid s) = \frac{L(M_a \mid s)}{L(M_0 \mid s)}$$

  The numerator and denominator may both be very small!

- The *log likelihood ratio* (LLR) is the logarithm of the likelihood ratio.

Weight Matrices for Site Models

- LR for sites: (prob under site model) / (prob under non-site (background) model)

$$\frac{P(s \mid M_{\text{site}})}{P(s \mid M_{\text{background}})} = \frac{\prod_{1 \le i \le n} P_i(s_i \mid M_{\text{site}})}{\prod_{1 \le i \le n} P_i(s_i \mid M_{\text{background}})}$$

- $\text{LLR} = \sum_{1 \le i \le n} \log(P_i(s_i \mid M_{\text{site}})) - \log(P_i(s_i \mid M_{\text{background}}))$
  - compute by reading from a *matrix* whose *i*-th column contains values $\log(P_i(r \mid M_{\text{site}})) - \log(P_i(r \mid M_{\text{background}}))$ for each residue *r* (with *r* labelling the rows).
    - We use $\log_2$.

Example: 3’ splice sites in *C. elegans*

- For *background distribution* take
  - genomic residue freqs computed from *C. elegans* chrom. I:

  A 4,575,132: 0.321

  C 2,559,048: 0.179

  G 2,555,862: 0.179

  T 4,582,688: 0.321

  - other choices are possible, e.g. composition of *transcribed regions*
- For the *site distribution* we take
  - site residue freqs from 8192 sites:

Weight Matrix – 3’ Splice Sites

SITE FREQUENCIES:

A	0.400	0.429	0.282	0.058	0.008	0.092	0.029	1.000	0.000	0.410	0.293	0.307
C	0.118	0.079	0.081	0.029	0.016	0.135	0.834	0.000	0.000	0.156	0.187	0.225
G	0.072	0.070	0.063	0.018	0.005	0.073	0.001	0.000	1.000	0.310	0.159	0.191
T	0.409	0.422	0.574	0.896	0.971	0.700	0.135	0.000	0.000	0.124	0.361	0.276

BACKGROUND FREQUENCIES:

A	0.321	0.321	0.321	0.321	0.321	0.321	0.321	0.321	0.321	0.321	0.321	0.321
C	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179
G	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179	0.179
T	0.321	0.321	0.321	0.321	0.321	0.321	0.321	0.321	0.321	0.321	0.321	0.321

WEIGHTS:

A	0.32	0.42	-0.18	-2.46	-5.29	-1.79	-3.45	1.64	-99.00	0.36	-0.13	-0.06
C	-0.60	-1.18	-1.15	-2.64	-3.51	-0.41	2.22	-99.00	-99.00	-0.20	0.06	0.33
G	-1.31	-1.35	-1.51	-3.35	-5.23	-1.30	-6.93	-99.00	2.48	0.79	-0.17	0.10
T	0.35	0.39	0.84	1.48	1.60	1.12	-1.24	-99.00	-99.00	-1.37	0.17	-0.22

Scoring a Candidate 3’ Splice Site

A	0.32	0.42	-0.18	-2.46	-5.29	-1.79	-3.45	1.64	-99.00	0.36	-0.13	-0.06
C	-0.60	-1.18	-1.15	-2.64	-3.51	-0.41	2.22	-99.00	-99.00	-0.20	0.06	0.33
G	-1.31	-1.35	-1.51	-3.35	-5.23	-1.30	-6.93	-99.00	2.48	0.79	-0.17	0.10
T	0.35	0.39	0.84	1.48	1.60	1.12	-1.24	-99.00	-99.00	-1.37	0.17	-0.22

T T C T T A C A G A A T

0.35 + 0.39 +-1.15 + 1.48 + 1.60 +-1.79 + 2.22 + 1.64 + 2.48 + 0.36 +-0.13 +-0.22 = 7.23

- General def.: a *weight matrix* W has

  entries w_{rj} indexed by residues $r \in A$, and $1 \le j \le n$

- *score* of a sequence $s = (s_1 s_2 \ldots s_n)$ is

$$\sum_{1 \le j \le n} w_{s_j j}$$

- In the site case,

$$w_{rj} = \log(P_j(r \mid M_{\text{site}})) - \log(P_j(r \mid M_{\text{background}}))$$

Simple Hypothesis Testing

- Suppose we wish to decide between two models:
  - M_a (the *alternative hypothesis*), and
  - M_0 (the *null hypothesis*)

  using an observation s from a sample space S. (e.g.
  - s a sequence,
  - M_a a site model
  - M_0 a "background" (non-site) model.
- Strategy:
  - choose a subset $C \subset S$, called the *critical region* for the comparison.
  - If s falls within C, reject M_0 (accept M_a),
  - otherwise accept M_0 (reject M_a).

