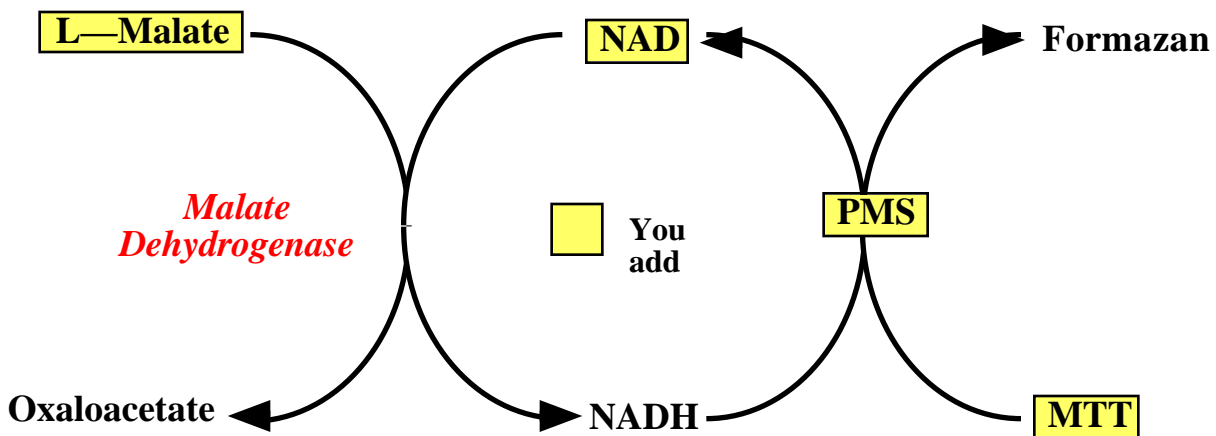
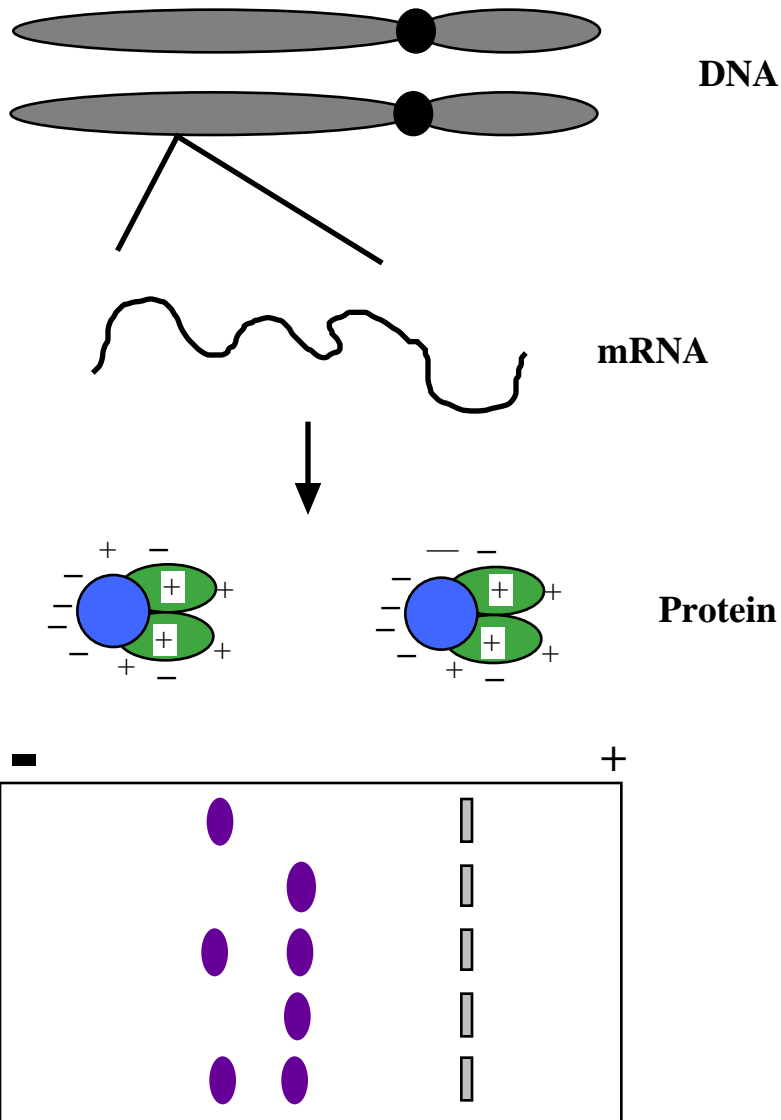
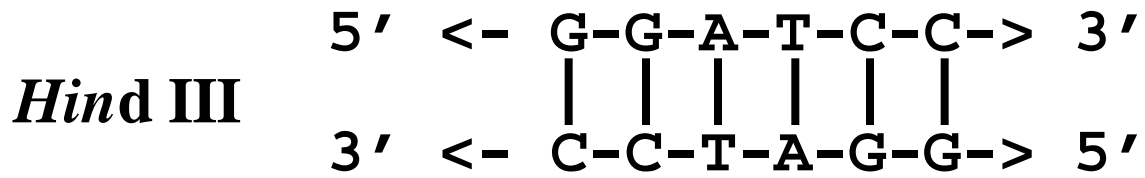