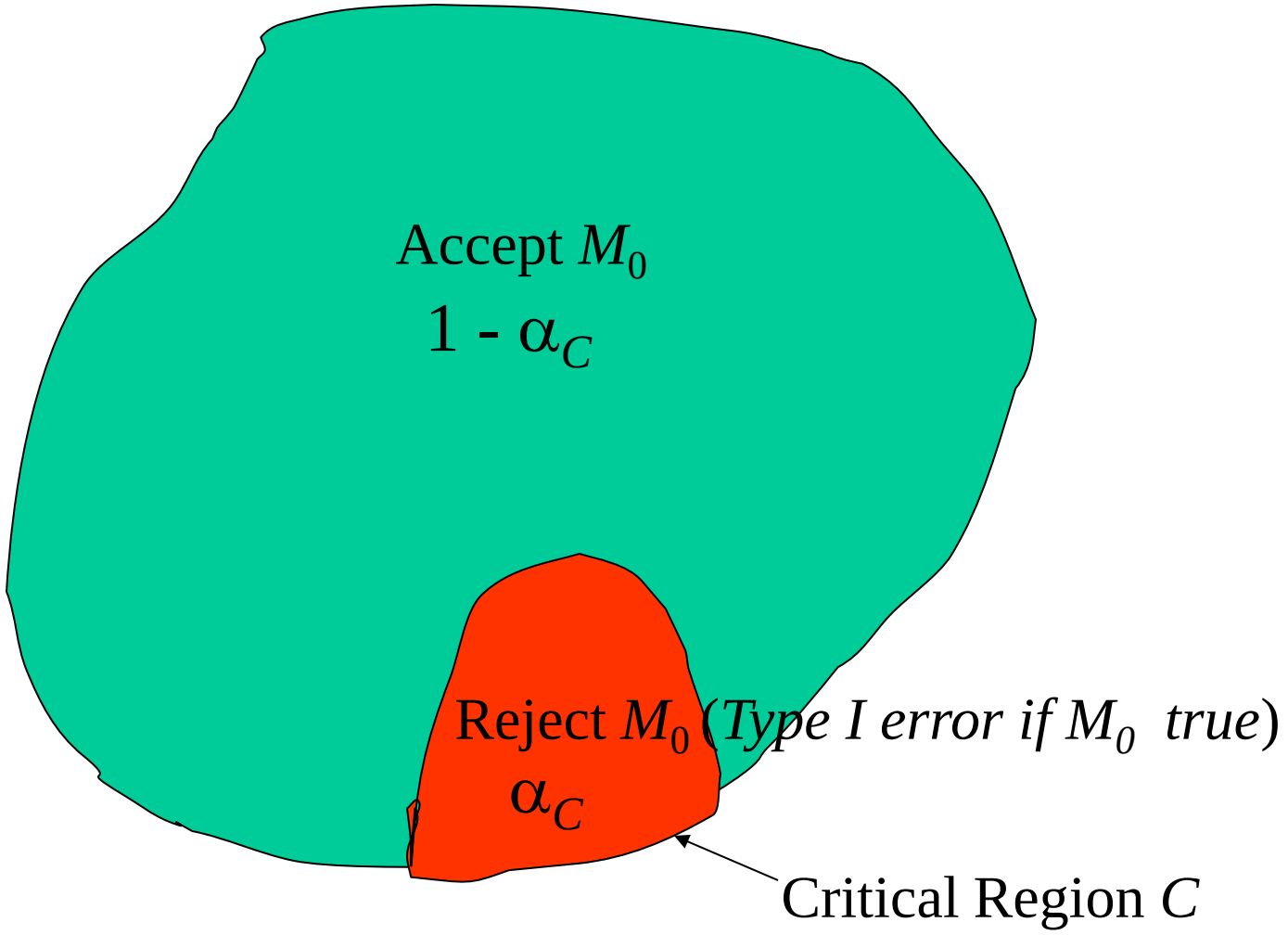
Types of Errors with Hypothesis Test

- a *Type I error* occurs if we reject M_0 when it is true.
  - For a given critical region C, the prob of committing a Type I error is denoted α_C

$$\alpha_C = P(C \mid M_0) = \Sigma_{s \in C} P(s \mid M_0)$$

- α_C is called the *significance level* of the test

Sample Space S – probabilities under M_0

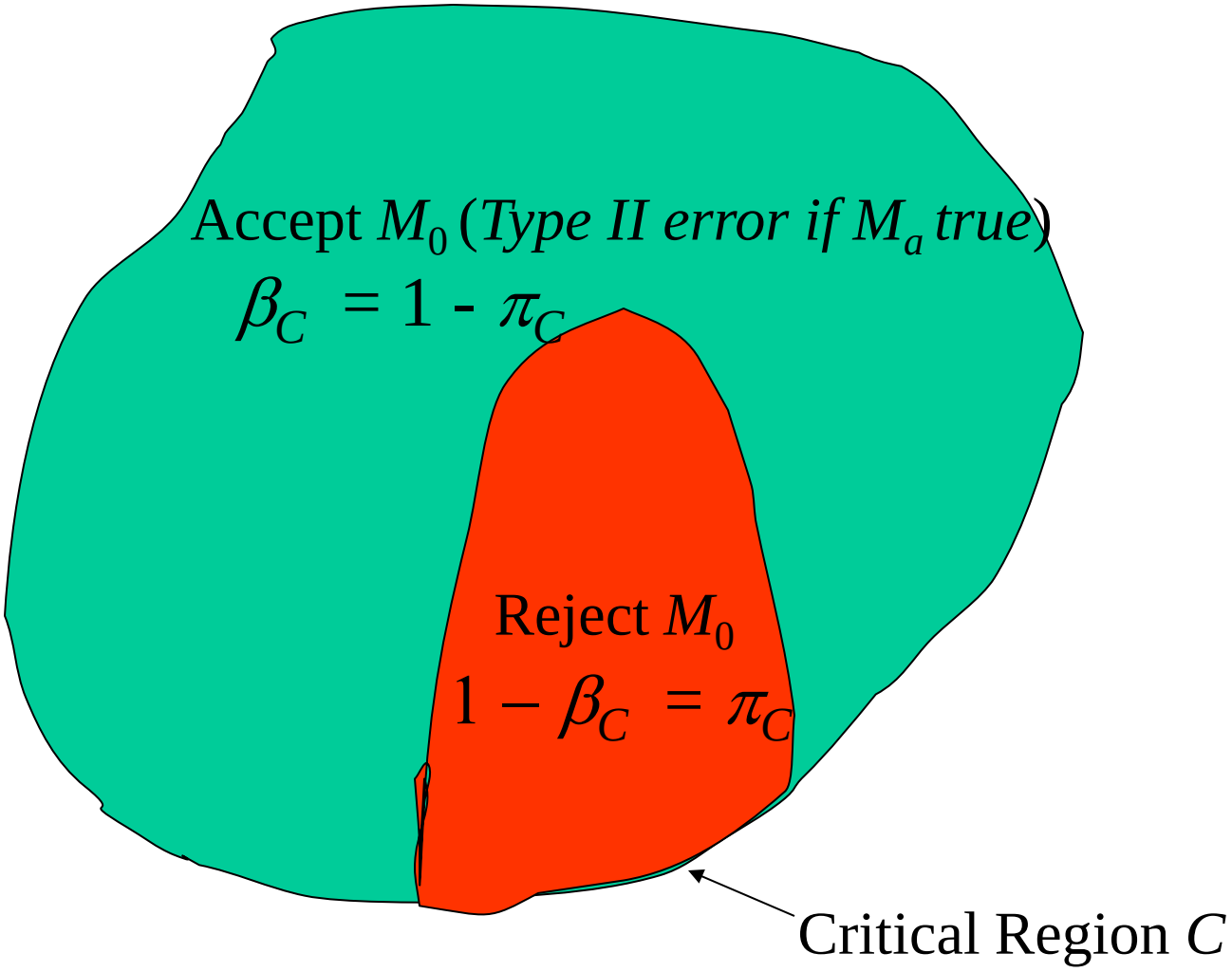


- a *Type II error* occurs if we accept M_0 when it is false.
  - For a given C, prob of committing a Type II error is denoted β_C

$$\beta_C = \Sigma_{s \notin C} P(s \mid M_a) = 1 - P(C \mid M_a)$$

- $\pi_C = 1 - \beta_C$ is called the *power* of the test.

Sample Space S – probabilities under M_a



- Designing a test involves a tradeoff between significance and power
  - smaller C gives smaller Type I error but larger Type II error (lower power).

Likelihood Ratio Tests

- A *likelihood ratio test* of models M_a and M_0 is a hypothesis test of the two models, with critical region C defined by

$$C = C_\Lambda = \{s \mid LR(M_a, M_0 \mid s) \geq \Lambda\}$$

for some non-negative constant Λ, the *cutoff value*.

- Neyman-Pearson lemma motivates use of the *likelihood ratio* as an optimal *discriminator*, or "score"
  - even in contexts where we aren't explicitly testing hypotheses.
- any monotonic function $f(LR)$ of likelihood ratio has equivalent optimality properties
  - because defines the same set of critical regions:

$$LR(M_a, M_0 \mid s) \geq \Lambda \Leftrightarrow f(LR(M_a, M_0 \mid s)) \geq f(\Lambda)$$

- convenient to take f to be the log function, in which case we get the *log likelihood ratio*.

Neyman-Pearson lemma

Let M_a and M_0 be two models, and C_Λ the critical region defined by a likelihood ratio test of M_a vs. M_0 with

- cutoff value Λ,
- significance level α_Λ, and
- power $\pi_\Lambda = 1 - \beta_\Lambda$.

Then if C is any other critical region, we have

- If $\alpha_C < \alpha_\Lambda$, then $\pi_C < \pi_\Lambda$ (and $\beta_C > \beta_\Lambda$)
- If $\alpha_C = \alpha_\Lambda$, then $\pi_C \leq \pi_\Lambda$ (and $\beta_C \geq \beta_\Lambda$)

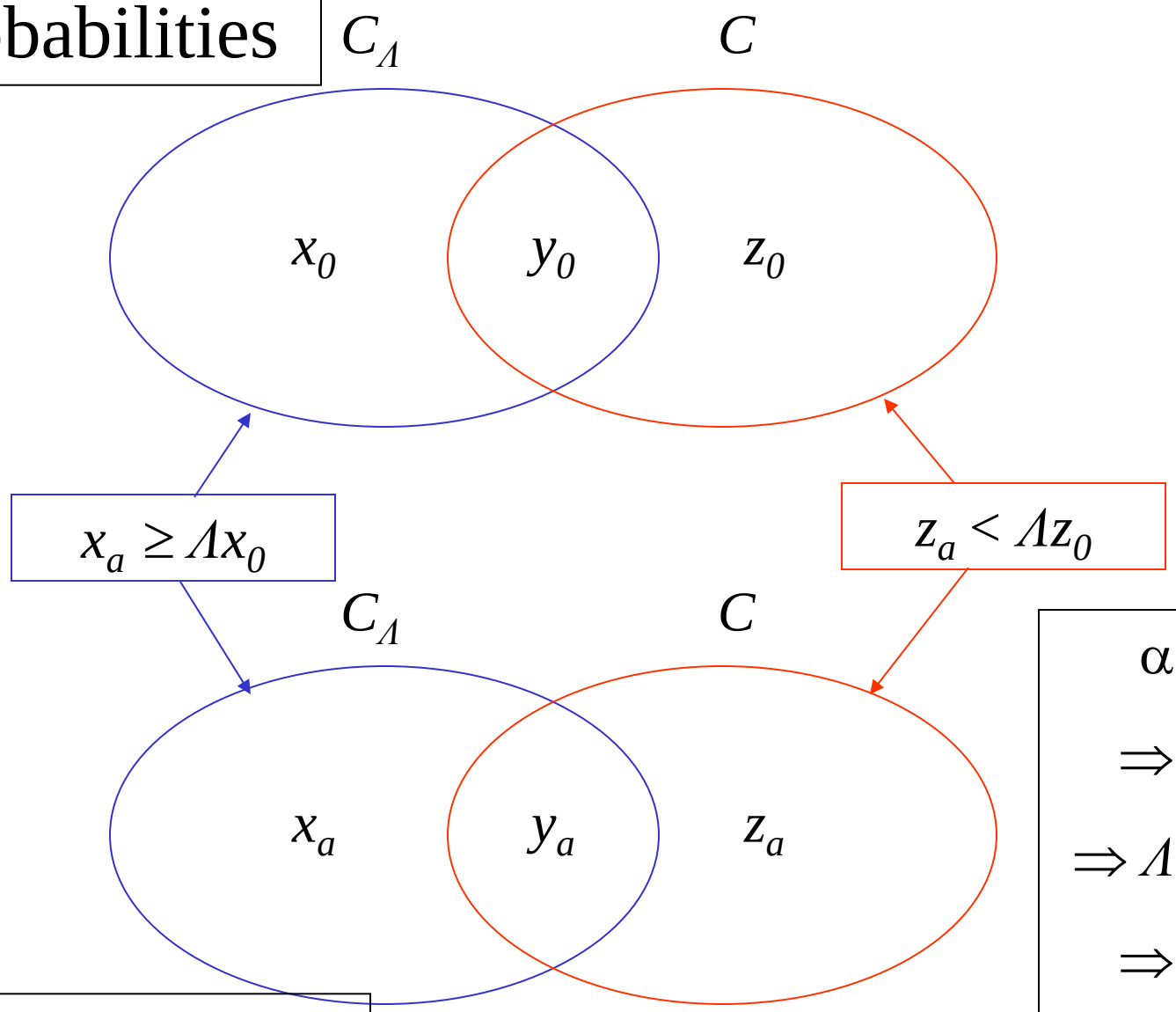
In other words, the likelihood ratio test with significance level α_Λ is the most powerful test

- (has the lowest type II error rate)

with that significance level.