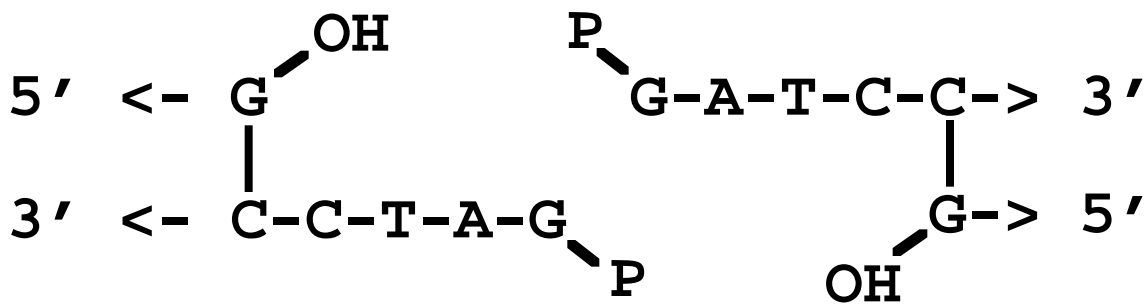
Allozyme Electrophoresis



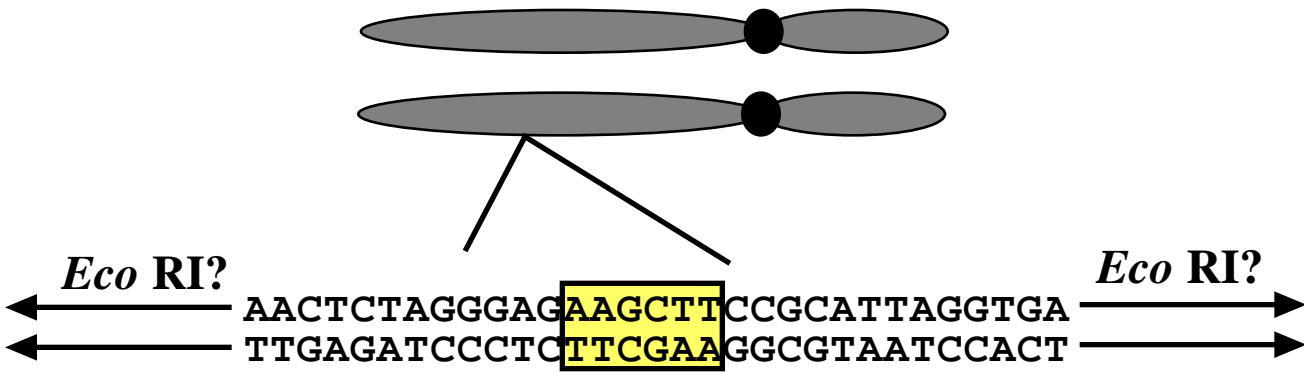
Restriction Endonucleases



Cut the DNA



Single locus RFLP

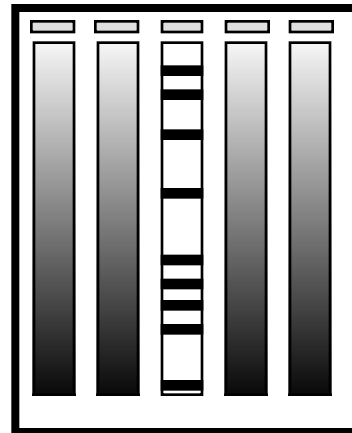
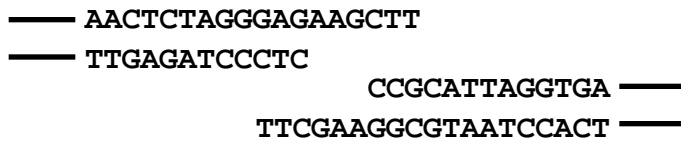