Idea of Neyman-Pearson lemma *proof*:

M_0 probabilities



M_a probabilities

$$\alpha_C < \alpha_\Lambda$$

$$\Rightarrow z_0 < x_0$$

$$\Rightarrow \Lambda\, z_0 < \Lambda\, x_0$$

$$\Rightarrow z_a < x_a$$

$$\Rightarrow \pi_C < \pi_\Lambda$$

- ***Proof***: Suppose $\alpha_C < \alpha_\Lambda$ .Then

$$\sum_{s \in C} P(s \mid M_0) < \sum_{s \in C_\Lambda} P(s \mid M_0)$$

Subtract from both sides the terms involving $s \in C \cap C_\Lambda$ This leaves

$$(1) \quad \sum_{s \in C \setminus C_\Lambda} P(s \mid M_0) < \sum_{s \in C_\Lambda \setminus C} P(s \mid M_0)$$

- By definition of the likelihood ratio test, for any observation s,

$$s \in C_\Lambda \Leftrightarrow P(s \mid M_a) \geq \Lambda P(s \mid M_0)$$

- From this, it follows that

(2) $$\sum_{s \in C \setminus C_\Lambda} \frac{1}{\Lambda} P(s \mid M_a) < \sum_{s \in C \setminus C_\Lambda} P(s \mid M_0)$$

and

(3) $$\sum_{s \in C_\Lambda \setminus C} P(s \mid M_0) \leq \sum_{s \in C_\Lambda \setminus C} \frac{1}{\Lambda} P(s \mid M_a)$$

- Combining (2), (1), and (3)

$$\sum_{s\in C\setminus C_\Lambda}\frac{1}{\Lambda}P(s\,|\,M_a) < \sum_{s\in C\setminus C_\Lambda}P(s\,|\,M_0) < \sum_{s\in C_\Lambda\setminus C}P(s\,|\,M_0) \le \sum_{s\in C_\Lambda\setminus C}\frac{1}{\Lambda}P(s\,|\,M_a)$$

so (cancelling the common factor $1 / \Lambda$)

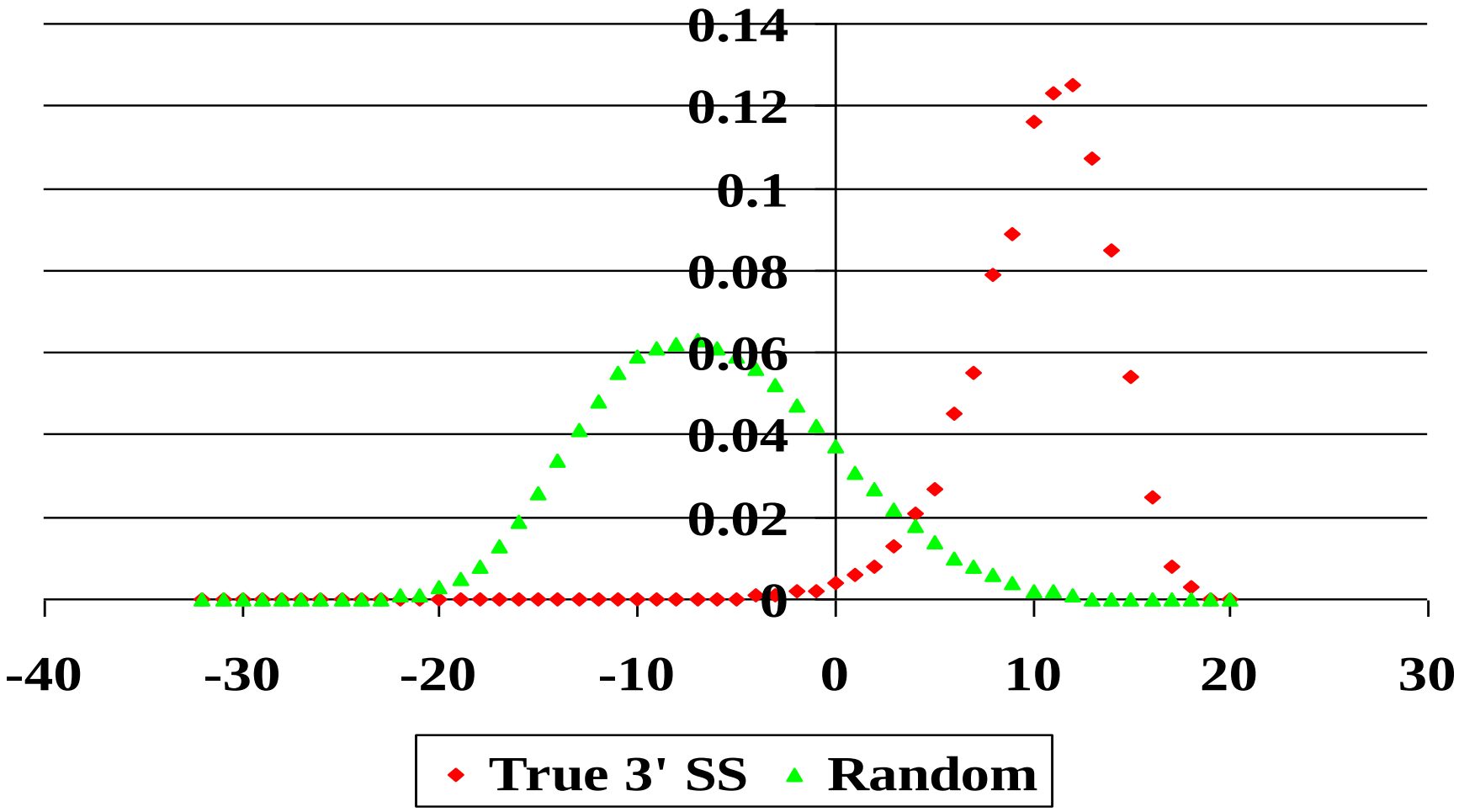
$$\sum_{s\in C\setminus C_\Lambda}P(s\,|\,M_a) < \sum_{s\in C_\Lambda\setminus C}P(s\,|\,M_a)$$

so, adding in the terms corresponding to $s \in C \cap C_\Lambda$

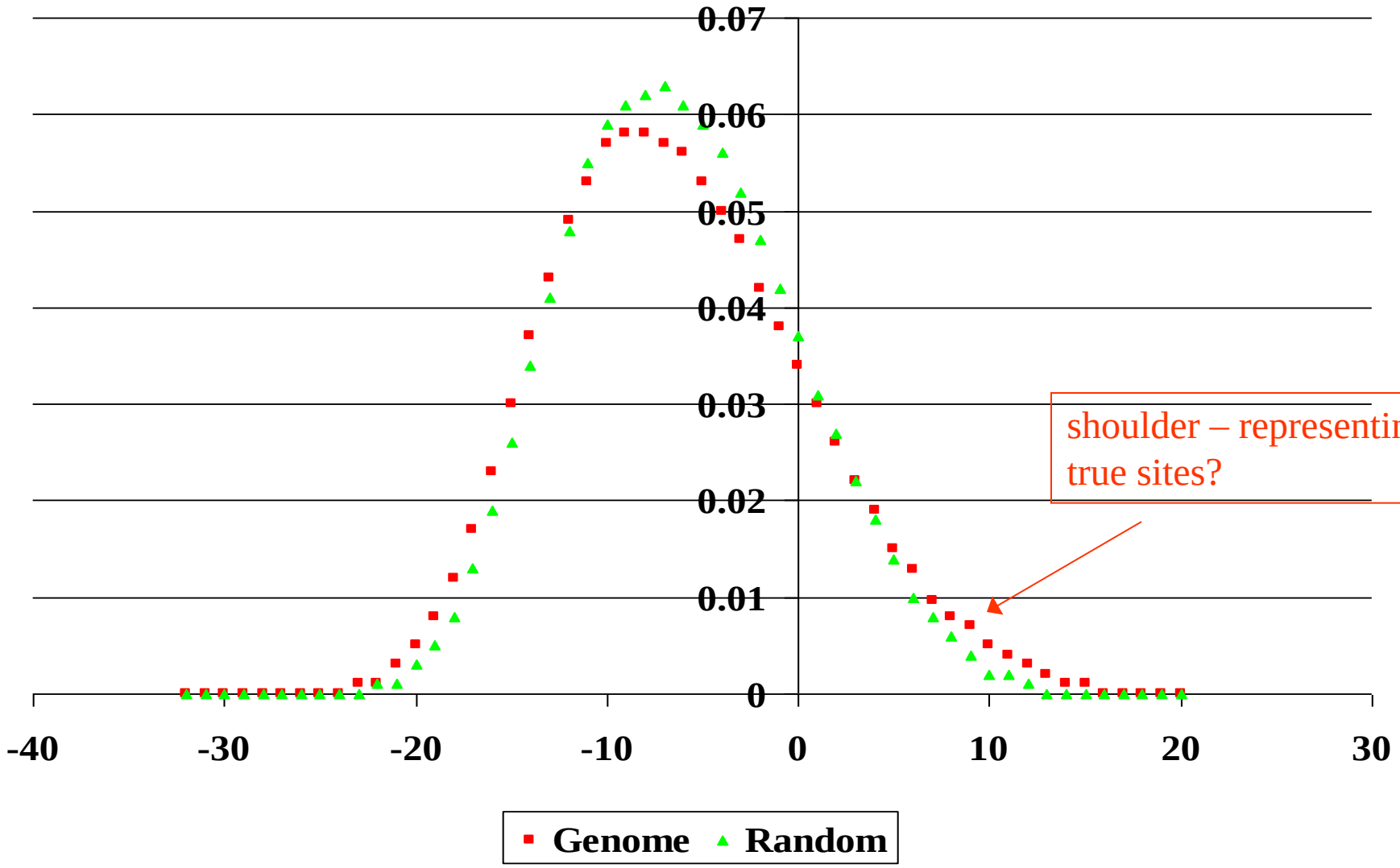
$$\sum_{s\in C}P(s\,|\,M_a) < \sum_{s\in C_\Lambda}P(s\,|\,M_a)$$

i.e $\pi_C < \pi_\Lambda$ The other part of the lemma ($\pi_C \le \pi_\Lambda$ if $\alpha_C = \alpha_\Lambda$) is proved similarly.