Eco RI

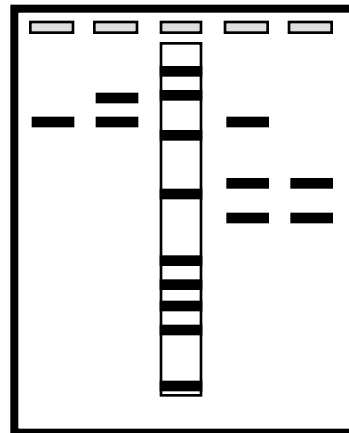
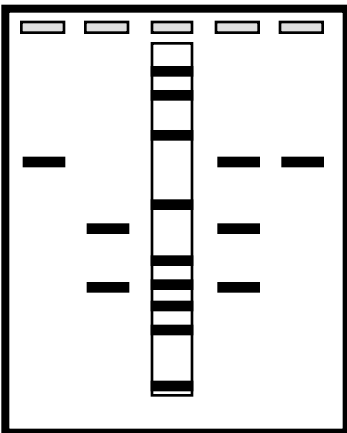
PCR Amplify

Digest total cell DNA with *Eco RI*

Digest Amplified DNA with *Eco RI*



Hybridize with Radioactively Labeled DNA



Sequence Alignment for Bonefish

brazil A			
brazil B	-----A-----	-----A-C-----	-----T-----
brazil C	-----A-----	-----A-C-----	-----T-----
brazil D			
brazil E			
brazil F			
belize A	-----A-A-GA-:--G		-----A-----G-----C-G--
belize B	-----A-A-GA-:--G		-----A-----G-----C-G--
caribbean A	-----A-A-GA-:--G		-----A-----G-----C-G--
caribbean B	-----A-A-GA-:--G		-----A-----G-----C-G--
florida A	-----A-A-GA-:--G		-----A-----G-----C-G--
florida B	-----A-A-GA-:--G		-----A-----G-----C-G--
florida C	-----A-A-GA-:--G		-----A-----G-----C-G--
florida D			
florida E			
florida G			
florida H			
florida I			
florida J			
florida K			
florida L			

Numbers of Oysters Exhibiting Indicated Genotypes at Four scnDNA Loci in 15 Locales

Locus	Population														
	MA*	SC*	GA*	SA	FL	SM	SM2*	OH	DP	PI	SE	VB	FP	ST	ST2*
CV-32															
AA	20	7	18	11	7	10	18	6	2	1	2	1	4	0	2
AB	8	12	8	5	8	9	6	9	3	5	7	7	5	4	11
BB	3	3	4	3	4	2	2	4	12	12	13	12	11	16	15
CV-19															
AA	2	2	6	1	1	5	3	2	5	6	6	3	8	6	10
AB	7	10	10	8	8	10	10	9	8	6	8	9	4	5	8
BB	7	11	14	7	10	6	16	9	5	5	8	8	8	9	9
CV-195															
AA	11	3	3	1	1	0	4	3	2	0	4	4	3	2	0
AB	21	13	17	14	14	16	19	10	7	10	14	8	7	9	13
BB	1	5	11	3	5	5	6	7	10	7	3	8	8	8	15
CV-7.0															
AA	25	20	29	19	15	21	27	15	10	8	11	12	12	9	14
AB	1	2	1	1	5	1	3	3	2	6	8	3	3	2	3
BB	0	0	2	0	0	0	0	0	6	4	2	4	5	6	9
CV-7.7															
AA	28	19	22	16	12	19	18	17	1	3	7	5	6	6	4
AB	3	4	10	4	8	3	10	2	10	10	14	10	9	6	11
BB	0	0	0	0	0	0	0	0	6	5	2	4	5	7	14