Score Distributions (AG sites)– 3’ SS Weight Matrix



Score Distributions (AG sites)– 3’ SS Weight Matrix



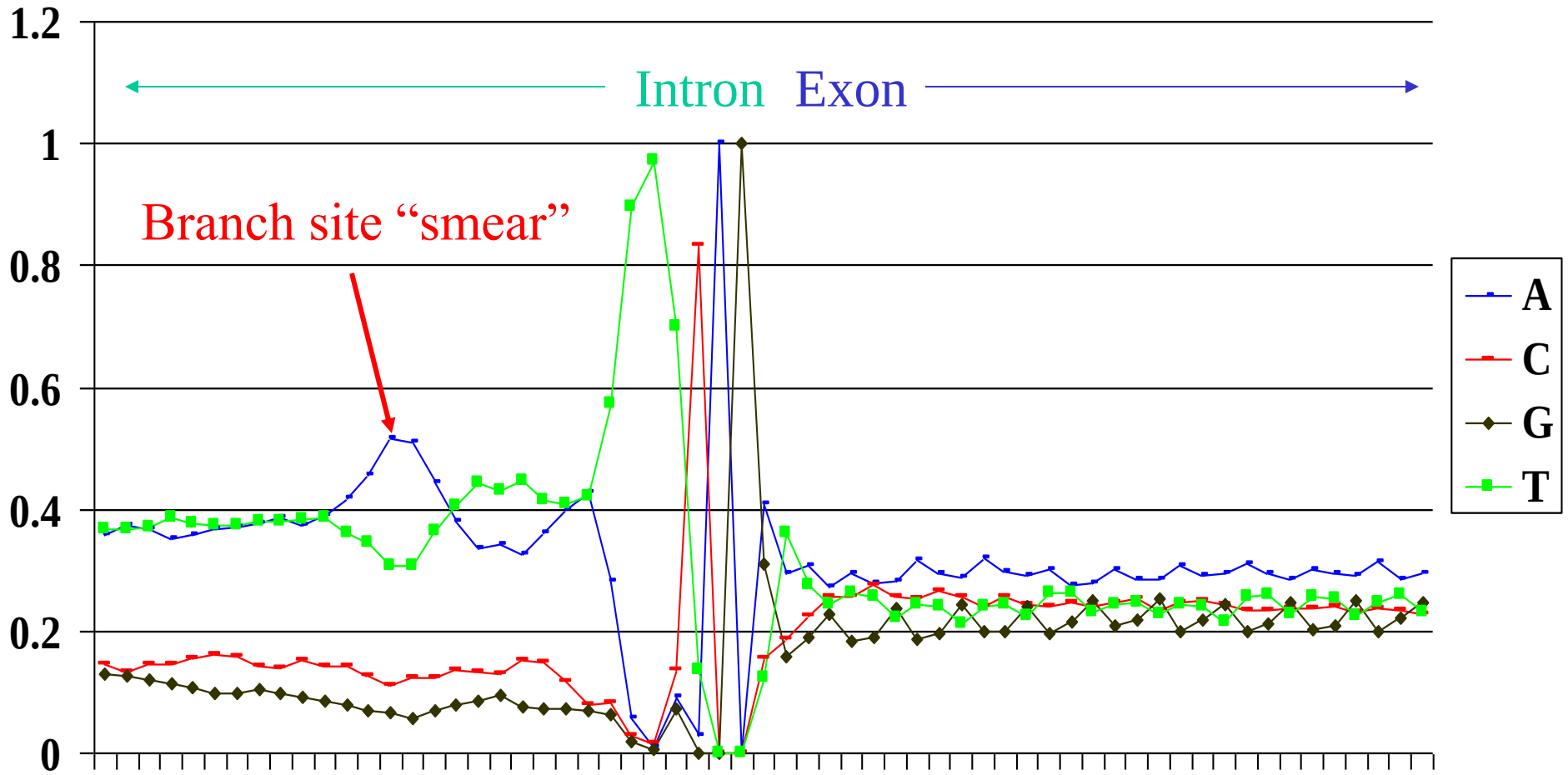
Some Issues for Site Weight Matrices (to be discussed later)

- Can derive *theoretical* probability distribution for scores, and compare with above *empirical* distributions
- Small sample correction to frequencies: pseudocounts
- Avoiding *overfitting* (e.g. using too large a window)

Limitations of Site Models

- Failure to allow indels means variably spaced subelements are “smeared”, e.g.:
  - branch site, for 3’ splice sites;
  - coding sequence, for both 3’ and 5’ sites
    - not really an indel issue -- could make reading-frame-specific matrices
- Independence assumption
  - usually OK for protein sequences (after correcting for evolutionary relatedness)
  - often fails for nucleotide sequences: examples:
    - 5’ sites (Burge-Karlin observation);
    - background (dinucleotide correlation)

3’ Splice Sites – *C. elegans*



Nucleotide Counts for 8192 *C. elegans* 5' Splice Sites

A	3404	4644	1518	0	0	4836	5486	837	1632	2189	2278	2355
C	1850	1224	583	0	14	118	588	237	801	771	889	986
G	1562	912	4891	8192	0	1890	672	6164	589	962	1056	827
T	1376	1412	1200	0	8178	1348	1446	954	5170	4270	3969	4024

CONSENSUS	x	a	g	G	T	a	a	g	t	t	w	t

A	0.416	0.567	0.185	0.000	0.000	0.590	0.670	0.102	0.199	0.267	0.278	0.287
C	0.226	0.149	0.071	0.000	0.002	0.014	0.072	0.029	0.098	0.094	0.109	0.120
G	0.191	0.111	0.597	1.000	0.000	0.231	0.082	0.752	0.072	0.117	0.129	0.101
T	0.168	0.172	0.146	0.000	0.998	0.165	0.177	0.116	0.631	0.521	0.484	0.491

Failure of independence for 5' splice sites: G vs. H ('not G') at position -1

H in position –1 :

A	1434	1664	1518	0	0	2032	2662	98	479	694	783	912
C	633	546	583	0	5	36	177	22	225	250	350	393
G	628	553	0	3301	0	943	187	3063	134	329	405	279
T	606	538	1200	0	3296	290	275	118	2463	2028	1763	1717

A	0.434	0.504	0.460	0.000	0.000	0.616	0.806	0.030	0.145	0.210	0.237	0.276
C	0.192	0.165	0.177	0.000	0.002	0.011	0.054	0.007	0.068	0.076	0.106	0.119
G	0.190	0.168	0.000	1.000	0.000	0.286	0.057	0.928	0.041	0.100	0.123	0.085
T	0.184	0.163	0.364	0.000	0.998	0.088	0.083	0.036	0.746	0.614	0.534	0.520