MA:

$$F(A) = \frac{(2 \times 20) + 8}{62} = 0.7741$$

$$F(B) = \frac{(2 \times 3) + 8}{62} = 0.2259$$

$$\hat{p} = 0.7741$$

$$SE = \sqrt{\frac{(0.7741) \times (0.2259)}{62}} = 0.0531$$

FP:

$$F(A) = \frac{(2 \times 4) + 5}{40} = 0.325$$

$$F(B) = \frac{(2 \times 11) + 5}{40} = 0.675$$

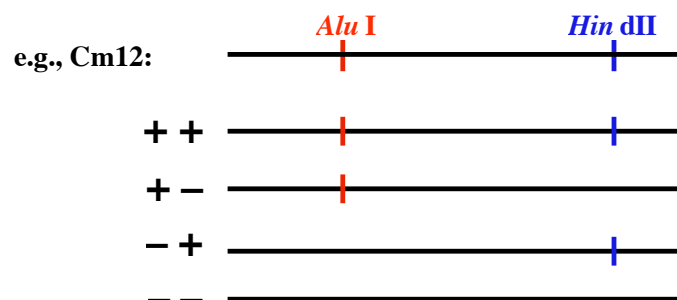
Table 3. Allele frequency, mean sample size (N), direct count heterozygosity (h), average heterozygosity over all loci (H), average number of alleles (A), and percent loci polymorphic (P)^a. (SD = standard deviations).

Locus ^c	Population ^b														
	ATLANTIC									PACIFIC					
	ASC	CSR	SUR	FLA	MEX-A	VEN	BRA	AFR	CYP	JAP	HAW	AUS	MEX-B	GAL	OMA
N	45.2	21.8	14.2	23.0	6.8	8.8	16.8	17.6	9.0	19.0	21.8	15.8	7.0	7.6	14.4
(SD)	(1.1)	(0.4)	(0.8)	(0.0)	(0.2)	(0.2)	(0.2)	(3.1)	(0.0)	(0.0)	(0.2)	(0.2)	(0.0)	(0.4)	(0.4)
CM-12															
- +	0.75	0.89	0.90	0.96	0.93	1.00	0.79	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00
+ -	0.25	0.11	0.10	0.04	0.07	0.00	0.21	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
h	0.43	0.23	0.20	0.09	0.14	0.00	0.41	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CM-14															
- +	0.16	0.44	0.17	0.52	0.86	0.44	0.30	0.05	0.00	0.71	0.84	0.53	0.21	0.44	0.40
+ -	0.50	0.12	0.26	0.09	0.00	0.00	0.41	0.47	0.06	0.00	0.00	0.00	0.22	0.06	0.07
+ +	0.34	0.44	0.57	0.39	0.14	0.56	0.29	0.48	0.94	0.29	0.16	0.47	0.57	0.50	0.53
h	0.57	0.70	0.53	0.61	0.29	0.67	0.59	0.63	0.11	0.37	0.32	0.56	0.43	0.38	0.40
CM-39															
- -	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.06	0.00
- +	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.87	1.00	0.94	1.00
h	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.00	0.13	0.00
CM-45															
- -	0.77	0.77	0.73	0.70	0.79	0.56	0.65	0.66	0.67	0.79	0.59	0.50	0.14	0.25	0.40
- +	0.23	0.23	0.27	0.30	0.21	0.44	0.35	0.34	0.33	0.21	0.41	0.50	0.86	0.75	0.60
h	0.30	0.46	0.40	0.44	0.14	0.67	0.47	0.37	0.44	0.32	0.73	0.75	0.29	0.25	0.40
CM-67															
- -	0.67	0.19	0.47	0.33	0.29	0.50	0.94	0.58	0.28	0.69	0.88	0.28	0.93	0.75	0.43
- +	0.21	0.05	0.03	0.11	0.07	0.00	0.03	0.21	0.05	0.26	0.02	0.44	0.07	0.00	0.32
+ -	0.12	0.76	0.50	0.56	0.64	0.50	0.03	0.21	0.67	0.05	0.10	0.28	0.00	0.25	0.25
h	0.35	0.48	0.60	0.61	0.71	0.50	0.13	0.67	0.44	0.47	0.14	0.69	0.14	0.17	0.29
H	0.34	0.37	0.35	0.35	0.26	0.37	0.32	0.38	0.20	0.23	0.24	0.44	0.17	0.18	0.22
SD	0.08	0.12	0.11	0.13	0.12	0.15	0.11	0.13	0.10	0.10	0.14	0.14	0.08	0.06	0.09
A	2.4	2.2	2.2	2.2	2.0	1.6	2.2	2.2	1.8	1.8	1.8	2.0	1.8	2.0	2.0
SD	0.2	0.4	0.4	0.4	0.3	0.2	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.3	0.4
P	100	80	80	80	80	60	80	80	60	60	60	80	60	80	60

^a Excluding non-variable enzymes and loci.