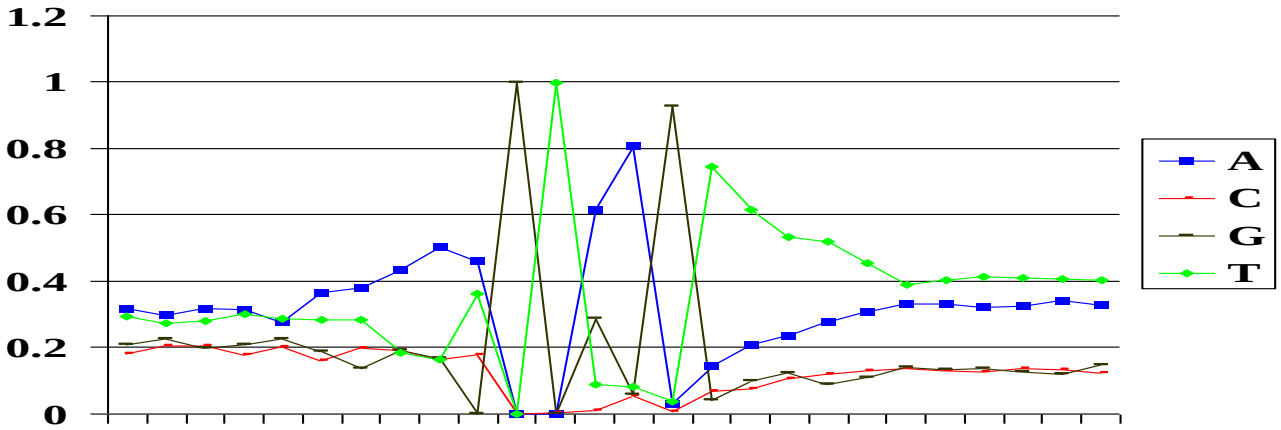
G in position –1 :

A	1970	2980	0	0	0	2804	2824	739	1153	1495	1495	1443
C	1217	678	0	0	9	82	411	215	576	521	539	593
G	934	359	4891	4891	0	947	485	3101	455	633	651	548
T	770	874	0	0	4882	1058	1171	836	2707	2242	2206	2307

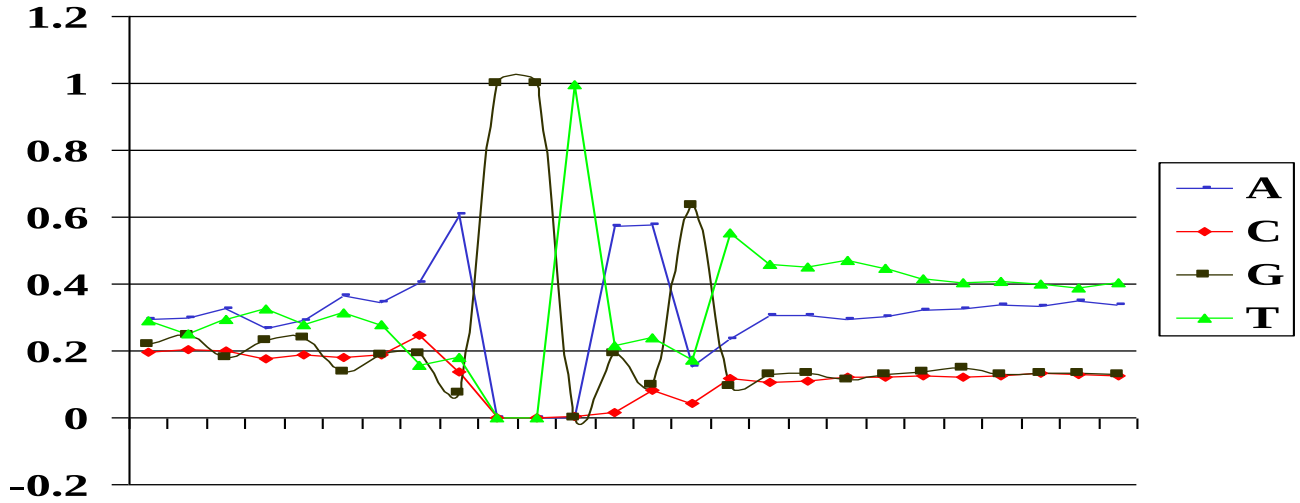
A	0.403	0.609	0.000	0.000	0.000	0.573	0.577	0.151	0.236	0.306	0.306	0.295
C	0.249	0.139	0.000	0.000	0.002	0.017	0.084	0.044	0.118	0.107	0.110	0.121
G	0.191	0.073	1.000	1.000	0.000	0.194	0.099	0.634	0.093	0.129	0.133	0.112
T	0.157	0.179	0.000	0.000	0.998	0.216	0.239	0.171	0.553	0.458	0.451	0.472

5’ Splice Sites – *C. elegans*

H at –1:



G at –1:



Why the correlation?

- Splicing involves pairing of a small RNA (U1 RNA) with the transcript at the 5’ splice site (positions -2 to +7).
- The RNA is complementary to the 5’ ss consensus sequence.
- A mismatch at position –1 tends to destabilize the pairing, & makes it more important for other positions to be correctly paired.