^b See legend to Figure 1 for location abbreviations.

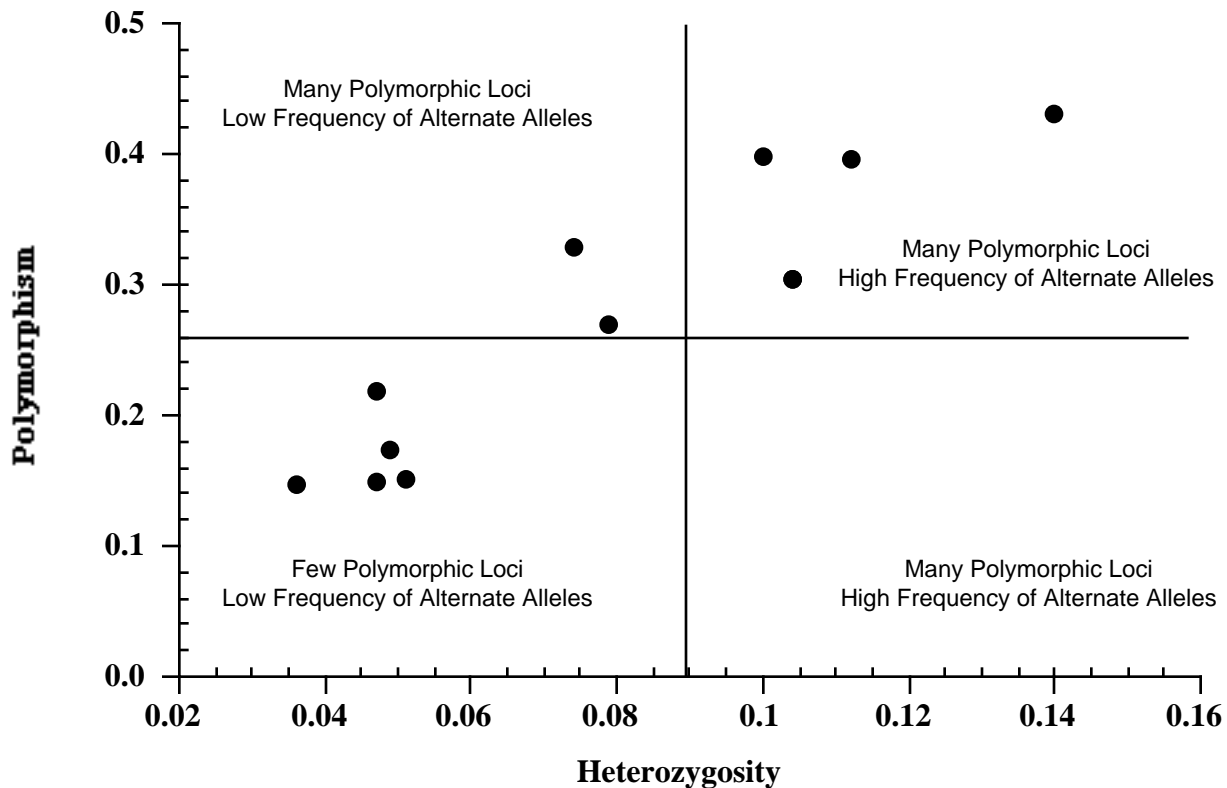
^c See footnote to Table 2 for allele designations.



The heterozygosity for 71 allozyme loci in humans (Harris and Hopkinson, 1972)

Locus	Heterozygosity
51 Monomorphic Loci	0.00
Peptidase C	0.02
Peptidase D	0.02
Glutamate-oxaloacetate transaminase	0.03
Leucocyte Hexokinase	0.05
6-Phosphogluconate dehydrogenase	0.05
Alcohol dehydrogenase-2	0.07
Adenylate kinase	0.09
Pancreatic amylase	0.09
Adenosine deaminase	0.11
Galatase-1-phosphate uridyl transferase	0.11
Acetyl cholinesterase	0.23
Mitochondrial malic enzyme	0.30
Phosphoglucomutase-1	0.36
PeptidaseA	0.37
Phosphoglucomutase-3	0.38
Pepsinogen	0.47
Alcohol dehydrogenase-3	0.48
Glutamate-pyruvate transaminase	0.50
RBC acid phosphatase	0.52
Placental alkaline phosphatase	0.53

Estimated levels of heterozygosity and proportion of polymorphic loci from allozyme studies (Nevo, 1978)



Theoretical Population Genetics

Homework I:

Calculation of π :

Allele	Variable Position															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	T	C	T	A	C	C	T	C	C	T	C	G	G	T	T	A
B	T	C	C	T	A	C	C	T	C	C	T	G	G	T	T	T
C	C	T	C	C	C	C	C	T	C	T	T	T	G	C	T	A
D	C	T	C	C	C	C	C	T	T	C	T	G	A	C	T	T
E	C	T	C	C	C	T	C	T	T	T	T	G	G	C	C	A

Assume:

Entire sequence is 500 bp and
there were: 10 allele A

5 allele B

2 allele C

2 allele D

1 allele E

Total 20 sequences

$$p_{ij} = \frac{p_{ij}}{m_t}$$

$$\hat{\pi} = \frac{n}{n-1} \sum_{i=1}^{n-1} \sum_{j=i+1}^n x_i x_j$$

$$\text{Var}(\hat{\pi}) = \frac{n+1}{3(n-1)m_t} \hat{\pi} + \frac{2(n^2 + n + 3)}{9n(n-1)} \hat{\pi}^2$$

Where: p_{ij} = number of nucleotide differences between sequence i and J ;

m_t = total number of nucleotides;

n = number of sequences;

x_i = frequency of the i^{th} sequence;

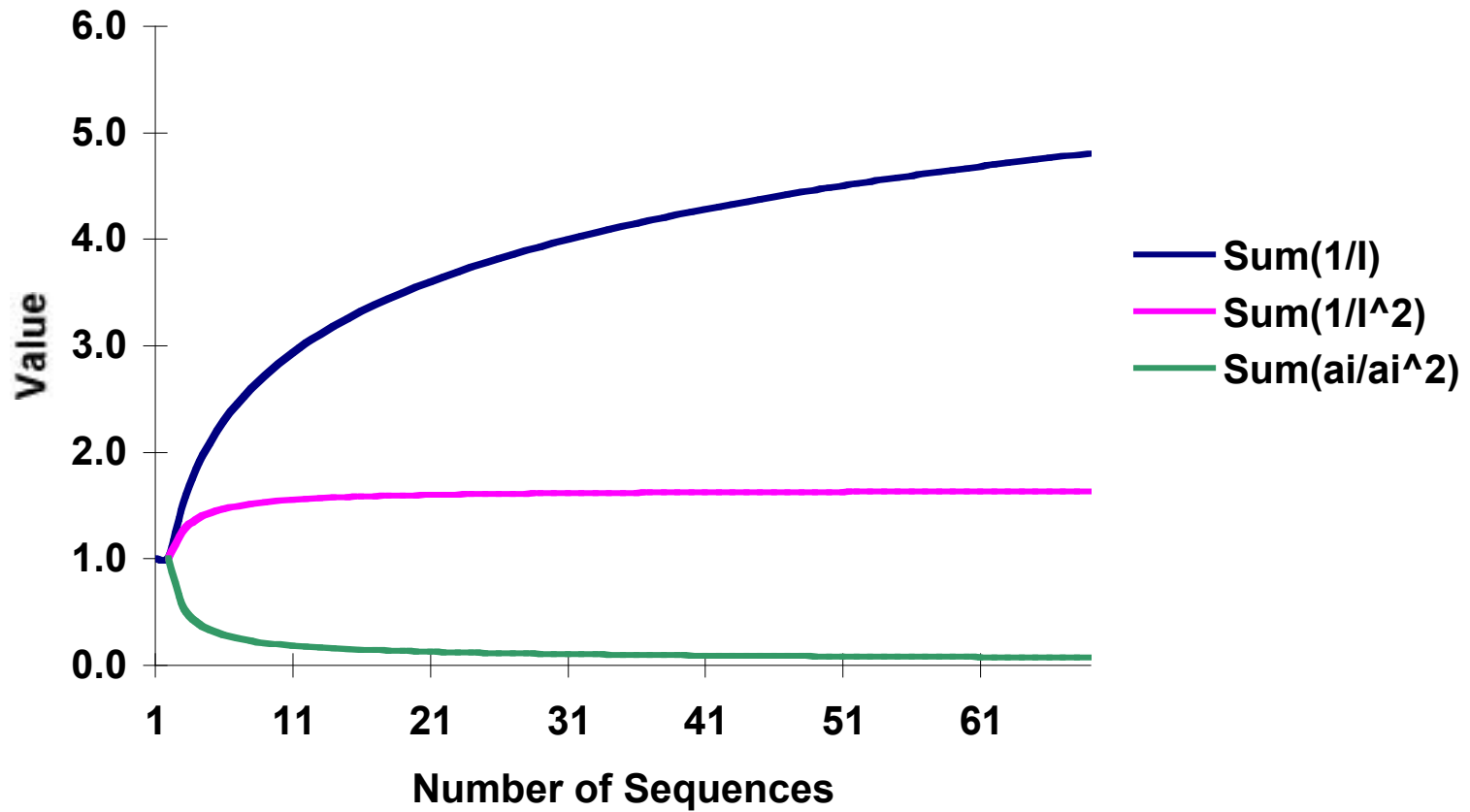
x_j = frequency of the j^{th} sequence;