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C	1850	1224	583	0	14	118	588	237	801	771	889	986
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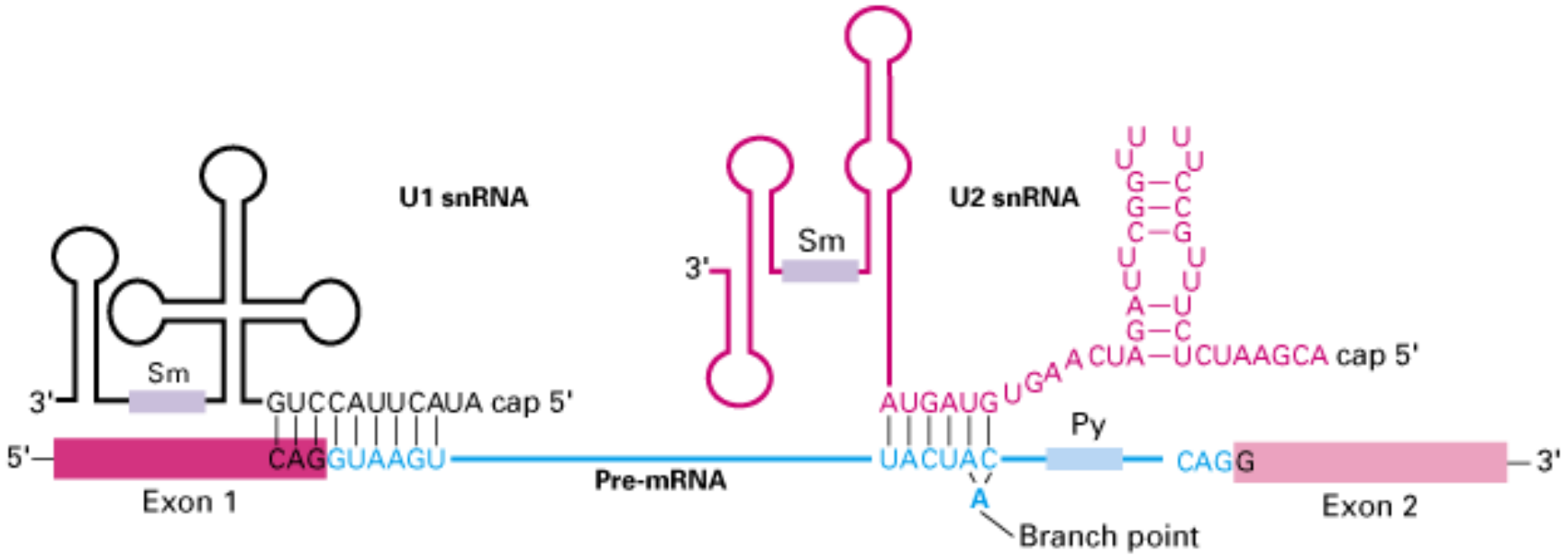
T	1376	1412	1200	0	8178	1348	1446	954	5170	4270	3969	4024

CONSENSUS	x	a	g	G	T	a	a	g	t	t	w	t
A	0.416	0.567	0.185	0.000	0.000	0.590	0.670	0.102	0.199	0.267	0.278	0.287
C	0.226	0.149	0.071	0.000	0.002	0.014	0.072	0.029	0.098	0.094	0.109	0.120
G	0.191	0.111	0.597	1.000	0.000	0.231	0.082	0.752	0.072	0.117	0.129	0.101
T	0.168	0.172	0.146	0.000	0.998	0.165	0.177	0.116	0.631	0.521	0.484	0.491

complementary to portion of U1 RNA



from http://departments.oxy.edu/biology/Stillman/bi221/111300/processing_of_hnrnas.htm

(Jonathon Stillman, Grace Fisher-Adams)

Failure of independence for 'background'

Nucleotide Freqs (*C. elegans* chr. 1):
A 4575132 (.321) ; C 2559048 (.179) ; G 2555862 (.179); T 4582688 (.321)

dinucleotide frequencies (5′ nuc to left, 3′ nuc at top – e.g. obs freq of *ApC* is .047): (Note "symmetry"!)

	Observed				Expected (*under independence*)			
	A	C	G	T	A	C	G	T
A	0.135	0.047	0.051	0.088	0.103	0.057	0.057	0.103
C	0.061	0.035	0.033	0.051	0.057	0.032	0.032	0.058
G	0.063	0.034	0.034	0.047	0.057	0.032	0.032	0.057
T	0.061	0.064	0.061	0.135	0.103	0.058	0.057	0.103

Observed / Expected

	A	C	G	T
A	1.314	0.818	0.885	0.853
C	1.055	1.075	1.031	0.886
G	1.106	1.062	1.074	0.818
T	0.597	1.105	1.056	1.313

Failure of independence for background (cont'd)

Conditional probability (in *C. elegans*) of a given nucleotide (top) occurring, given the preceding nucleotide (left)

	A	C	G	T
A	0.421	0.147	0.159	0.274
C	0.338	0.193	0.185	0.284
G	0.355	0.190	0.192	0.263
T	0.191	0.198	0.189	0.421

Deviations From Expectation

- Underrepresentation of *T*p*A*: found in nearly all genomes;
  - reason unknown:
    - neutral (mutation patterns)?
    - selection?
- Overrepresentation of *A*p*A*, *T*p*T*, *C*p*C*, *G*p*G* – also frequently observed in other organisms.
- Unlike mammalian genomes, no underrepresentation of *C*p*G*
  - *C*p*G* not methylated in *C. elegans* (or most other non-vertebrates).

Dinucleotide Freqs – *H. sapiens* Chr.21

Nucleotide Freqs:

A	10032226	0.297;	T	9962530	0.295
G	6908202	0.204;	C	6921020	0.205

Entropy: 1.976 bits

Observed Dinuc Freqs

	A	C	G	T
A	0.099	0.051	0.069	0.078
C	0.073	0.052	0.011	0.069
G	0.059	0.043	0.052	0.050
T	0.066	0.059	0.072	0.098

Expected (*under independence*)

	A	C	G	T
A	0.088	0.061	0.061	0.087
C	0.061	0.042	0.042	0.060
G	0.061	0.042	0.042	0.060
T	0.087	0.060	0.060	0.087

Observed / Expected

	A	C	G	T
A	1.124	0.839	1.139	0.891
C	1.204	1.243	0.260	1.139
G	0.974	1.025	1.245	0.839
T	0.752	0.976	1.204	1.125

Dinucleotide Freqs – *H. sapiens* Chr.22

Nucleotide Freqs:

A	8745910	0.261;	T	8720493	0.261
G	7999585	0.239;	C	7997931	0.239

Entropy: 1.999 bits

Observed Dinuc Freqs

	A	C	G	T
A	0.077	0.051	0.075	0.058
C	0.077	0.071	0.016	0.075
G	0.061	0.057	0.071	0.051
T	0.047	0.061	0.077	0.076

Expected (*under independence*)

	A	C	G	T
A	0.068	0.062	0.062	0.068
C	0.062	0.057	0.057	0.062
G	0.062	0.057	0.057	0.062
T	0.068	0.062	0.062	0.068

Observed / Expected

	A	C	G	T
A	1.125	0.817	1.205	0.855
C	1.233	1.236	0.285	1.206
G	0.975	0.989	1.237	0.818
T	0.684	0.977	1.233	1.124