e.g.,

$$p_{AB} = \frac{8}{500} = 0.016$$

$$\hat{\pi} = \frac{5}{4} [(0.5 \times 0.25 \times \pi_{AB}) + (0.5 \times 0.2 \times \pi_{AC}) + (0.5 \times 0.2 \times \pi_{AD}) + (0.5 \times 0.1 \times \pi_{AE}) + \dots]$$

Nucleotide Polymorphism



Theoretical Population Genetics

Examples of Naturally Occurring Genetic Variation

Estimate of proportion of polymorphic loci and average heterozygosity of human enzymes.

Number of Loci	P	H	Reference
10	0.300	0.099	Harris 1966
26	0.308	0.072	Harris 1966
71	0.282	0.067	Harris and Hopkinson 1972
104	0.231	0.063	Harris and Hopkinson 1972

Average heterozygosity at allozyme loci whose products have different metabolic functions

Organism	Variable Substrate	Enzyme Regulatory Enzymes	Category Non-regulatory Enzymes	Average
Other Insects	0.289	0.281	0.094	0.221
Drosophila	0.205	0.210	0.086	0.167
Amphibians	0.118	0.227	0.062	0.137
Non-insect invert.	0.169	0.100	0.122	0.130
Birds	0.088	0.096	0.151	0.112
Fish	0.063	0.110	0.066	0.080
Reptiles	0.079	0.039	0.039	0.052
Mammals	0.048	0.059	0.032	0.046
Average	0.175	0.161	0.073	0.136

General Allozyme Studies

Group	# species	P	H
Giant Clam	3	85	0.35
Starfish	1	87.75	0.2665
Sponges	3	75	0.26
Krill	3	47	0.141 (0.026)
Foraminiferan	1	75	0.125
Salmon	1	41.7	0.124
Polycheate (Temperate)	1	28.2	0.073 (0.007)
Polycheate (Antarctic)	1	11.7	0.038 (0.018)
Amphipods & copepods	6	39.87	0.054 - 0.241
Insects			
Solitary	37	-	0.069 (0.0009)
Eusocial	73	-	0.036 (0.004)
Social parasites	5	-	0.010 (0.002)

Nuclear DNA Loci

Group/Method	# Species	Loci	H
nDNA RFLP			
Cod	1	24	0.328 (0.035)
Scallop	1	11	0.221 (0.040)
Microsatellite			
Advanced Eusocial	4	4	0.700 (0.069)
Primitive Eusocial	8	5.4	0.338 (0.055)
Drosophila	1	18	0.504
Brown Trout	1	5	0.636 (0.112)